Effetcs of root zone composition and nitrogen and phosphorus rates on mycorrhizal colonization in different turfgrass species on sand-based golf greens in Scandinavia

Effekter av rotsonens sammensetning og nitrogen og fosfor nivåer på mykorrhiza kolonisering i ulike arter av sportsgress på sandbaserte golf greener i Skandinavia

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Preface

This thesis is a result of my five years of biology studies here at the Norwegian University of Life sciences (UMB), and also marsk the end of a two-year master's program in microbiology. It was created thanks to some dedicated and benevolent researchers at the Norwegian Institute for Agricultural and Environmental Research (Bioforsk), who met me with open arms and immediately responded positively to my request of collaboration on an external master thesis involving environmental microbiology. The topic is of interest to me because of my passion for nature and its impressive mechanisms in general, and I also wanted to write a thesis from my home town that later could give me the opportunity to work on related topics in the same region.

The work with the thesis was carried out in the period from winter/spring of 2012 to the spring of 2013, at the Norwegian Institute for Agricultural and Environmental Research (Bioforsk). The thesis was written for the Department of Chemistry, Biotechnology and Food Science (IKBM) at the Norwegian University of Life sciences (UMB). My official supervisor at UMB was Arne Tronsmo, and my practical advisors from Bioforsk were Trygve S. Aamlid, Tatsiana Espevig and Erik J. Joner.

The task of writing this thesis has been a great challenge, especially since I have never before written such an extensive paper. There have been times where I have felt tired and frustrated, but I have also felt encouraged that I am part of an important process to provide useful knowledge for future use of land in cultivation. During my work with the thesis I have learned a lot about turfgrass management as well as the mycorrhizal associations in these grasses. I have also gained insight in how research takes place, from planning and performing an experiment, to presenting and interpreting results. The importance of reading updated literature, discussing results with others and being open about own research so that others can make use of the information in their studies, has become very clear.

First of all, I would like to thank Erling Stubhaug, Trygve S. Aamlid, Tatsiana Espevig and Erik J. Joner for believing in me and my work already from our first meeting, and for being so open to new activity in their research institutions. I would also like to thank Aamlid, Espevig, Joner and Agnar Kvalbein for valuable discussions and comments on the topic ‘mycorrhizal colonization in turfgrass’.

A special thank you goes to Aamlid and Espevig for helping me with statistical data analyzes, for giving good and constructive reviews of the thesis, and for always finding the time to answering my questions and sharing their great knowledge and experiences within the turfgrass science. You have taught me to trust my own data, and to not give up on finding logical explanations to why results are as they are. I would also like to thank Joner for rewieving the thesis, for answering my questions about mycorrhizal associations, teaching me the methodology of the mycorrhiza assays, and for guiding me

through the greenhouse experiment. An additional person who deserves a special thank you is Theo Ruissen, for help with identifying mycorrhizal diversity, photography through the microscope and for providing significant amounts of relevant litterature and information on mycorrhiza in general.

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Last but not least, I would like to thank the rest of my 'colleagues' at Bioforsk, Landvik for their encouragement and contributions to a good and healthy social environment, and for making my semester here so pleasant. Thanks to my dear parents for always supporting and believing in me, and to my boyfriend for bearing over with my varying mood and lack of prescence during the most stressfull periods. I would also like to thank my fellow students at UMB for the many hours of collaboration, discussions and social gatherings that have made my time as a student an unforgettable experience.

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Abstract

Mycorrhiza is an ancient and widespread form of symbiosis that takes place when specialized, soil-living fungi colonize plant roots, where they exchange nutrients like nitrogen (N) and phosphorus (P) in return of carbon sugars. Besides enhanced nutrient uptake, mycorrhizal relationships often provide additional benefits like increased resistance to drought, chilling, salinity and pathogens, to the host. In this context, the golf industry, which is challenged by the need to restrict inputs of fertilizers, pesticides and energy for irrigation and mechanical maintenance, is interested in utilizing these symbiotic associations to improve green quality in a more environmentally friendly and cost-effective way. Little is known about mycorrhizal colonization in turfgrasses, but since greens are believed to be generally poor in mycorrhizal forming fungi compared to natural soil habitats, there is focus on applying commercial inocula to 'boost' the extent of colonization in these plants. The ultimate goal is that inoculation will improve green quality through early establishment, enhanced growth and stress tolerance. In addition, the potential in controlling the invasive annual bluegrass (*Poa annua*) by means of mycorrhizal inoculation is under investigation, because this species have been found to benefit less from these symbiotic interactions than other, frequently used turfgrasses on golf greens.

The objective of this thesis was to investigate how green management practices affect mycorrhizal colonization in different turfgrass species. More specifically; I studied how N/P rates, type of growth medium and inoculation with a mycorrhiza product affected arbuscular mycorrhizal colonization in different turfgrass species on sand based golf greens, as well as how the symbiosis affected green quality in interaction with these management practices.

The research was conducted on two already established greens at Bioforsk Landvik, Grimstad, that were testing effects of different green management on turf quality, playability and competition against annual bluegrass on Scandinavian golf greens. Root samples were harvested from both fields, and analyzed for percent mycorrhizal colonization before a suggestion of possible fungal genera were made based on observed mycorrhizal structures. Root weights were also recorded. In addition, data analyses and visual data that had been recorded on turf quality through the whole growth season in conjunction with the main projects were obtained for both fields. An initial greenhouse experiment was performed at the Center for Climate Controlled Plant Research at Ås, Akershus, where the same growth media, mycorrhizal inoculum and turfgrass species as in the field trials were tested over a thirteen week period. Collection of clippings and visual data recordings were performed at regular intervals. By the end of the experiment, the roots from each pot were harvested and analyzed in the same manner as with the samples from the field studies. Total above-ground material was also collected from each pot and weighed.

Turf quality increased with increasing N availability. At the higher N-levels, the incidence of disease and annual bluegrass competition was limited, while the general impression was better. This was also true for root zones amended with compost instead of peat, the former releasing nutrients more easily. However, in the greenhouse study, plants grown with peat as organic amendment had the best growth, due to excessive N-fertilizing in these pots that was overshadowing the effect of compost amendment. There were no significant effects of phosphorus on either turf quality or mycorrhial colonization. Mycorrhizal colonization was high in all turfgrass species, except for the greenhouse experiment, where the inoculum tested showed no significant effects. The bentgrasses seemed to be the most mycorrhizal species, but the difference in colonization between red fescue and annual bluegrass was somewhat unclear. Moreover, these results were not significant. There were no significant effects of N/P rates on mycorrhizal colonization either, but a tendency pointed towards increasing colonization with decreasing N amounts. Furthermore, colonization rates increased when compost was incorporated into the root zone instead of peat. There were no significant effects of inoculation in the field studies, although a small tendency pointed towards a reduction in annual bluegrass, as well as increased P removal from the soil, in inoculated plots.

The results showed several interactions between species and N-level, and between species and growth medium. This underlines that processes related to plant growth and quality as well as symbiosis development is largely controlled by N availability, but also that growth responses to this element are related to individual species growth potentials. Mycorrhizal growth responses was probably neutral or could not be detected in the different species because the high nutrient availability in the greens was overshadowing any mycorrhizal effect, and this further indicates that direct uptake of available N is the primary cause of increased plant fitness in greens. The lack of significances when testing the mycorrhiza inoculum illustrates that successful incorporation of such commercial products into the field may be a challenge. However, use of compost in the root zone of the youngest green seemed to increase the colonization rates, and if simple measures like this can accelerate the establishment of nautral, mycorrhizal fungal populations in the future, inoculating greens during construction may not be necessary.

Sammendrag

Mykorrhiza er en gammel og utbredt form for symbiose som skjer ved at spesialiserte, jordlevende sopper koloniserer planterøtter, hvor de utveksler næringsstoffer som nitrogen (N) og fosfor (P) til gjengjeld for karbohydrater. Foruten økt næringsopptak tilbyr mykorriza ofte flere fordeler for verten, slik som bedre toleranse for tørke, kulde, saltholdighet og patogener. Golfindustrien utfordres av behovet for å begrense mengder av gjødsel, sprøytemidler og energi brukt til vanning og mekanisk vedlikehold, og er i denne sammenheng interessert i å utnytte disse symbiotiske interaksjonene til å vedlikeholde greener på en mer miljøvennlig og kostnadseffektiv måte. Lite er visst om mykorrhiza - kolonisering i sportsgress, men siden greener antas å inneha generelt lite mykorrhizasopper i forhold til naturlige jord habitater, fokuseres det på bruk av kommersielle inokulater for å øke omfanget av kolonisering i disse plantene. Det endelige målet er at inokuleringen skal forbedre greenkvaliteten gjennom tidlig etablering, forbedret vekst og stresstoleranse. I tillegg blir det forsket rundt potensialet i å kontrollere tunrapp (*Poa annua*) ved hjelp av mykorrhiza inokulering, fordi denne arten har vist seg å ha mindre nytte av symbiosen enn andre gressarter som er ønskelige i golfgreener.

Formålet med denne avhandlingen var å undersøke hvordan skjøtsel av greener påvirker mykorrhiza - kolonisering i ulike arter av sportsgress. Mer spesifikt studerte jeg hvordan N/P nivåer, type vekst - medium og inokulering med et mykorrhiza produkt påvirket arbuskulær mykorrhiza kolonisering i ulike sportsgressarter på sandbaserte golfgreener, samt hvordan symbiosen påvirket greenkvalitet i samspill med disse skjøtselsfaktorene.

Forskningen ble gjennomført på to allerede etablerte greener hos Bioforsk Landvik, Grimstad, som tester effekter av ulike skjøtselsregimer på gressets kvalitet, spillbarhet og konkurranseevne mot tunrapp i typiske Skandinaviske golfgreener. Rotprøver ble høstet fra begge feltene, og analysert for prosent mykorrhiza kolonisering før en antydning av mulige soppslekter ble gjort, basert på observerte mykorrhiza strukturer. Rotvekter ble også registrert. I tillegg ble dataanalyser og visuelle data som hadde blitt registrert på gressets kvalitet gjennom hele vekstsesongen i forbindelse med de to hovedprosjektene skaffet for begge feltene. Et innledende drivhusforsøk ble utført ved Senter for Klimaregulert Planteforskning i Ås, Akershus, hvor de samme vekstmediene, mykorrhiza inokulumet og gressartene som i feltforsøket ble testet over en tretten-ukers periode. Samling av avklipp og visuelle dataregistreringer ble utført med jevne mellomrom. Ved slutten av eksperimentet ble røttene fra hver potte høstet og analysert på samme måte som med prøvene fra feltforsøkene. Alt overjordisk materiale ble også samlet fra hver potte, og veid.

Gresskvaliteten økte med økende N tilgjengelighet. Ved de høyere N-nivåene ble forekomsten av sykdom og konkurranse fra tunrapp begrenset, mens det generelle inntrykket av gresset var bedre. Dette gjaldt også for rotsoner som inneholdt kompost i stedet for torv, fordi kompost frigav næring lettere. I veksthusforsøket viste imidlertid planter dyrket i sand og torv den beste veksten, og dette

skyldtes at en overdreven N-gjødsling i disse pottene overskygget effekten av kompost. Det var ingen signifikante effekter av fosfor på hverken gresskvalitet eller mykorrhia kolonisering. Koloniseringen var høy i alle gressartene, bortsett fra i drivhusforsøket, hvor det testede inokulumet ikke viste noen signifikante effekter. Kveinartene så ut til å være mest mykorrhizadannende, mens forskjellen mellom kolonisering i rødsvingel og tunrapp var noe uklar. Ingen av disse resultatene var signifikante. Det var heller ingen signifikante effekter av N/P nivåer på mykorrhiza kolonisering, men en tendens pekte mot økende kolonisering med synkende N-konsentrasjon i jorda. Videre økte koloniseringsraten når kompost ble inkorporert i rotsonen istedet for torv. Det var ingen signifikante effekter av inokulering i feltstudiene, selv om en liten tendens pekte mot en reduksjon av tunrapp, samt økt opptak av P fra jord, i inokulerte ruter.

Resultatene viste flere samspill mellom arter og N-nivå, og mellom arter og vekstmedium. Dette understreker at prosesser knyttet til plantevekst og kvalitet, samt utvikling av symbiose, i stor grad er styrt av N-tilgjengelighet, men også at vekstresponser på dette elementet er knyttet til de individuelle arternes vekstpotensiale. Vekstresponsene på mykorrhiza var trolig nøytrale eller kunne ikke påvises hos de ulike artene fordi det høye næringsinnholdet i greenene maskerte eventuelle mykorrhiza-effekter, og dette indikerer videre at direkte opptak av tilgjengelig N er den primære årsaken til økt planteproduksjon og fitness i greener. Mangelen på signifikanser under testingen av mykorrhiza-inokulumet illustrerer at vellykket inkorporering av slike kommersielle produkter i felt kan være en utfordring. Imidlertid virket bruk av kompost i rotsonen til den yngste greenen ved å øke både gresskvalitet og koloniseringshastigheten, og hvis enkle tiltak som dette kan fremskynde etableringen av naturlige mykorrhizapopulasjoner i fremtiden, vil det kanskje ikke være nødvendig å inokulere nye greener under konstruksjon.

Contents:

1 Introduction

Golf is a popular sport, played by approximately 900 000 people in Scandinavia. In the five Nordic countries there are about 1000 golf courses, and based on membership, golf is the third largest sport in Norway (Norges Golfforbund 2013). This makes golf an important part of recreation and sport, not only in Norway, but in the whole of Scandinavia. Golfers spend much time on the greens, and want high standards regarding playability and general appearance. Course quality is usually evaluated from the quality of the greens, because a high amount of shots are played from here. Moreovert the primary criteria for how players perceive playing quality on golf greens are smoothness and consistency of the turf (Jensen 2010).

The main turfgrass species seeded on Norwegian putting greens are creeping bentgrass (*Agrostis stolonifera L.),*used on 40 % of the courses, a mixture of fine leaved red fescue (*Festuca rubra)* and colonial bentgrass (*Agrostis capillaris L.),* used on 60% of the courses, and velvet bentgrass (*Agrostis canina L.*), used on 3 – 4 courses. In practice, many greens are dominated by annual bluegrass *(Poa annua),* which is very invasive and can become a troublesome weed where it is not meant to be a part of the sward (Gange et al. 1999b). From an environmental point of view; red fescue is the most desirable species on golf greens because it has low requirements to water and nitrogen, good winter hardiness, and high resistance against all turfgrass diseases except red thread (*Laetisaria fuciformis) (Kvalbein & Aamlid 2012).*

Many golf clubs need to reduce their maintenance costs regarding fertilizers, pesticides, energy for irrigation, mowing and mechanical maintenance. The golf sector is also challenged by national and EU legislations that are restricting the use of pesticides and irrigation water, and movement of fertilizer that is not utilized into the groundwater can be a serious problem. In this context, the characteristics of red fescue makes it the most economic and environmentally friendly turf grass species because it can lead to more sustainable golf management, and in particular; reduction in fungicide use (STERF 2011). However, there are also many challenges related to sustainable greens with pure red fescue as the predominant grass species, and the fact that most turfgrass research in Scandinavia has been devoted to creeping bentgrass (STERF 2013) leaves many questions regarding red fescue management open. Red fescue greens have low wear tolerance, poor color and other low quality parameters, and they require a different style of playing because of their hardness and low resistance to ball roll (Kvalbein & Aamlid 2012). Due to low density, red fescue greens are also highly susceptible to invasion by annual bluegrass. Because of all this, greens in Norway are often sown with mixtures of 90 % red fescue and 10 % colonial bentgrass, following the British tradition of using bentgrasses in combination with a dominant share of red fescue (Perris & Evans 1996). Red fescue in mixtures with velvet bentgrass might be an even better combination, as velvet bentgrass also requires little water and fertilizer. The species also has great winter hardiness, competes well against annual bluegrass, and tolerate wear stress due to its high density (Espevig 2011). Thus; greens consisting of

red fescue and velvet bentgrass could, in theory, be maintained as pure fescue greens, ensuring a sustainable management of Nordic golf courses and satisfying quality requirements at the same time.

Another strategy for sustainable management of cultivated land which is becoming more and more relevant to the golf industry, is the utilization of symbiotic microorganisms that, among other benefits, increase the water and nutrient uptake in their hosts and may act as biological control agents; the mycorrhizal forming fungi (Gange & Case 2003; Newsham et al. 1995; Smith & Read 2008; Smith & Smith 2011). Extended knowledge about these organisms' ecological role in nature and how they function in concert with selected host plants in different environments, could ultimately lead to a more efficient and environmentally friendly management of high-quality golf courses (Amaranthus 2001; Koske et al. 1995).

1.1 Definitions and abbreviations

Apoplast: The free diffusional space outside the plasma membrane.

Aseptate: Not containing septae.

Axenic culture: Not contaminated by or associated with any other living organisms.

Biotrophic: A symbiotic organism that obtains nutrients from the living cells of its partner.

Coenocytic: Multiple nuclei within the same cell.

Dimorphic: Existing or occurring in two distinct forms.

ER cisternae: Interconnected, flattened vesicles or tubules comprising the endoplasmic reticulum.

Heterokaryon: A cell having two or more genetically different nuclei.

Microfilament: Strong, but flexible, linear polymer of actin subunits and component of the cytoskeleton.

Microtubules: Fibrous, hollow rods that function primarily to help support and shape the cell.

Monophyletic phylum: A group of organisms descended from a single ancestor.

Mutualism: A symbiosis that is beneficial to both partners.

Necrotroph: A parasitic organism that kills the living cells of its host and then feeds on the dead matter.

Obligate biotroph: An organism that is unable to complete a reproductive cycle in the absence of a living host.

Parasitism: A form of symbiosis in which one organism is fed by another, usually at the expense of that other another organism.

Photoautotroph: An organism that is capable of synthesizing its own food from inorganic substances using light as an energy source.

Plasmalemma: The semipermeable membrane enclosing the cytoplasm of a cell (cell membrane).

Propagule: A source of mycorrhizal colonization (e.g. spores, root pieces with living AM structures).

Saprotroph: An organism that lives and feeds on dead organic matter.

Symbiosis: The living together of two dissimilar organisms.

Thatch: A tightly intermingled layer of living and dead stems, leaves and roots which accumulates between the layer of actively growing grass and the soil underneath.

Turfgrass: Any of various grasses grown to form turf (short, thick and even grass).

AM: Arbuscular mycorrhiza

AMF: Arbuscular mycorrhizal fungi

DP: Direct pathway

MGR: Mycorrhizal growth response

MP: Mycorrhizal pathway

RNS: Root nodule symbiosis

C: Carbon

K: Potassium

N: Nitrogen

P: Phosphorus

P_i: Inorganic phosphorus

1.2 Theory/literature

1.2.1 Mycorrhiza – 'the roots of the roots'

Mycorrhiza, often referred to as 'the root of the roots' (Joner 2012), is a symbiotic relationship between a fungus that is specialized for a life in soil and the root of a plant. The term comes from the Greek words 'mycos' and 'rhiza', meaning 'fungus' and 'root'. Certain soil living fungi colonizes the roots of a host plant and extends its root system, thus enabling the plant to absorb more water and nutrients from the soil in exchange of carbon sugars (Parniske 2008; Smith & Smith 2011). The mycorrhizal symbiosis is an ancient phenomenon that is estimated to have existed for over 450 million years, and played a major role during terrestrial plant colonization, according to molecular phylogeny and fossil evidence (Read et al. 2000; Remy et al. 1994; Smith & Read 2008). A closely related fungus that form unique symbiosis with some cyanobacteria species from the genus *Nostoc*, where the fungus itself is the macro symbiont, has further created the hypothesis that even more ancient fungus-autotroph symbioses occurred between fungi and algae/cyanobacteria in aqueous environments, before the fungi eventually colonized the primitive plants and let them evolve towards a complex life on dry land (Pirozynski & Malloch 1975; Schussler et al. 2001; Schussler 2002; Smith & Read 2008). More than 80% of the now living vascular plant species (including some non-vascular bryophytes) form mycorrhizal associations, and with a co-evolution of plants and fungi in mind; the few non-mycorrhizal species that exist must have evolved away from the symbiosis, from obligate mycorrhizas through facultative mycorrhizas, and ultimately to non – mycorrhizas (NM), while developing other mechanisms for nutrient uptake under extreme conditions (Trappe 1987; Wang & Qiu 2006).

What are the benefits of having mycorrhizal symbiosis?

Despite the fact that information about ecology and molecular data that is revealing the evolution, biodiversity and distribution of mycorrhizal fungi is continuously being produced, their roles in plant ecosystems are still somewhat diffuse (Opik et al. 2010; Stockinger et al. 2010). A well known feature of the mycorrhizal fungi is their ability to take up nutrients from the soil that otherwise are unavailable to the plant. Many soil types are limited in nutrients, and while plants can only absorb nutrients in the form of inorganic ions, these are often immobilized through chemical processes. Sufficient amounts of inorganic phosphorus (Pi) being released from organic forms by soil microorganisms is especially important for plants in active growth, but these anions get strongly bound to iron (Fe) and aluminium (Al) cations in acidic soil. At high pH, Pi is bound to almost insoluble calcium phosphates. Nutrients of low solubility are also highly immobile, and a depletion zone (rhizosphere) will quickly form around plant roots because of the slow ion replacement by diffusion from bulk soil. To be able to continue its uptake of nutrients, the plant can either extend its root system, or form mycorrhizas that utilize undepleted zones in the rhizosphere. Roots are, indeed, more efficient in nutrient uptake than

fungal hyphae, but root production also require a larger C-investment. Thus; many more hyphae can be formed in a particular soil volume compared to roots, and with a narrow, extensive mycelium branching into soil compartments that are unreachable to the root epidermis and hairs, the harvest efficiency of scarce ions like Pi is maximized in the plant. Other nutrients that have low mobility in soil and that are known to be taken up by mycorrhiza are nitrogen (N), zink (Zn) and copper (Cu). The fungi can further form hyphal networks that connect roots of several host plants of same or different species, making the nutrient uptake even more efficient, and carefully coordinated at the same time. (Joner 2012; Ruissen 2012a; Smith & Read 2008; Smith & Smith 2011).

The large percentage of land plants forming mycorrhizal symbioses suggests that plants rely strongly on this symbiosis to be able to live without artificial nutrition. The principal role of mycorrhiza is popularly thought to be faciliation of P uptake in plants, based on many studies that have calculated P mobility in soil or quantified the amount of P that are taken up via mycorrhizal pathways, using tracers (Finlay 2008; Sanders & Tinker 1973; Smith et al. 2003; Smith et al. 2004; Smith & Read 2008). Many research papers are also reporting positive effects of mycorrhizal colonization on plant P nutrition and growth responses, especially those related to contrived greenhouse studies or field experiments in the tropics and other areas with strongly P – limited soils (Caravaca et al. 2002; Howeler et al. 1982; Howeler et al. 1987; Ortas et al. 2011). However, field evidence from temperate ecosystems is more rare, and mycorrhizal colonization have, in fact, shown to trigger a wide range of growth responses, from highly positive to neutral or even negative (Facelli et al. 2010; Fitter 1985; Gange & Ayres 1999; Johnson et al. 1997; Smith & Read 2008; Smith & Smith 2011). When putting this in context with the widespread ocurrence of mycorrhiza (Wang & Qiu 2006), it is likely that the symbioses may provide other properties to the plant than just altered plant growth and nutrient uptake (Newsham et al. 1995). In fact, plants colonized by mycorrhizal fungi have shown increased fitness and tolerance to other biotic and abiotic stress factors such as drought (Auge 2001; Gemma et al. 1997b; Querejeta et al. 2007), heavy metal poisoning and other environmental pollution (Hildebrandt et al. 1999; Leyval et al. 1997), chilling (El-Tohamy et al. 1999) and infection by fungal pathogens, including nematodes (AzconAguilar & Barea 1996; Gange & Case 2003; Johansson et al. 2004; Liu et al. 2007; Newsham et al. 1995; Vos et al. 2012). Mycorrhiza may also reduce nutrient leaching from fertilized soils and into the ground water (Asghari et al. 2005), influence host diversity (van der Heijden et al. 1998) improve plant tolerance to salt stress and transplanting shock, accelerate flowering and improve soil structure (Aroca et al. 2007; Auge 2004; Koske & Gemma 2005; Ortas et al. 2011; Wilson et al. 2009). Because they operate a bidirectional transport of nutrients between soil and plants, they also contribute significantly to global cycling of P, N and CO_2 (Kruger et al. 2009).

On the basis of these beneficial effects, it is clear that complex AMF communities have a profound influence on both plant community structure and productivity in ecosystems (van der Heijden et al. 1998). AMF are fundamental regarding the soil fertility of both natural and agricultural ecosystems (Smith & Read 2008), and are thus ecologically and economically important organisms,

whether they pose negative, positive, direct or indirect effects. Despite the many unanswered questions existing about mycorrhizal biology and the wide range of host growth responses that have been documented, exploiting and managing this symbiosis have numerous consequences. Especially in the tropics, the coastal Mediterranean and other areas that are poor in nutrients and organic matter, exploiting natural plant mechanisms in cultivated crops could significantly reduce the need for P fertilizer and other artificial nutrient inputs. Moreover, fumigation with harmful substances such as methyl bromide is a is a common way to combat the troublesome weeds, soil pathogens and nematodes that are prevalent in the same areas, and this consequently reduces plant growth and nutrient uptake because also desirable organisms such as the mycorrhizal fungi are killed. Applying biological control agents like mycorrhizal fungi to horticultural and agricultural systems seems like a more efficient and environmentally friendly solution that, in addition to keeping pathogens at bay, preventing resistance development to pesticides and enhancing natural uptake of nutrients, could reduce the great amount of energy and costs that are currently invested in irrigation and other maintenance practices around the world. (Charron et al. 2001; Harrier & Watson 2004; Howeler et al. 1987; Michelsen & Rosendahl 1990; Ortas et al. 2002; Ortas et al. 2011; Vos et al. 2012).

Classification

Mycorrhizal fungi are best known as obligate biotrophs that form mutualistic symbioses within their hosts, but they can also form endophytic, antagonistic and necrotrophic (although their saprotrophytic abilities are limited) interactions with host or non-host plants (Brundrett 2004; Hobbie et al. 2001). A few species even form 'exploitative' symbiosis with parasitic orchids, and degrade soil organic matter for nourishment (Deacon 2006). Several types of mycorrhiza exist, and they are classified according to which plants and fungi that are involved in the symbiosis, and what kind of soil - and nutritional factors that dominate the place (Joner 2012). Most of them will not be discussed here, but the main forms are the 'endomycorrhiza' and 'ectomycorrhiza'. The latter predominantly occurs in woody plants of temperate forests, and twith fungal hyphae always growing outside of the root cells, delivering and receiving nourishment through a network of hyphae growing between the cells of their host. Endomycorrhiza is more widespread, and have parts of the hyphae that penetrate cells of their host for nutrient exchange (Parniske 2008). One special group of fungi forming this type of symbiosis is the arbuscular mycorrhizal fungi (AMF), which are of great ecological and economical importance because they are very common in tropical and temperate ecosystems, and occur in many many soil - and horticultural crops. (Joner 2012; Ruissen 2012a). As much as 85% of surveyed plant families form arbuscular mycorrhiza (AM), while 10 % form ectomycorrhizal types (Wang & Qiu 2006). Consequently, AMF are often the organisms that give rise to discussions about the many benefits that mycorrhiza can offer plants. Throughout this thesis, AM will be the symbiosis in focus.

Based on molecular, morphological and ecological characteristics, all AMF (including the *Geosiphon pyriformis*, forming endosymbiosis with cyanobacteria) are placed in a monophyletic phylum; the *Glomeromycota* (Figure 1). These fungi probably share common ancestors with the basidiomycetes and ascomycetes, and form the most ancient and widespread terrestrial plant symbioses (Schussler et al. 2001; Smith & Read 2008). AMF are unusual in their conserved, genetic make-up that has resulted in a virtually unaltered morphology that actually makes these organisms living fossils. However, in addition to the association formed between a glomeromycetous fungus and some photosynthetic and nitrogen fixing *Nostoc* species, most AMF harbor a wide spectrum of endosymbiotic bacteria. This suggests that there have been multiple, independent uptake events of symbiotic bacteria in ancient fungi, and that the glomeromycetous fungi evolved before land plants and their ultimate AM symbiosis ocurred (Naumann et al. 2010; Parniske 2008). Since AM is the ancestral type of mycorrhiza in plants, evolution of this association must have led to derivation of all other known mycorrhizas, whose fungal symbionts belong to *Basidiomycota* and *Ascomycota.* While development of ectomycorrhizal types may have been a response by plant and fungal partners to a constantly changing environment, AM represent a long – term evolutionary strategy in that they helped primitive plants, lacking a root system, to solve their problem of water and nutrients – deficiency when first invading land. In fact, parallel evolution let ectomycorrhiza and its derivatives, including the nonmycorrhizal condition, evolve independently from AM many times. Regarding the fact that there is a great number of species in plant families that mainly possess ectomycorrhiza or its derivatives, and that basidiomycetous and ascomycetous mycorrhizal fungi have a high host specificity compared to the AMF, co-evolution between these plant hosts and their fungal symbionts is probably responsible for much of the diversity found among both symbiotic partners today. Because of their low host specificity, AMF cannot have contributed significantly to the diversification of glomeromycetous fungi. A single root can even inhabit several AMF species at the time; their nutrient acquisition activities then seeming to be complementary. Anyhow, there is still a positive correlation between the biodiversity of AMF and plant communities, making host preference important in natural ecosystems (Brundrett 2002; Koide 2000; LePage et al. 1997; Maherali & Klironomos 2007; Newton & Haigh 1998; Read et al. 2000; Remy et al. 1994; Santos-Gonzalez et al. 2007; Stubblefield et al. 1987; Trappe 1987; Vandenkoornhuyse et al. 2003; Wang & Qiu 2006).

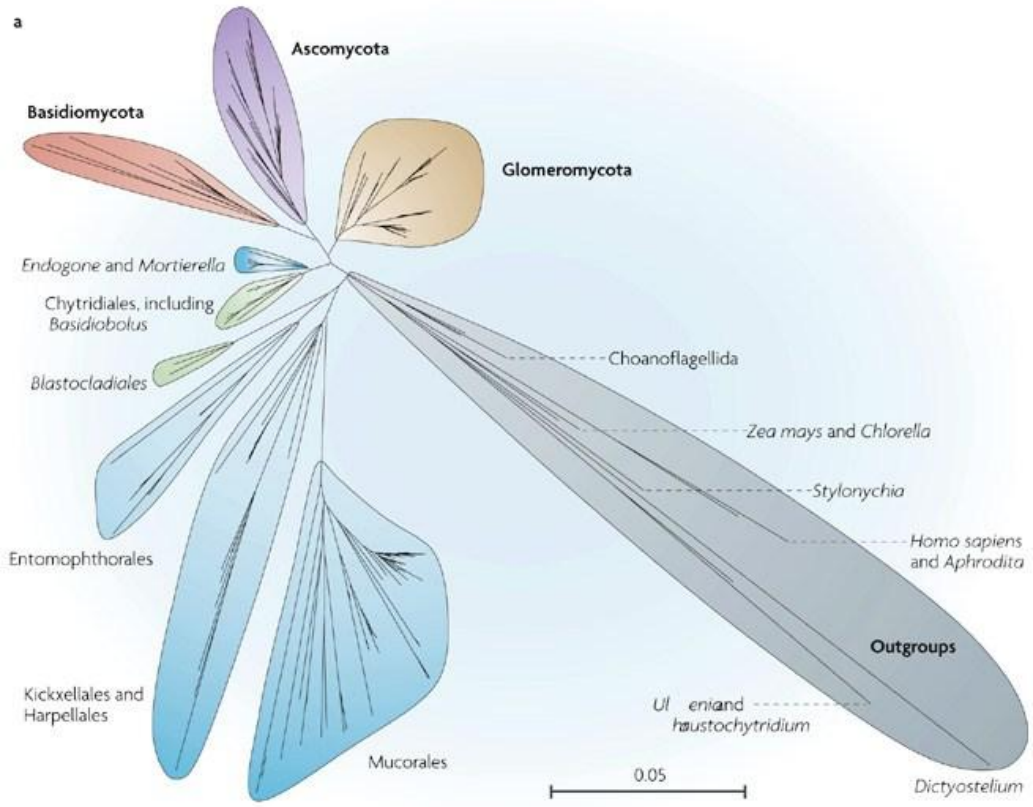

Figure 1: Phylogeny of the main fungal lineages, showing the relative position of Glomeromycota. Zygomycota (blue) and Chytridiomycota (green) are non-monophyletic. Source:(Parniske 2008)

The *Glomeromycota* with its resolved orders, families and genera are presented in Table 1. AMF live "exclusively as obligate symbionts of photoautotrophs" (James et al. 2006), and form asexual, multinucleate spores of great size. The hyphal network is aseptate and coenocytic at all stages during their life cycle. Both cytoplasm and individual spores may contain hundreds of genetically different nuclei, a phenomenon called heterokaryosis (Hijri & Sanders 2005; Parniske 2008; Reinhardt 2007). Currently, there are 230 described species (Kruger et al. 2012). This is definitely an underestimate of the real diversity because many species are still difficult or impossible to grow in axenic cultures, and among the > 200 000 plant species forming symbioses with AMF in the field, most individuals are colonized by multiple fungal species. The number of acquired molecular sequences deriving from different AMF species have, indeed, already exceeded the diversity found in available cultures. Taxonomic classification should include both morphological and molecular expertise. Unfortunately, extended species recognition is often difficult due to unavailable or lacking biological material of more defined cultures and single spore – isolates. Moreover, the hidden, asexual and obligate biotrophic lifestyle of AMF, their few morphological characters, the many DNA – sequence variants within a single cell, and potential formation of dimorphic spores, all makes identification difficult (Clapp et al. 2003; Kruger et al. 2009; Kruger et al. 2012; Parniske 2008; Young 2012).

Members of the Glomeromycota were previously called vesicular arbuscular mycorrhiza (VAM), due to the many families forming vesicles, which are thin-walled, lipid containing bodies, or storage organs, that possibly exist for maintenance and regrowth of the fungal organism after roots have ceased metabolic function. Vesicles are usually terminal, but may also be formed intercalary. (INVAM 2013; Redecker 2004). When AM symbiosis is formed, parts of the fungal hyphae penetrate the cortex cells of their host and develop tree-shaped arbuscules, which are probably main structures for nutrient exchange between the plant and the fungus (Parniske 2008). It has recently been demonstrated that other AM structures, like hyphal coils and intercellular hyphae, also have functional interfaces with plant cells that can be of significant importance in some species, and the number of AM forming plant species in nature may thus be underestimated due to the traditional dependence on present arbuscules for identifying a root as colonized by glomeromycetous fungi (Dickson et al. 2007; Genre et al. 2008; Karandashov et al. 2004; Smith & Smith 1997).

Table 1: The phylum Glomeromycota, as it appears in the present. Relevant genera are highlighted.

Phylum *Glomeromycota*		
Class *Glomeromycetes*		
Orders (4)	**Families (11)**	**Genera (17)**
Glomerales	***Glomeraceae***	***Glomus*** *Funneliformis (former Glomus Group Aa, 'Glomus mosseae clade')* *Rhizophagus (former Glomus Group Ab, 'Glomus intraradices clade')* *Sclerocystis (basal in former Glomus Group Ab)*
	Claroideoglomeraceae	*Claroideoglomus (former Glomus Group B, 'Glomus claroideum clade')*
Diversisporales	***Gigasporaceae***	***Gigaspora*** ***Scutellospora*** *Racocetra (including Racocetra weresubiae)*
	Acaulosporaceae	***Acaulospora*** *(including the former Kuklospora)*
	Entrophosporaceae	***Entrophospora*** *(with unclear phylogenetic affiliation)*
	Pacisporaceae	*Pacispora*
	Diversisporaceae	*Diversispora (former Glomus Group C, including several former Glomus species)* *Otospora (unclear phylogenetic affiliation)*
Paraglomerales	***Paraglomeraceae***	***Paraglomus***
Archaeosporales	*Geosiphonaceae*	*Geosiphon*
	Ambisporaceae	*Ambispora*
	Archaeosporaceae	***Archaeospora*** *(including the former Intraspora)*

The varied range of structures that can be formed by AMF is demonstrated in the following description and classification (Figure 2) of the genera most relevant for this study:

Glomus

This genus contain the most diverse species of *Glomerales*. Vesicles usually stain darkly in trypan blue or other stains, and are generally thin – walled and oblong to ellipsoid. They can be highly dispersed in the colonized root, but abundance and timing of appearance varies with species and the environmental conditions of the host. Arbuscules generally have cylindrical or flared trunks, with branches progressively tapering in width toward tips. They can be faintly staining in a few species, but generally stain darkly. Older colonization consists mostly of hyphae, but vesicles can also be abundant, if present. Most of the hyphal biomass in this family is found within roots. Intraradical hyphae usually stain darkly and grow parallel to the root longitudinal axis, with cross-connecting branch hyphae at varying angles. They can be coiled at entry points. Infection units often merge to form continuous colonization. Extraradical hyphae are highly varied in abundance, distribution and morfologi among species, but the great diversity of the genus is primarily due to a high degree of plasticity in number, phenotypes, and position of layers in the spore wall. Spores develop through blastic expansion of a hyphal tip (Figure 2), and some species can, on rare occasions, form spores intercalary. Otherwise, they are produced close to the root, on profusely branching hyphae. They are produced singly, in aggregates, or in a hyphal matrix, with layers of the spore wall usually continuous with a wall of the subtending hypha. Some species form spores within the roots of their host, possibly as a substitute or replacement for vesicle development (INVAM 2013; Parniske 2008; Ruissen notes, 2012d).

Enthrospora, Acaulospora

These genera stain weakly in more than 50 % of their colonization, and the staining intensity is generally very variable. They form intraradical vesicles that vary considerably in shape, often having knobs and concavities on their surfaces. When formed abundantly, the vesicles tend to be localized in entry regions. Here they often form from coils of the penetration hyphae prior to, or concurrent with, arbuscule formation. Arbuscules are generally similar to those of *Glomus.* Intraradical hyphae can be straight or coiled, but coiled hyphae are usually formed at entry points. These are wider than hyphae growing parallel to the root axis, which are interconnected to neighboring hyphae by angled branches. Infection units may merge but often remain isolated, giving colonization a patchy ditribution. Extraradical hyphae are generally thin, but profuse around roots. The overall spore development is quite similar in the two genera, but *Acaulospora* develop its spores laterally from the neck of a pre-differentiated 'sporiferous saccule' that is formed terminally on a fertile hypha, while in *Enthrospora*, spores are borne from within the neck of the saccule (Figure 2). Spores are produced singly, and on rare occasions in loose aggregates. There is an outer layer to the spore wall, which is continuous with the wall of the saccule neck. This layer usually sloughs with age or manipulation, leaving the spores without hyphal attachment (INVAM 2013; Ruissen notes, 2012a).

Archaeospora, Paraglomus

Arbuscules and intraradical hyphae consistently stain lightly, and definite vesicles are not formed in any of the species examined to date. *Archaeospora* have a patchy distribution of arbuscules, which consist of narrow trunk hyphae with fine branching near tips in both genera. Intracellular hyphae are often tightly coiled, but can also coil more infrequent and looser, with irregular branching. The hyphae have variable widths, depending on their growing pattern, and spread both intra - and inter-cellularly in *Archaespora*. Extraradical hyphae stain darkly, and are often in profuse abundance around the roots. The spores of *Paraglomus* develop terminally on a cylindrical to slightly flared subtending hypha. They are produced singly, and on rare occasions in loose aggregates of a few spores. The sub-cellular spore structure and development is identical to that of *Glomus* species, having layers of the spore wall that are continuous with layers of the subtending hypha after blastic expansion of the hyphal tip. In some species the subtending hypha of mature spores is so thin that it is hard to see or separate from the spore. *Archaeospora* spores are also produced singly and more rarely in loose aggregates, and originate laterally from the neck of a sporiferous saccule that is formed terminally on a fertile hypha. Spore development can be similar to that of *Acaulospora* species, with spores eventually detaching from their hyphae and remaining sessile in the soil. The spores can also develop similar to that of *Glomus* species, on a hyphal 'pedicel' branching from the subtending hypha of the soporiferous saccule (Figure 2). In the latter case, *Glomus* – like spores may also be formed from external hyphae, some of which also can form soporiferous saccules (INVAM 2013; Ruissen notes, 2012b).

Gigaspora, Scutellospora

These genera do not form intraradical vesicles, and are thus not 'VAM fungi'. Instead, auxiliary cells (thin–walled cells which compartmentalize lipids) are formed singly or in clusters by branching from external hyphae, often differentiating on germ tubes from spores prior to establishment of mycorrhizal colonization. Colors are hyaline to dark brown. The cells are very abundant around roots during early colonization, but become less frequent and sometimes absent as sporulation increases, suggesting that one of their tasks is to provide carbon macromolecules independent of the host during spore formation. *Gigaspora* have auxiliary cells with a spiny surface, while *Scutellospora* form broad concavities to varying degrees that makes the cells appear almost smooth to having wide knobs. Arbuscules stain darkly. They generally have swollen trunks with branches tapering abruptly at tips, and the network can be abundant for a long time after the roots have ceased growth. With arbuscule senescence, the fine tips are degraded but the trunk may remain intact in cells as tightly packed coils. Intraradical hyphae are often coiled throughout the root, but their coiling is most prominent at entry points. The hyphae vary in width, but often appear knobby or have projections. Infection units merge and form a uniform colonization throughout the root cortex. Extraradical hyphae are either coarse and wide, or fine hyphae. Both are abundant during auxiliary formation, whereas fine hyphae are less evident in older cultures. Most of the fungal biomass is found in the external hyphae, which are bridging over

long distances with few deriving branches. *Gigaspora* and *Scutellospora* form large spores that are usually > 200 µm after maturation, and range from white to dark red in color. The spores develop singly and blastically, from the tip of a bulbous, sporogenous cell that is formed terminally on a fertile hypha growing relatively distant from the root (Figure 2). Subcellular organization consists only of a bilayered spore wall. In *Gigaspora*, germ tubes arise from a thin papillate, or warty, layer developing from the inner surface. Spores are without ornamentations. Some *Scutellospora* species, on the other hand, have ornamentations of the outer layer of their spore wall, and the inner layer may vary in color. Germ tubes arise from a plate–like germination shield that is associated with the flexible inner wall (INVAM 2013; Parniske 2008; Ruissen notes, 2012c; Ruissen notes, 2012e).

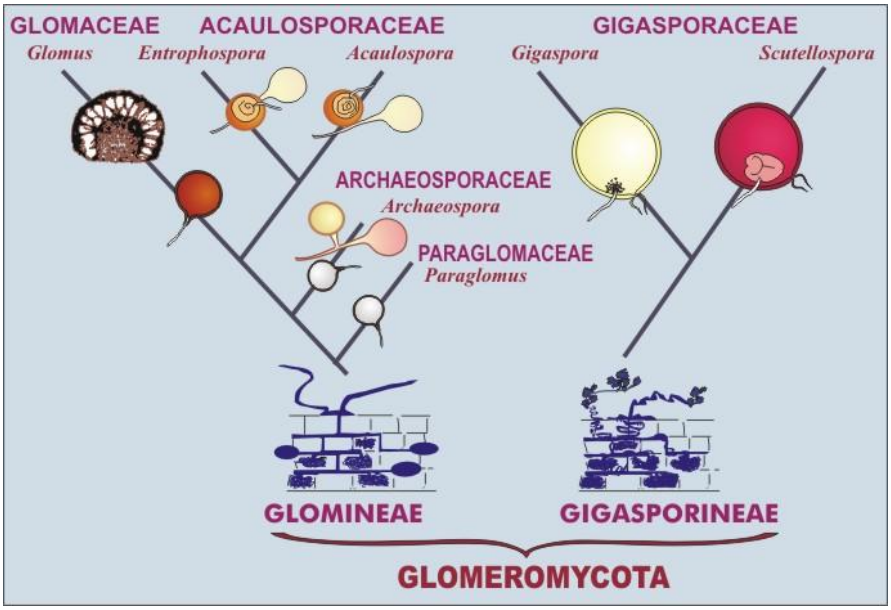

Figure 2: Classification and illustrated spore formation in the two glomeromycetous sub orders; Glomineae, which form vesicles, and Gigasporineae, forming auxiliary cells. Both morphologial and molecular characters have been taken into account. Source: (INVAM 2013).

Fine endophytes

Another group of fungi that form symbiosos within same hosts as AMF are called fine endophytes, formerly named *Glomus tenue* and classified as glomalean fungi. The function and taxonomical status of these fungi is an unsolved mysterium, but they seem to be more frequent in cold or harsh environments than AMF, which can be abundant in antarctic cold areas but have a very low frequency at the more polar sites. Typical AM forming plants are often abundant in such harsh environments, but their lack of AM shows that the symbiosis is less important in arctic and alpine ecosystems than it is in temperate ecosystems. The relatively high frequency of fine endophytes at high latitudes compared to that of AMF indicates that these fungi are better adapted for establishing successful symbioses under adverse conditions that inculde short growth seasons (Christie & Nicolson 1983; Olsson et al. 2004; Thippayarugs et al. 1999).

How does AM form, and how does it work?

When a fungus and a plant host are going to form AM symbiosis, both parts have to recognize and accept each other before intimate associations that involve penetration of plant tissue and invasion of individual host cells can be established. First, the plant roots release root exudates that are recognized by the AMF spores and hyphae, which starts to grow, branch and alter their physiological activity, once they perceive this signal. The substance released are called strigolactones, because when they were discovered 50 years ago, this class of compounds was found to induce seed germination of the parasitic plant genus *Striga*. Faster growth of the AMF hyphae increases the chance of encountering a host, but the hyphae can also be stimulated to grow chemotropically towards a root, because strigolactones hydrolyze quickly in soil and form a steep concentration gradient in the rhizosphere that makes its perception a reliable guide towards the root. Other microbes are also able to recognize inducing signals released from the plant, which therefore must be able to to recognize their rightful symbionts and reject saprotrophs or potential pathogens at the same time. This problem is solved by means of unknown, diffusible symbiosis signals called 'Myc factors' that are emitted from AMF hyphae growing towards the roots in response to the plant initial signals. The Myc factors initiate a cascade of signals in the plant cells that leads to altered metabolism and transcription of symbiosis – related genes in the plant, which then actively helps the fungus colonize its roots. Pathogenesis – related proteins may also be released from the plant during early stages of AMF colonization, as a defence reaction to unspecific microbial signals (Parniske 2008; Reinhardt 2007).

Once the fungus comes into contact with the root surface, infection structures named appressoria, or hyphopodia in the case of AMF, is formed outside the epidermal cell layer. The underlying epidermal cell responds to mechanical stimulation together with a local signal emitted from the fungus that contains information about the exact position of the appressoria, and start reorganizing its cell components to form an aggregation of microtubules, actin filaments and ER cisternae; the pre – penetration apparatus (PPA). A fungal hypha that extends from the hyphopodium then penetrate the epidermal cell through the trajectory formed by the PPA, while the cell membrane invaginates, leaving the fungus in an apoplastic pocket that contains remnants of the plant cell wall. The PPA guides the fungus through epidermal cells and towards the cortex, where the hypha leaves the plant cell and enters the apoplast. Lateral, intercellular growth and branching along the root axis eventually lead to the hyphae inducing formation of PPA – like structures in inner cortical cells, through a similar process as with the epidermal cells. The hyphae enter these cells, where they develop into a highly ramified structure with fine terminal tips; the arbuscule. This structure is separated from the host cytoplasm by a plant – derived periarbuscular membrane (PAM) that is continuous with the plasmalemma, the fungal plasma membrane, and the periarbuscular space between them, containing remnants of the fungal cell wall and apoplastic material of the plant. The way that AM develops in a plant root are illustrated in Figure 3.

The arbuscule has a high surface – to volume ratio, which makes this symbiotic interface a

perfect site for exchanging nutrients and symbiotic signals. Local cell autonomous signals that are produced by the fungus activate expression of genes that, among others, code for transporter proteins mediating this metabolite exchange. Arbuscules generally have a short lifetime, but a single host cell may undergo several rounds of successive fungal invasions. Early degradation of arbuscules is probably a way of discriminating between efficient and inefficient fungal species, because research has suggested that the lifetime of an arbuscule is influenced by its ability to deliver nutrients. A short arbuscule lifetime thus ensures constant renewal of the hyphal network, while connections are made to the most efficient nutrient providers (Javot et al. 2007; Parniske 2008; Reinhardt 2007).

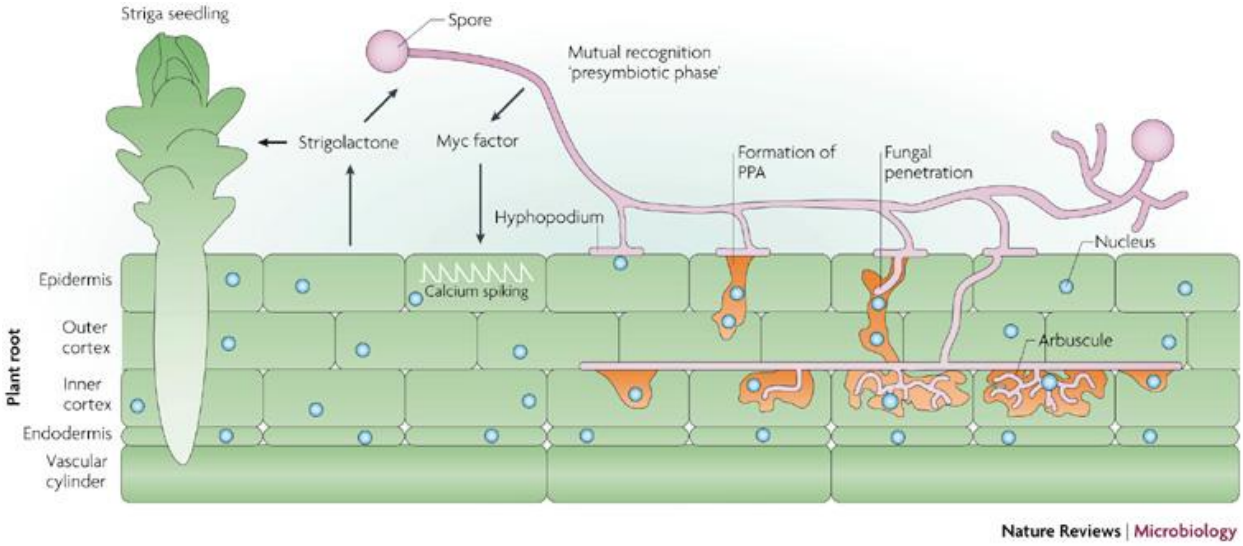

Figure 3: Development of AM symbiosis in a plant root. Fungal spores germinate, and hyphae start branching and growing towards the root when perceiving strigolactones; initial signals that are released from the plant. When the hyphae encounter the root, Myc factors act as a recognition signal to the plant, which then starts to express symbiosis-related genes and alter their metabolic function. A fungal hypha extending from a hyphopodium infection structure penetrates the epidermal cell layer through a pre – penetration apparatus (PPA) formed by cellular reorganization, and guides the fungus into the cortex. Here, the fungus enters the apoplast and grows laterally along the root axis before it invades the inner cortical cells in a similar manner as with the epidermal cells, and branches extensively to form arbuscules. The endodermis is never penetrated. Source: (Parniske 2008).

Due to improved molecular methods, it has now become clearer how AM symbiosis develops, how nutrients are taken up by external soil hyphae, and how these substances are translocated on to the internal hyphae before leaving the fungus through structures that are well adapted to nutrient exchange with the host plant. Yet, the major changes in fungal and plant gene expression that leads to AM formation are not fully resolved. Important details about the physiological mechanisms underlying signalling pathways and nutrient transport are missing, and there is also need for identification of all single components involved (Balestrini & Lanfranco 2006; Parniske 2008; Reinhardt 2007).

Mycorrhizal plants have two ways of taking up nutrients: through the roots and root hairs, also called the direct pathway (DP), or through the mycorrhizal pathway (MP). The pathways have different biochemistry, and the fact that plants often favor the MP for nutrient uptake might be due to a higher efficiency of fungal nutrient transporters. However, when nutrients are absorbed through the MP, they first have to pass a boundary between the soil and fungal hyphae, before transport through the intraradical hyphae eventually leads them to a second boundary between the fungus and plant which must also be passed before nutrient uptake is complete. The DP only requires that nutrients pass the soil-plant cell boundary, but since depletion zones develop quickly in the rhizosphere, and inorganic ion replacement from bulk soil is slow and inefficient, choice of pathways for nutrient uptake is probably related to the ability of root systems to access nutrients from undepleted soil. In this case, the MP is most efficient because of the ability of AM hyphae to comb the soil for nutrients that are placed far away from where the root hairs can reach. (Ruissen 2012a; Smith & Smith 2011)

The varying mycorrhizal growth responses (MGR) that may occur to AM colonization are often discussed in relation to a mutualism – parasitism continuum (Johnson et al. 1997). Positive MGRs are usually due to increased nutrient uptake via the MP, but many factors, both molecular and ecological, can influence the final response in the plant. For example; fungal growth and capacity of nutrient uptake/delivery, efficiency of nutrient exchange interfaces, root morphology and ability to produce nutrient mobilizing root exudates, as well as environmental factors, all may influence MGRs. Thus; the conventional explanation to why plants sometimes show neutral or negative MGRs is that physiological features in both the plant and and the fungus makes the MP less efficient than the DP alone, and maintaining the fungus becomes negative to the plant because the P benefit is lower than the C cost, especially when nutrient levels are high. In this case, the fungus could be regarded as a parasite. However, the plant never eliminates its fungal partner to save photosyntates, although colonization and MP operation may be suppressed with strongly elevated P levels in the soils that make the symbiosis redundant (Amijee et al. 1989; Joner 2012; Nagy et al. 2009; Smith & Smith 2011).

Since the plant maintain AM symbiosis despite the fact that it is largely in control of the fungal colonization through genetic programming, the many suggestions that AMF provides benefits to the plant that are not related to nutrient uptake remains relevant. At the same time, P tracking have shown that the MP is always operational in mycorrhizal plants and makes a major contribution to P uptake, regardless if the plant growth responses are positive or negative (Smith et al. 2003; Smith et al. 2004). A designation of AMF as parasites in cases of negative MGRs could then be discussed. A parasite is, indeed, fed by another organism, but since the fungus constantly delivers P in exchange for C, regardless of the type of MGRs, the relationship between plant and fungus is, strictly said, a mutualistic interaction. Moreover; growth depressions are not always associated with high fungal C costs or large extent of colonization, as negative MGRs occur also when colonization is low (Johnson et al. 1997; Jones & Smith 2004; Li et al. 2008; Reinhardt 2007; Ruissen 2012b; Smith et al. 2009;

Smith & Read 2008; Smith & Smith 2011).

AM plants often reduce DP contributions to P – uptake in a greater or lesser extent, and sometimes this is not compensated for by the MP because of the strong functional diversity in AM symbioses. The AMF also have different efficiencies regarding uptake and delivery of P, and thus; imbalance in P-uptake between the DP and MP is likely the main reason to neutral or negative MGRs in AM plants. The DP contribution may be suppressed also in plants showing positive MGRs, but in these cases, the MP contribution is so efficient that the plant ends up with a P – profit despite lower DP activity. The contributions of the DP and MP are never additive because of the variation in total P delivered by the two pathways, and the 'hidden' MP contribution cannot be determined from total plant P content without using tracking methods, due to the fact that the DP may be much less active in AM plants compared NM plants of same kind (Facelli et al. 2010; Li et al. 2008; Munkvold et al. 2004; Poulsen et al. 2005; Smith et al. 2009; Smith & Read 2008; Smith & Smith 2011).

NM plants can have a more efficient P – uptake and thus show a better growth response than AM plants, but this does not necessarily mean that colonization is not beneficial to the plant. In the field, different AMF colonize the same roots, and their nutrient acquisitions are likely to complement each other. Moreover, hidden MP contribution that makes a plant show zero or negative MGRs in greenhouse experiments could be an advantage in competition against NM plants in a field situation. If situations occur where the fungus take more energy from the plant than it is able to compensate for due to environmental factors etc., these tend to be temporary, and the interaction will eventually stabilize again. Finally, the MGR of a plant is not a good indicator of how dependent it is on AM colonization, because the magnitude of MGRs varies with how the specific NM plant responds to different environmental conditions. A NM plant might experience increased or decreased growth when subjected to a new environment, but the growth of the respective AM plant will not necessarily change. Overall growth should always be taken into account when discussing plant dependence on AMF (Facelli et al. 2010; Janos 2007; Koide 2000; Ruissen 2012a; Smith & Read 2008; Smith & Smith 2011).

Extended knowledge about why and how the AM plants suppress DP contribution to nutrient uptake is important, because cultivated plants showing neutral or negative MGRs could ultimately increase their P – uptake if the DP and MP were made additive. Manipulating plants to eliminate their DP down-regulation should still be made with caution as there might be important reasons to why the pathway is suppressed, that are not discovered yet. For example; reducing expression of genes coding for nutrient transporters in the DP could be a way of saving energy for use in other processes that affect fitness. Oxidizers like arsenate (AsO_4^{3-}) also enters the plant via Pi transporters in the DP, and decreased nutrient uptake through this pathway limit the amount of harmful substances entering the plant. Disregarding the risk of tampering with natural systems that are not completely understood, research on both ecological and molecular biological levels is important if we are to exploit the AM symbiosis to our advantage in the future. Knowledge of the mechanisms and reasons behind all

processes involved is essential to maintain a maximum, but still sustainable, utilization of the interaction between different organisms, such as the one between plants and AMF (Rae et al. 2004; Smith & Read 2008; Smith et al. 2010; Smith & Smith 2011).

Why is AM symbiosis so evolutionary persistent?

The *Glomeromycota* is the only fungal phylum where all single members inhabit the same ecological niche; namely as obligate biotrophs with a photosynthetic partner. Their impressive age and the discovery of colonization in the earliest, primitive land plants suggests a strong conservation of the genetic program that controls development of the AM symbiosis in existing AM plants. Common evolutionary and developmental aspects between the AM and the root nodule symbiosis (RNS) formed by nitrogen-fixing bacteria in legumes have, indeed, been discovered. The AM and RNS share several symbiosis genes that are related to signaling pathways in PPA formation, and thus; these genes must be part of an ancient program that evolved in AM plants before the angiosperms diverged, and must later have been recruited for the RNS and possibly other plant – microorganism symbioses. It is worth mentioning that some of the events during PPA formation in the AM and RNS also have independent signaling pathways. This is probably to maintain the level of specificity that is necessary when initiating peculiar developmental events in the two different forms of symbiosis (Kistner & Parniske 2002; Parniske 2004; Parniske 2008; Reinhardt 2007).

An evolutionary link has also been suggested between parasitic and symbiotic fungi because many important fungal plant pathogens are biotrophs living within photosynthesizing hosts, like AMF. However, most pathogenic fungi belong to the *Basidiomycota* or *Ascomycota*, and diverged about 240 million yeras after the Glomeromycetous fungi. Convergent evolution that has led to a number of similarities between mycorrhizal colonization processes and pathogen infection are more likely than overlapping of accommodation programmes within plants harbouring these organisms (Parniske 2000; Parniske 2008; Ruissen 2012b).

Evolutionary advantages associated with AM must have been the reason why this symbiosis program is preserved in almost all lineages. For example; hidden P transfer via the MP gives an indication of why the AM symbiosis has been so evolutionary persistent also in plants displaying neutral or negative MGRs. On the other hand, the question of how AMF remain heterokaryons despite their conserved asexual, coenocytic life cycle, is currently not completely solved. It is suggested that the many existing polymorphic DNA sequences are maintained through indigenous mutations, combined with recombination of genetic material that is exchanged by means of temporary hyphal fusion in a process called anastomosis. The next question that then arises is why, or *how,* this genetic diversity is important for symbiotic development. Plasticity allowing AM development in different hosts, or dependence of a fungal multi-genome in order to survive as a result of degradation of individual genomes, could be possible explanations. (Giovannetti et al. 1999; Hijri & Sanders 2005;

Parniske 2008; Reinhardt 2007; Sanders 2002; Smith & Smith 2011).

1.2.2 Mycorrhiza and turfgrass

Control of annual bluegrass on golf greens is a challenge. In Norway, there are no selective herbicides approved against the species in turf, and even though such herbicides exist in other countries, the use of them is difficult, costly and not environmentally friendly (Gange 1998). Studies have shown that annual bluegrass requires more water and nutrients than other turfgrass species (Aamlid 2006; Blombäck 2009; Lodge & Lawson 1993). Thus; besides nitrogen, phosphorous may be an important nutrient governing the competition between red fescue and annual bluegrass on golf greens. In context of this theory, there is interest in adding compost products to the growth medium instead of peat, which is the most commonly used organic amendment at present (Koske et al. 1997b). Green Mix is a composted garden litter product that has a low C/N and C/P ratio, and thus release nutrients more easily compared to peat (Aamlid et al. 2009). Moreover, AMF seems to thrive in the presence of organic materials that are easily degradable (Hrselova et al. 1999) and colonization of turfgrass is not significantly altered by application of fungicides (Bary et al. 2005; Frank 1984; Hartin et al. 2005). The roots of annual bluegrass are less extensive (Perris & Evans 1996; Vargas Jr & Turgeon 2004), and have been shown to benefit less from AM colonization than roots of various bentgrasses (Baker et al. 2006; Gange 1994; Gange 1998; Gange et al. 1999b). If these observations hold true, the red fescue, with its deep and extended root system (Kvalbein & Aamlid 2012), might have an advantage in utilizing compost as a slow – release nutrient source in that it has a deep and extended root system, and thus the potential to get colonized by AMF to a great extent. Gange et al. (1999) found that bentgrasses were the most strongly mycorrhizal turfgrasses in comparison with annual bluegrass and red fescue, but red fescue was second of the three. Thus; red fescue has the potential to benefit more from AM colonization than annual bluegrass, and might be able to outcompete this invasive species when growing in soils amended with compost, if the growth medium enhances AMF development.

Because growth media consisting of sand and peat are less rich in mineralized nutrients than media consisting of sand and compost (Aamlid et al. 2009), they consequently demand a higher amount of fertilizer. In addition to the fact that large amounts of fertilizer create better growing conditions for annual bluegrass, very heavy phosphorus fertilization can reduce mycorrhizal colonization (Gange et al. 1999b; Smith & Read 2008), so utilization of mycorrhizal fungi in sand based greens with peat would probably not be as successful as with compost. Another advantage of using recycled organic waste instead of peat is that compost can suppress several grass diseases (Boulter et al. 2002; Espevig 2011), and that peat moss has an important ecological function in nature . Peat harvesting from bogs release heavy amounts of carbon into the environment, and the world's resources of peat are still declining rapidly. A combination of amendment with compost and inoculation with AMF could thus function as a good replacement of readily available P fertilizer, especially in USGA sands that are very low in microbial life (Gemma et al. 1997a; Koske et al.

1997b). This may also help shifting the competition in favor of red fescue and bentgrasses so that chemical control of annual bluegrass no longer is necessary (Gange 1998; Gange et al. 1999b). This is a highly desirable scenario because of the aforementioned challenges regarding control of annual bluegrass while also restricting use of fertilizers, pesticides, energy for irrigation and mechanical maintenance.

Their high prevalence and the many benefits which AMF can offer their hosts are well documented. Yet, relatively little is known about mycorrhization of turfgrasses. This is mainly due to the old assumption that plants with finer, extensive roots and abundant root hairs, or plants that are grown in highly maintained and fertilized environments, are less dependent on mycorrhizal symbiosis to obtain sufficient water and nutrients. Few studies have been performed on which fungal species are involved in AM formation, as well as on their biology and impact on preferred host plants in the turf environment, although the interest in this subject has increased after it was known that many turf grass species form abundant and diverse mycorrhizas from which they may achieve a number of benefits. Furthermore, little is known about how green management affects AMF colonization, and how the individual fungal species respond to various environmental factors. For allowing an optimal interaction of these organisms with amenity turfgrass, and to be able to find suitable combinations of AMF species that provides positive growth responses and which may be used in the production of any high quality inoculation products for turfgrass in the future, more field studies addressing the current issues are necessary (Frank 1984; Gange et al. 1999b; Gange & Case 2003; Gemma et al. 1997a; Gemma et al. 1997b; Hartin et al. 2005; Koske et al. 1995; Koske et al. 1997a; Koske et al. 1997b; Koske & Gemma 2005; Newsham et al. 1995; Pelletier & Dionne 2004)

1.3 Hypotheses and objectives

In the context of this background, the following hypotheses were set up:

1. The extent of AM colonization on frequently used turfgrass species in Scandinavia varies in the order: annual bluegrass < red fescue < colonial/velvet bentgrass.

2. AM colonization levels will decrease with increased N and P fertilization.

3. Use of Green Mix (compost) in the growth medium will lead to more extensive AM colonization.

4. Inoculating the green with a mycorrhiza product while using lower inputs of fertilizer will enhance the AM colonization, and thus provide better field quality in terms of less disease, less invasion of annual bluegrass and a higher general impression.

5. A complex fungal community contributes to AM colonization of golf greens.

Objectives

The main objective was to *find out more about how N/P rates and growth medium affect AM colonization in different turfgrass species on sand based golf greens in Scandinavia.*

Sub goals

1. To quanify the extent of AM colonization on roots of red fescue, velvet bentgrass, colonial bentgrass and annual bluegrass.

2. To determine the effects of N and P rates (including inoculation with a mycorrhiza product) on AM colonization.

3. To compare AM colonization levels in red fescue on sand-based rootzones amended with either peat or composted garden waste (Green Mix).

4. To examine the effects of turfgrass species, fertilization, growth medium, mowing height and mycorrhiza inoculation on turf quality, to get a more complete picture of how management and growth responses are connected.

5. To examine the AMF diversity in 'natural' versus inoculated Scandinavian golf greens.

The experimental data for this thesis were derived from a pot study and from two independent field studies that investigated effects of different green management on turf quality, playability and competition against annual bluegrass on Scandinavian golf greens. The aim of the first project (later referred to as 'study 1') was to study the effect of mowing heights, nitrogen and phosphorus rates as well as mycorrhizal inoculation, on green quality and competitiveness against the invasive annual bluegrass on greens dominated by red fescue. The aim of the second project ('study 2') was to study the effect of a well – defined compost made from garden waste ('Green Mix', Høst A/S, Grimstad, Norway) in the root zone or in the topdressing sand, on red fescue turf quality, disease incidence and competition against annual bluegrass.

A thirteen week experiment ('study 3') was set up in a greenhouse, to practice methods related to staining and root analysis, and to learn a little about direct effects of different input factors under controlled conditions. The aim of the project was to measure the effects of root zones amended with peat or Green Mix, as well as an AMF inoculation product, on growth and AM formation in the current turfgrass species. As with the field study, AMF colonization of the roots was examined and estimated, and visual plant performance was recorded. All practical work related to the thesis was carried out in 2012. The pot experiment was conducted as an initial project in the winter / spring, while the practical work related to the field trials took place in the fall.

2 Materials and methods

2.1 Field studies

2.1.1 Location and weather conditions

The research was performed during the late summer and autumn of 2012 on two already established and maintained greens at Bioforsk Landvik, Grimstad (58° N lat, 12 m above sea level), that belonged to two on-going experiments . Landvik is situated at a coastal location far south in Aust Agder county, have a temperate, coastal climate, and lies in the southern zone for turfgrass variety testing (Aamlid & Molteberg 2011). Table 2 shows the mean monthly temperature and monthly precipitation from establishing the first of the two field projects in August 2010 and to the end of the growth season in October 2012.

Table 2: Mean monthly temperature and precipitation at Landvik, from August 2010 to October 2012. Data are compared with thirty year normal values (1961 – 90).

	Temperature, °C				Precipitation, mm			
	2010	**2011**	**2012**	**Normal**	**2010**	**2011**	**2012**	**Normal**
January		-2.0	0.1	-1.6		104	144	113
February		-1.9	-0.5	-1.9		141	14	73
March		1.9	6.8	1.0		52	27	85
April		9.3	5.2	5.1		8	130	58
May		10.3	11.8	10.4		95	53	82
June		15.2	12.9	14.7		106	118	71
July		17.0	15.8	16.2		157	83	92
August	16.0	15.5	15.8	15.4	130	188	106	113
September	11.7	12.9	11.6	11.8	121	234	132	136
October	6.9	8.9	6.8	7.9	154	74	217	162
November	-0.2	6.5		3.2	117	53		143
December	-8.3	2.1		0.2	50	155		102
Average	5.2	8.0	8.6	6.9				
Sum					575	1372	1028	1230

2.1.2 Study 1: The Niblick experimental green

Establishment and experimental design

The experiment was conducted from August 12th to November 1st in 2012, on a green constructed in summer 2007 according to specifications of United States Golf Association (USGA) (USGA 2004). The green consisted of a 30 cm sand–based root zone amended with 10 % (v/v) sphagnum peat moss underlying by 10 cm gravel. In July 2010, the 4 cm top layer of a 3 year old green was replaced with the same root zone as an initial type. Soil samples taken to 20 cm depth prior to the sowing had an average pH of 5.8, measured by extraction with H_2O, and an P-Al content of 1.39 and 1.63 mg/100 g, tested by the Gilford method and the ICP method, respectively.

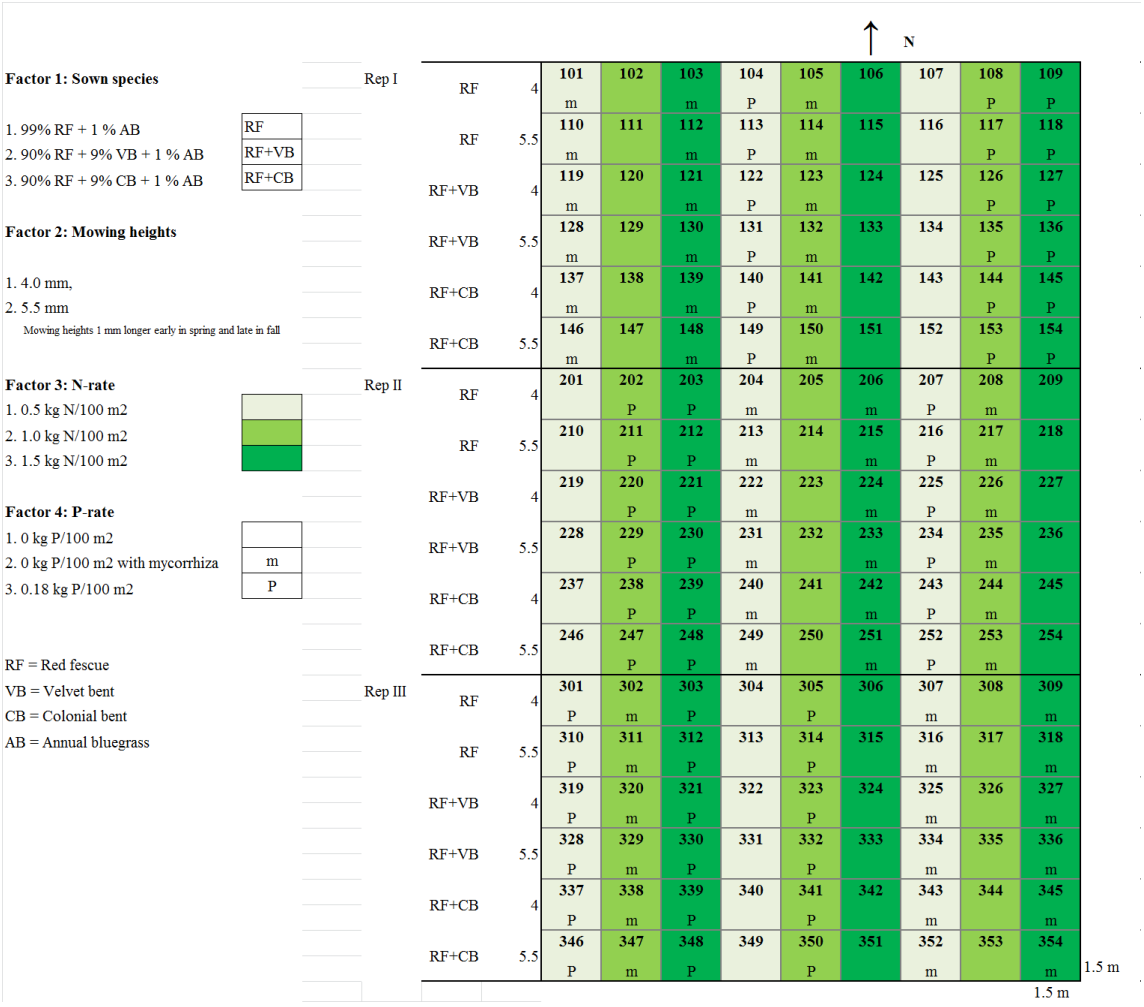

Figure 4: Field map and experimental factors of the Niblick experimental green.

The trial was lied out according to a split-split-block design with 4 experimental factors and 3 blocks. The experimental factors are shown next to the field map in Figure 4. The plot size was 2.5 m^2, and the boundary area around the trial was 30 cm and 100 cm at the western and northern side, respectively.

With respect to mowing, fertilizing and other practical reasons, plots within each block could not be completely randomized. They were placed so that all plots in one vertical row had the same N and P treatment, and so that all plots in one horizontal row had the same mowing height and species combination.

Species: The green was seeded on August 12th 2010. All seed mixtures were sown at a rate of 2.5 kg/100 m^2, using a drop seeder. See Appendix 1 for species varieties used in the mixtures. Reseeding of approximately 5g/m^2 to winter damaged spots were performed on the 15th and 29th of April 2011. Additional 2g/m^2 of annual bluegrass was sown on November 9th the same year. Due to the generally poor growth of this species, a pure annual bluegrass cylindrical core sample of 5.5 cm in diameter was planted in each plot on August 18th 2011.

Mowing: The grass was mowed to either 4.0 or 5.5 mm three times a week (Monday, Wednesday and Friday), using a John Deere 220A (Moline, IL) or Allett (Allet mowers LTD, Arbroath) walk – behind green's mower. The experimental mowing heights were gradually raised and lowered by up to 6 mm in early spring and late fall, respectively.

Experimental fertilization and biological treatments: N was applied every other week from June 3rd to November 1st in 2011 and from March 29th to November 11th in 2012, in the form of liquid ammonium nitrate (NH_4NO_3). P was applied simoultaneously with N, in the form of 85 % liquid phosphoric acid (Figure 5). Total input of N and P to plots treated with different N – levels and normal P fertilizer in 2011 and 2012, are shown in Table 3. Hollow tine ventilation (coring) was performed at the experimental startup on June 1st 2011, and on May 14th 2012, using a John Deere Aerator 800 (Moline, IL) mounted with 6 mm hollow tines to decompress and aerate the soil to a depth of 6 cm (Figure 6). On June 2nd in 2011 and May 15th in 2012, the mycorrhiza inoculum SYMBIVIT® (Norsk Mykorrhiza, Oslo, Norway/Symbiom Ltd., Sazava 170, Czech Republic) was applied to the field at a product rate of 150 g/ m^2 using a solid fertilizer machine, and raked into holes from the coring performed the day before (Figure 7AB). SYMBIVIT® contains fragments of colonized roots and mycelium, as well as spores, from six mycorrhizal species of the *Glomus* genus (on average 250.000 infective propagules per L), and natural ingredients that support mycorrhiza, such as humates, ground minerals and extracts from sea organisms. The product also contains natural clay carriers and degradable granules of a water retaining gel (Symbiom 2012).

Table 3: Total input of N, P and K during the experimental season of 2011 and 2012.

	N			**P**	**K**
	0.5	**1**	**1.5**		
	kg/100m²				
2011	0.39	0.77	1.15	0.13	0.75
2012	0.50	1.00	1.50	0.18	0.98

Figure 5: Applying liquid fertilizer to plots with same N/P treatments. Photo: Tatsiana Espevig.

Figure 6: Coring at experimental startup on June 1st, 2011. Photo: Tatsiana Espevig.

Figure 7: Applying mycorrhiza inoculum as surface dressing with a solid fertilizer machine (A), and raking the granules into holes from coring performed the previous day (B). Photo: Tatsiana Espevig.

Plots's general maintenance

Fertilization: All fertilizers were applied in liquid form. The total amount of N, P and K applied during during grow – in from August to October 2010 was 0.56, 0.06 and 0.43 kg/100 m^2, respectively. During this period, the green was fertilized every week for four weeks, and then with a two – and three week interval, respectively. For the experimental periods, micronutrients were applied five times from June to October in 2011 and seven times from April to September in 2012, in form of Potassium chloride, Magnesium sulfate, Rexolin Ca and Rexolin APN. Their volumes were calculated from the highest nitrogen level used in the trial. Total input of N, P, K and micronutrients, as well as the total input of each fertilizer type during the experimental season of 2011 and 2012, are shown in Appendix 2. Also see Table 3 for both years total input of N, P and K.

Grooming: On occasional Mondays in 2011, grooming was done in conjunction with mowing, using a John Deere grooming attachment mounted on a John Deere 220A that was adjusted to a depth of 0.1 – 0.2 mm from the surface.

Rolling: At the end of May 2011, before the first mowing that year, the field was subjected to rolling. A friction wear roller without spikes was then pulled over the field to make the ground more compact and smooth.

Vertical cutting: In the middle of August 2011, vertical cutting was performed to 2 mm depth, using an Aztec verticutter pod mounted on an Aztec drive unit (Allett mowers LTD, Arbroath, Scotland).

Wear: Starting in May 2012, a friction wear drum with soft spikes was pulled over the plots one time three times a week, always after mowing. This corresponded to approximately 25.000 rounds of golf per year.

Topdressing: Topdressing was applied every Friday after mowing and wear infliction, and after spiking and vertical cutting, by pulling a centrifugal spreader over the field. Total amount of topdressing applied in 2011 and 2012 was 9.50 and 9.25 mm, respectively.

Irrigation: the field was irrigated to the field capacity each time the soil humidity at 12 cm was 8 % or less, measured with a portable time-domain reflectrometer (TDR) (Campbell Scientific ltd.,Thuringowa, Australia). Five mm was also applied after fertilizing if the weather was dry, and after mycorrhizal inoculation.

Other mechanical treatments

Spiking to 6 cm depth, with 8 mm firm tines mounted to the John Deere aerator, was performed at the beginning of November 2011 and in the middle of August 2012. Deep ventilation was implemented in the middle of November 2012, by driving a tractor with a mounted VertiDrain machine (AJ & R Scambler & sons ltd., Cambridgeshire, UK) over the field (Figure 8). Firm 12 mm tines of 25 cm length were used, to aerate and 'open' the subsoil for better drainage, minimal thatch, deeper rooting and a thriving grass, among others.

Figure 8: Tractor mounted VertiDrain machine used for deep ventilation. Photo: Tatsiana Espevig

2.1.3 Study 2: The red fescue experimental green

Establishment and experimental design

This experiment was seeded on August 17th 2011, in a USGA Green field lysimeter facility. The 30 cm root zone was amended with 17 % (v/v) sphagnum peat moss or 17.5 % composted garden waste ('Green Mix'- Høst A/S, Grimstad, Norway) in alternating plots. The existing turf and root zone had been removed down to the gravel before new material was added (Figure 9). Results from analysis of the two root zone materials, sampled prior to establishment, are shown in Table 4. Based on previous trials, the volume percentage had been adjusted to a target ignition loss of 2.5 (w/w). As shown in the table, the realized values were 2.63 % and 2.85 % w/w in soil with peat and Green Mix, respectively. The volumetric soil water capacity in the 3-1500 kPa tension range, determined in undisturbed soil cores taken in autumn 2011, were 19.2 % for peat and 21.1 % for Green Mix.

Figure 9: One of the plots before establishing the red fescue green in 2011. Old turf and growth root zone have been removed. The 0.4 m deep lysimeters are seen sticking up from beneath the ground in each plot. The tanks are 0.45 m deep with a drain line located in the middle. Photo: Trygve S. Aamlid.

Table 4: Chemical soil analyses of rootzone materials at establishment, and of topdressing in 2012.

Parameter	Unit	Root zone, August 2011		Topdressing sand, 2012		Reference/ method
		Peat	Green Mix	Pure sand	Green Mix	
Volume weight	kg/L	1.5	1.5	1.7	1.6	
pH		5.6	7.8	6.5	8	H_2O
P-AL	mg/100g	1.7	6.4	< 1.0	5.9	AL
K-AL	mg/100g	2.3	25	< 2.0	24	AL
Mg-AL	mg/100g	2.4	6.8	< 1.0	4.9	AL
Ca-AL	mg/100g	14	95	< 10	111	AL
Na-AL	mg/100g	2.2	3.1	< 5.0	< 5.0	AL
K-HNO_3	mg/100g	47	72	-	-	
Mineral N	mg/100g	0.06	3.0	0.17	3.2	
Loss on ignition	% DW*	2.63	2.85	0.1	0.95	
Total N	% DW	< 0,1	0,14	< 0.11	<0.11	CENTS15104/1540
Total C	% DW	1.5	1.9	< 0.5	0.58	CENTS15104/1541
Cu	mg/kg	0.26	1.3	< 0.20	0.84	EDTA
B	mg/kg	< 0.10	0.56	0.36	<0.10	Hot water extr.
Fe	mg/kg	6.7	2.5	10	3.8	NH_4-acetate
Mn	mg/kg	4.5	0.60	< 0.50	<0.50	$Mg(NO_3)_2$
Zn	mg/kg	< 1.0	6.7	< 1.0	4.4	0.2 M HCl
Mo	mg/kg	< 0.20	< 0.20	< 0.20	<0.20	Tamms solution

*DM, dry weight (w/w)

The trial was laid out according to a completely randomized design, with 2 experimental factors and 4 replicates. The experimental factors were:

Factor 1: Root zone at construction in 2011

1. USGA – sand + sphagnum peat moss (OM = 2.63 % w/w).
2. USGA – sand + Green Mix (OM = 2.85 % w/w).

Factor 2: Topdressing material used in 2012

1. Pure sand
2. Green Mix (OM = 0.95 w/w).

With 4 replicates, this equaled 2 x 2 x 4 = 16 gross plots of 2 x 3 m. All registrations were done in the central net plots of 1 x 2 m, corresponding to the surface of the lysimeter. The trial was seeded with a drop seeder (Figure 10) at a realized seeding rate of 29.3 g/m^2. The seed mixture consisted of 97 % red fescue and 3 % unspecified annual bluegrass, where the red fescue varieties were: '*Cezanne'* (38.8%), '*Musica*', '*Bargreen*' and '*Calliope*' (19.4%).

Figure 10: Using a drop seeder for seeding a mixture of 97 % red fescue varieties and 3 % unspecified annual bluegrass. Photo: Trygve S. Aamlid.

Figure 11: Overview of the red fescue green on April 25th 2012. Visual differences between treatments have already started to show up. The central border area separating rep 1 and 2 (left) from rep 3 and 4 (right) was used for other experimental purposes. Photo: Trygve S. Aamlid.

Plots's general maintenance

Mowing: In 2011, the experiment was mowed for the first time to 10 mm on September 7th. On subsequent mowing two to three times per week, mowing height was gradually reduced to 6 mm on October 3rd, and brought up to 7 mm again at the last mowing on October 26th. In 2012, the green was mown every Monday, Wednesday and Friday in weeks without top dressing, but only on Monday and Friday in weeks with dressing. Mowing height started at 9 mm on March 26th, and was gradually lowered to 5 mm on May 7th, at which it was kept for the rest of the season until the last mowing on October 22nd.

Topdressing: Once the grass started to become established in 2012, dressing /dusting was implemented every other Tuesday. Dressing was applied by hand 13 times followed by brushing, the first time on April 25th and the last time on October 10th. 0.63 mm of topdressing, corresponding to 3.8 L, was applied to each plot. This equaled a total of 8.2 mm top dressing applied during the season.

Aeration and overseeding: The green was aerated with 6 mm solid spikes to 6 cm depth on April 23rd, June 5th and August 14th 2012. Following aeration on April 23rd and August 14th, the green was overseeded with 20 g/m^2 of the same seed mixture as used for establishment.

Wear: The green was subjected to artificial wear and compaction using a friction wear drum with golf spikes. Wear started once grow – in was complete in early July, and went on until early October. On average there were four passages (two times back and forth) per week over the fifteen week period, corresponding to approximately 10000 rounds of golf.

Irrigation: Because the calculated water capacity was similar for both root zones, all plots were irrigated once a week according to the same strategy. The green was given 5 mm of water every Tuesday after dressing or fertilizing, unless it had been at least 5 mm of natural rainfall. Beyond this, 10 mm was given each time the volume percentage in the upper 12 cm became lower than 10 %, as measured with a TDR probe (Delta T devices, Cambridge, UK).

Fertilization: To ensure fast and complete establishment of red fescue on both root zones, it was decided to use twice as high fertilizer rates on the peat root zone as on the GreenMix root zone during the grow – in phase. During the first five weeks of grow – in, fertilizer was applied every week, starting with the organic fertilizer 'Binadan (Binadan A/S, Nørre Snede, Denmark)' pre seeding, and then alternating between the liquid fertilizer 'Wallco (Cederroth International AB, Falun, Sweden)' and the granular fertilizer 'Andersons 13-2-13 (The Andersons, Maumee, Ohio, USA)' (Table 5). The rest of the season, fertilizer was given every other week. In 2012, fertilization was the same on all plots. As the turf cover was not mature until the middle of July, fertilizer was applied every week from March 20th to July 10th, and every other week for the rest of the season. The fertilizer types were all in liquid formulation, partly 'Greenmaster liquid NK 10-0-10 (Everris International AB, Geldermlsen, The Netherlands)'and partly 'Arena Calcium' in combination with 'Arena Crystal 19-2-15 (Yara AB, Landskrona, Sweden)'. The total input of N, P and K from March to October 2012 was 1.30, 0.06 and 1.05 kg/100 m^2, respectively. For total fertilizer inputs and amount of N, P and K given at each

application date during the experimental season, see Appendix 3.

Table 5: Total fertilizer inputs and amount of N, P and K given during grow – in from August to November 2011.

		kg/100m^2							
Week	**Fertilizer type**	**Total**		**N**		**P**		**K**	
		Peat	**Green mix**	**Peat**	**Green mix**	**Peat**	**Green mix**	**Peat**	**Green mix**
33*	Binadan organic†	10.00	5.00	0.850	0.425	0.100	0.050	0.750	0.375
34	Wallco liquid	2.35	1.18	0.12	0.06	0.024	0.012	0.101	0.051
35	Andersons 13-2-13	0.92	0.46	0.12	0.06	0.008	0.004	0.100	0.050
36	Wallco liquid	2.35	1.18	0.12	0.06	0.024	0.012	0.101	0.051
37	Andersons 13-2-13	0.92	0.46	0.12	0.06	0.008	0.004	0.100	0.050
39	Wallco liquid	2.35	1.18	0.12	0.06	0.024	0.012	0.101	0.051
41	Andersons 13-2-13	0.77	0.38	0.10	0.05	0.007	0.003	0.083	0.042
43	Wallco liquid	0.98	0.49	0.05	0.03	0.010	0.005	0.042	0.021
44	Andersons 13-2-13	0.38	0.38	0.05	0.05	0.003	0.003	0.040	0.040
SUM				**1.650**	**0.855**	**0.208**	**0.105**	**1.420**	**0.733**

* Pre-seeding.
† 50/50 mixture of Binadan Green 11-1-3 and Binadan Blue 6-1-12.

2.1.4 Collection of data

Visual observations and analysis obtained from the field studies

Visual assessments and other data collection were performed by researchers and technicians on both field studies through the whole growth season, lasting from March to October in 2012.

Soil characteristics (study 1): On October 23rd 2012, soil samples were taken to 20 cm depth from each plot containing pure red fescue mowed to 5.5 mm, and analyzed by Eurofins Food & Agro Testing (Moss, Østfold). New samples were taken from the same plots on April 24th 2013 and sent to the IJVF laboratory, belonging to the Institute of Plant and Environmental Sciences at the Norwegian University of Life Sciences in Ås, Akershus, for more accurate analyzes of P-Al. All grass was removed from the samples before analyzing, but the thatch layer was retained.

General impression (study 1 and 2) was estimated monthly, based on turf cover, density, color, amount of weeds (including invasive annual bluegrass), bare soil and diseases. Each plot was graded 1 – 9, where 9 is the best quality.

Development of take-all patch (study 1): The percentage of the plot covered with dead spots caused by the fungus *Gaeumannomyces graminis var. graminis*, was estimated monthly.

Competition against annual bluegrass (study 1 and 2): In study 1, a cylindrical core sample with annual bluegrass had been inserted in each plot on August 18th 2011. Sample diameters were recorded on the 19th - 20th of July and on the 11th of October 2012. At the same time, coverage percentage was estimated. In study 2, annual bluegrass was estimated as the percentage of plots covered in May, June and July.

Weight of clippings and nutrient removal (study 2): In 2012, clippings in the basket of the greens mower from an area of 0.56 x 2 = 1.12 m^2 were weighed every other Monday from March 26th to October 22nd. Dry weight was determined after drying at 60°C for 48 hours. Clippings were analyzed for total content of N, P and K by Eurofins.

Root mass (study 2): A cylindrical root sample of 56 mm in diameter was taken to 25 cm depth from each plot on April 18th and October 18th 2012. The roots were washed, dried to constant weight at 60°C, and their dry weight was recorded as dry matter in g/m^2.

Mycorrhiza assay

Two soil cylinders of 21 mm diameter and 20 cm depth were randomly taken from each plot (Figure 12AB). In study 1, samples were taken only from plots mowed to 5.5 mm (due to time pressure), on September 11th, September 24th and October 1st from blocks 1, 2 and 3, respectively. In study 2, samples for studies of mycorrhiza colonization were taken for the first time on October 18th 2011. In 2012, new samples were taken in conjunction with aeration on June 5th. These samplings were analyzed by another Bioforsk employee. At the first two samplings, there was still a significant amount of annual bluegrass in the green, and samples were taken both from this species and from red fescue to get a better basis for discussing competition between annual bluegrass and other turfgrass species. Later, the annual bluegrass was outcompeted because of its high demand for water and nutrients in the summer months, while the red fescue grew stronger. The last samplings on October 11th (rep 3 and 4) and on October 16th (rep 1 and 2) therefore included red fescue only. Dead spots, or spots which were obviously diseased with fungal attack or invaded by weeds, were always avoided when taking out samples. After harvesting, the samples were put in marked plastic bags to keep the soil moist, and stored at 4°C for maximum three days.

Cylinders were brought to the lab, and the whole thatch layer (Figure 12B) was cut off. Roots were first roughly washed from the cylinder, to remove most of the sand and other coarse particles. The root lump were then cut into 0.5 – 1cm pieces with a scissor, and rinsed twice to remove remaining sand and organic material. A sieve with 63 µm aperture size was used for rinsing out the finest particles without losing any roots, and for recovering the root pieces between rinsing and cutting steps. The roots were squeezed free from excess water, before as much as possible of the remaining water was pressed out by blotting with paper towels.

Figure 12: A: Harvesting of soil samples from the Niblick green (study 1) with a soil drill made for the purpose. B: One of the root cylinders. This was taken from plot number 147, containing red fescue and colonial bentgrass. The grass on this plot was mowed to 5.5 mm, given no phosphorous, and fertilized with the intermediate nitrogen level. The distinction between old growth mass from previous experiments (lowest arrow), new growth mass (middle arrow) and the layer of turfgrass thatch (top arrow), is very clear. Photo: Tina E. Andersen.

The roots were split into 2 – 3 parts. For each sample, one part was weighed out for staining, and put in a 20 mL flask. A similar share of the root lump as the one taken out for staining was then weighed out from one out of two parallels, put in an envelope made out of filter paper and stored in a marked zipper bag that was filled with 30 - 40 g silica gel. Remaining roots from all samples were weighed and dried to constant weight at 37°C for 48 hours. Based on their dry weight and the raw weight of stored roots, the total root dry weight in each sample was calculated. The dry matter of the two samples taken from each plot were averaged, and converted to g/m^2.

The roots on 20 mL flasks were covered with 6 – 10 mL 5% KOH using an automatic pipette. Lids were put on loosely, and the flasks were carefully heated in a boiling water bath for 13 – 15 minutes to clear the root tissue (Figure 13A). The now transparent roots wee rinsed free of KOH (Figure 13B), and put back into the flasks with a minimum of water. Six to eight mL of 0.05 % Trypan Blue staining solution was then carefully added (Figure 14A), and the roots heated in a water bath for approximately 60 seconds. This stain had been prepared in a 1:1:1 (v/v/v) mixture of water, glycerol

and lactic acid. Most of the solution was poured off through the sieve, and collected in a jar for hazardous waste (Figure 14B). The roots were rinsed to remove all remaining stain, put back into the flask with a minimum of water, and covered with 70 % lacto – glycerol (prepared in a 1:2:1 (v/v/v) mixture of lactic acid, glycerol and water) for storage (Figure 15A). The roots were now ready for analysis (Figure 15B). Methodology associated with root staining corresponded to that of (Koske & Gemma 1989)

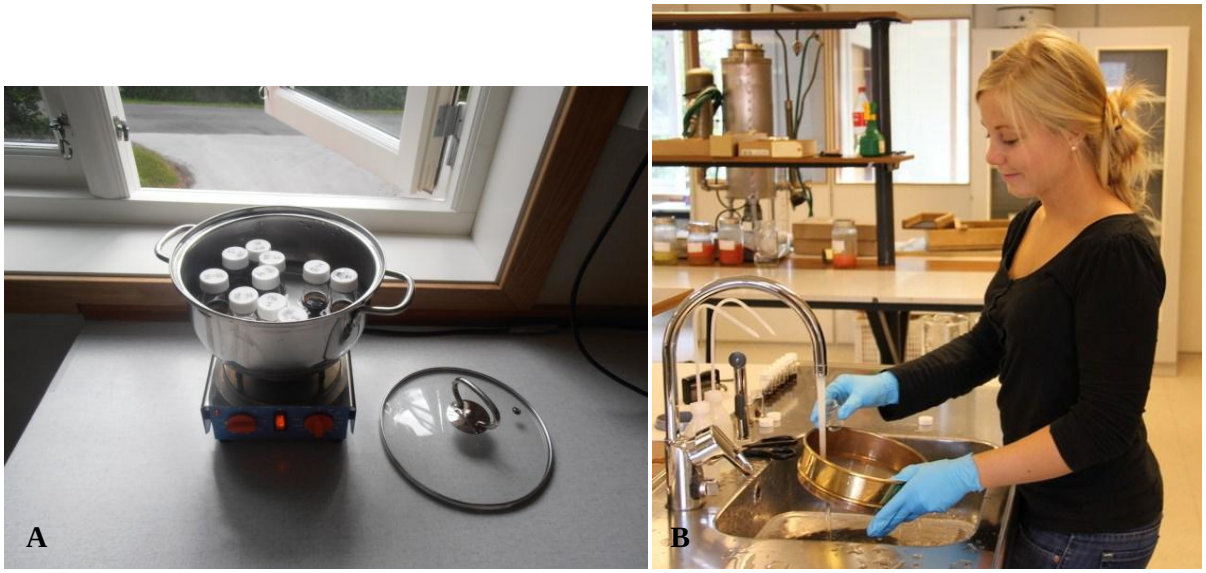

Figure 13: A: Boiling the roots with KOH in a water bath, to make their cells transparent and ready for staining. Flasks were cooled a couple of minutes before rinsing the roots. B: Rinsing roots free of KOH with the fine sieve. Photo: Tina E. Andersen (A) and Anne A. Steensohn (B).

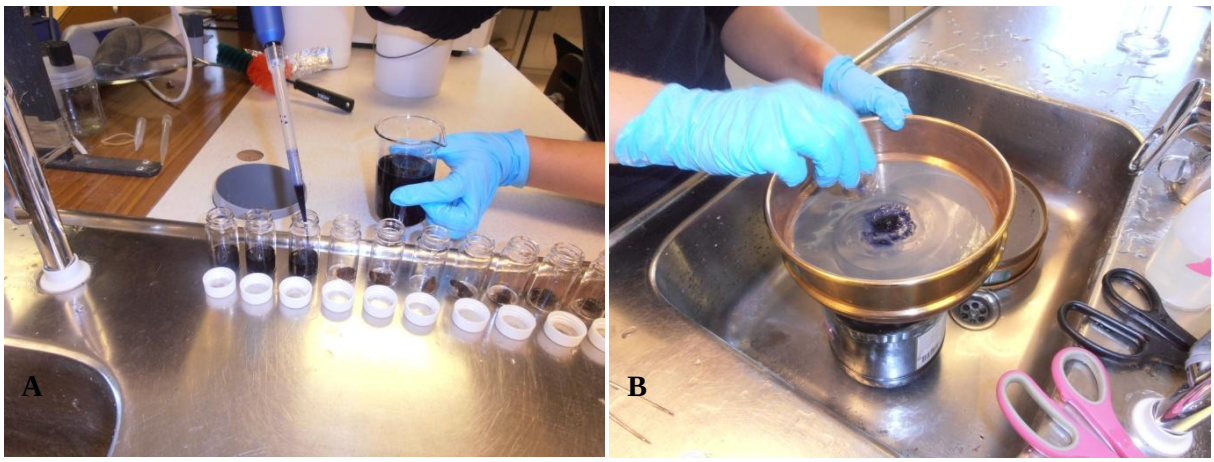

Figure 14: A: Adding Trypan Blue to stain the roots. B: Pouring of most of the heated staining solution into a jar for collecting hazardous waste. Photo: Geir Solgaard (A) and Tatsiana Espevig (B).

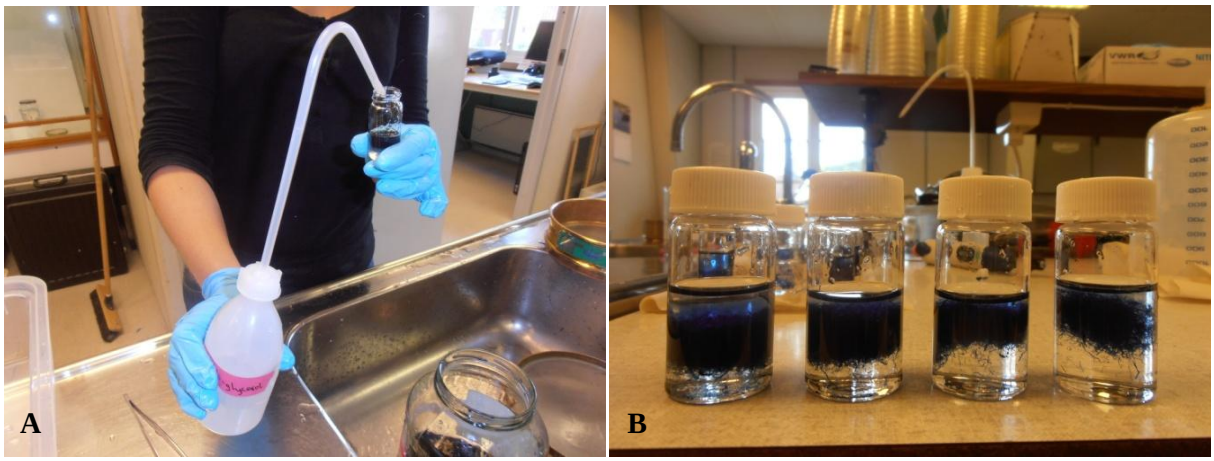

Figure 15: A: Adding lacto – glycerol until the roots are well covered. B: Stained roots, stored in lacto – glycerol and ready to be analyzed. Photo: Tatsiana Espevig (A) and Tina E. Andersen (B).

AM infection was studied and assessed in a petri dish with a 1 cm grid drawn up in the bottom. A small amount of root pieces were evenly spread out in the dish, and the colonization was quantified through a binocular loupe (Leica S8AP0) according to the 'grid-line intersect' method (Giovannetti & Mosse 1980) (Figure 16A). Two hundred root transects was tallied, and scored as 'colonized' or 'not colonized' by AMF (Figure 16B). Roots that were crossing the line twice were tallied and scored at both points of intersection. The estimate of fungal abundance in each sample was expressed as the percentage of roots that were colonized, where the total number of root pieces containing vesicles, hyphae, arbuscules or other mycorrhizal structures was a percentage of the total observations. Data from the two cores taken per plot were averaged.

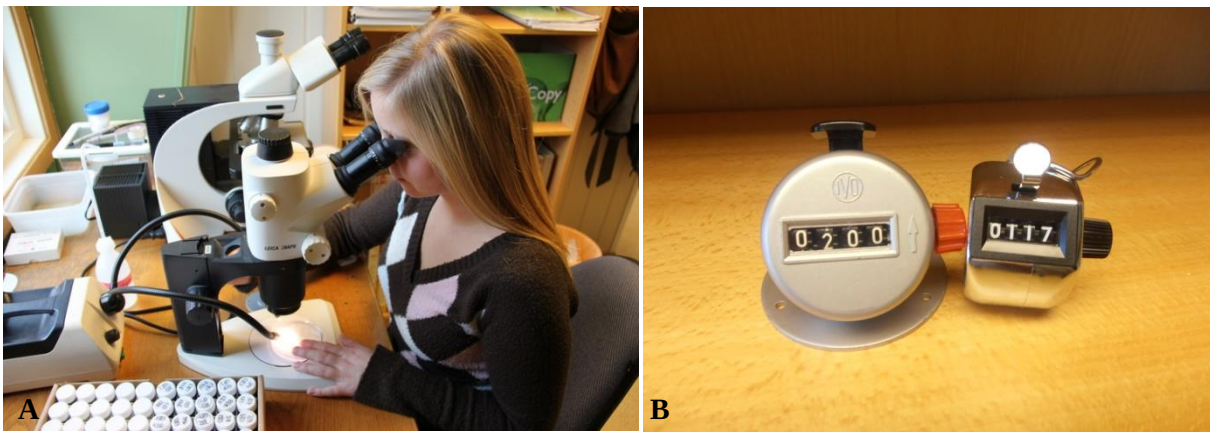

Figure 16: Scoring roots from a sample as 'colonized' or 'not colonized' through a binocular lupe (A), using two hand-held tally counters (B). Photo: Anne A. Steensohn (A) and Tina E. Andersen (B).

In lack of equipment for spore extraction as well as a microscope with sufficient resolution, the various AMF observed in the samples could not be determined beyond genera. A normal microscope did not show enough details to be able to distinguish between species, something that is also very difficult without investigating mature spores. Thus, a rough overview was taken of each sample through the binocular lupe, and AMF structures like hyphae, spores, arbuscules, vesicles and auxiliary cells were noted before a suggestion of possible present genera were made. Interesting structures were photographed, and examined as closely as possible with a normal upright 100x magnification light microscope (Leica DM2500).

2.1.5 Statistical analyses

Statistical analysis were performed using the SAS software package, version 9.2 (SAS institute, Cary, NC, USA). Significant differences among treatments were identified by Fisher's protected LSD test at the 0.05 probability level. Some effects within the 0.05 – 1.0 probability levels were expressed as tendencies, but the term 'significant' always refers to the 0.05 probability level throughout this thesis.

Study 1

Effects of experimental factors on general impression, take – all patch, plant coverage and diameter of annual bluegrass cylinders were tested using the PROC MIXED model statement. Three – factorial analysis was run for data on AM colonization and root dry weight, because these characteristics had been obtained from one mowing height only. Effects of N and P treatments on soil properties were analyzed using the PROC ANOVA statement.

Study 2

Effects of growth medium, top dressing and their interaction on (monthly assessments) of general impression, percent coverage of annual bluegrass, total nutrient removal in clippings, dry weight of roots per m^2 and percentage of AM colonization was analyzed using PROC ANOVA. The effect of grass species was also included in the ANOVA model for AM colonization.

2.2 Study 3: Greenhouse experiment

2.2.1 Location and environmental conditions

The experiment was performed from March 8th to June 5th 2012 in a greenhouse with polycarbonate plates in the walls and ceiling, located at the Center for Climate Controlled Plant Research at Ås, Akershus. Ventilation occurred in the roof ridge, and high pressure nozzles combined with fans in the ciling gave good air humidification. The temperature was set to 20°C during daytime and 15°C at night, with a 16 hour clock-controlled photoperiod lasting from 04.00 am to 20.00 pm. Additional light was given during dark periods and when the temperature was < 20°C, and consisted of seven lamps of the type Son-T LU400W/PSL/T/E40, which irradiated yellow photosynthetic light. The light intensity (photon flux density) was adjusted to 180µmol/m^2/s ± 20, and lamps were automatically disengaged if solar radiation exceeded 300W/m^2. Relative humidity was set to 70% during the establishment phase of about six weeks, and then lowered to 60% for two weeks.

Soil conditions

To match field conditions in study 2, two different growth media were used in the experiment. The media was prepared at Landvik, and as with the root zone on the red fescue green, USGA-sand was mixed with either sphagnum peat moss or composted garden waste (Green Mix). Soil analyzes performed by Eurofins (Table 6), showed that the organic matter content was very similar in both media. Loss on ignition was 1.2 % (w/w) in peat, and 0.96 % (w/w) in Green Mix. In addition, the Green Mix again showed the higher nutrient content when compared to peat.

Table 6: Soil analyzes of the two growth media used in the pot experiment.

		Growth medium		
Parameter	**Unit**	**Peat**	**Green Mix**	**Reference/method**
Volume weight	kg/L	1.6	1.7	
pH		5.8	6.5	H_2O
P-AL	mg/100g	< 2.0	3.1	AL
K-AL	mg/100g	< 2.0	3.2	AL
Mg-AL	mg/100g	1.3	1.6	AL
Ca-AL	mg/100g	< 10	17	AL
Na-AL	mg/100g	< 5.0	< 5.0	AL
K-HNO_3	mg/100g	47	54	
Loss on ignition	% DW*	1.2	0.96	
Total N	% DW	< 0.10	< 0.10	SS-EN15104/1540
TOC	g/100g DW	0.53	0.61	ISO 10694

*DW, dry weight (w/w)

2.2.2 Experimental design

The trial was lied out according to a completely randomized design, with 3 experimental factors and 3 replicates. The experimental factors were:

Factor 1: Growth medium

1. USGA – sand + sphagnum peat moss (OM = 1.2 % w/w)
2. USGA – sand + Green Mix (OM = 0.96 % w/w)

Factor 2: Inoculum

1. No mycorrhiza (NM)
2. Inoculation with "SYMBIVIT®" (M)

Factor 3: Turfgrass species combination

1. 100 % Annual bluegrass
2. 100 % Colonial bentgrass
3. 100 % Velvet bentgrass
4. 100 % Red fescue
5. 95 % Red fescue + 5 % Annual bluegrass

With 3 replicates, this equaled 5 x 2 x 2 x 3 = 60 pots with a surface area of 0,00785m^2, a 10 cm diameter and a 40 cm depth. The experiment also included two negative (one without inoculum and one with killed inoculum) and three positive (living inoculum) controls sown with subterranean clover (*Trifolium subterraneum*).

2.2.3 Preparations

Weighing of seeds

Seeds were weighed and prepared in advance, so that the accurate seed quantity for each species could be applied consecutively on the day of seeding. For annual bluegrass, colonial bentgrass, velvet bentgrass and red fescue, the seed amount per pot was 0.08, 0.05, 0.05, and 0.2 g, respectively. This corresponded to a respective seeding rate of 10, 7, 7 and 25 g/m^2.The seed amount for the species combinations was 0.19 g per pot for red fescue and 0.01 g per pot for and annual bluegrass, corresponding to a seeding rate of 25 g/m^2. When two species were sown in the same pot, they were spread individually to obtain an even distribution of both species. For the controls, 9 seeds were prepared per pot.

Preparation of fertilizer

Nutrient solutions for the two growth media were prepared individually. Because Green Mix releases more nutrients than peat, an additional amount of NH_4NO_3 was provided for the peat solution. This was in line with what was done in study 2 during grow – in, in order to ensure an even and well established turf. Thus; the pots containing peat were fertilized with twice as much N as the pots containing Green Mix. After 4 weeks, new nutrient solutions were prepared for the last eight weeks, but with higher concentrations, since fertilization was limited to every other week from then. Calcium fertilizer was prepared separately both for the first four – and for the last eight weeks, to avoid precipitation reactions in the main solution. All solutions were stored cool and dark, at 4C°.

Pot preparations

Five specially designed racks, each with 16 cylindrical pots made out of drain pipes and nylon mesh in the bottom for the purpose of growing turfgrass, were used after machine – washing at 80 °C to reduce microbial contamination. The nylon meshes were cleaned by hand and sterilized over night in 96 % ethanol. The meshes were then attached to the bottom of the pots by using plastic strips. Due to small amounts of available growth medium, the lower 12 cm of the totally 40 cm deep pots were filled with >1L of LECA pellets. This gave ~28 cm free space for filling with medium. Pots were filled with their respective growth medium and placed in racks in the greenhouse. First, medium was poured in until there were ~15 cm of free space left in the pot. While pouring, it was carefully packed and compressed. Five g of SYMBIVIT® inoculum was then evenly applied on the media surface, and more medium poured in and packed until there were ~ 5cm of free space left. Additional 5 g of SYMBIVIT® inoculum was applied, before a final layer of growth medium was poured in and the final height from the medium surface to the edge of the pot was ~1cm (Figure 17). The purpose of applying two layers of inoculum was to increase the chance that the roots would be extensively colonized, by letting them encounter the inoculum twice while growing. Non – mycorrhizal treatments received same amounts of inoculum that had been killed by heating at 90°C for one hour, to prevent possible effects on plant growth caused by product carrier ingredients from obscuring the results if only the mycorrhizal pots received the inoculum. SYMBIVIT® namely contain natural ingredients supporting mycorrhiza, such as humates, ground minerals and extracts from sea organisms. The product also contains natural clay carriers, and degradable granules of a water retaining gel (Symbiom 2012).

Finally, 500 mL of water was used to soak each pot by carefully pouring it into a small perforated pot with an acrylic cloth inside, before letting it drain into the dry growth medium. This prevented, to a certain extent, accumulation of water and swirling of pot contents in the top. The two negative and the three positive controls were made with excess Green Mix. All controls were prepared in the same manner as the experimental pots, but normal flower pots with drip trays underneath were used instead of the cylinders, and the bottom was not filled with LECA pellets.

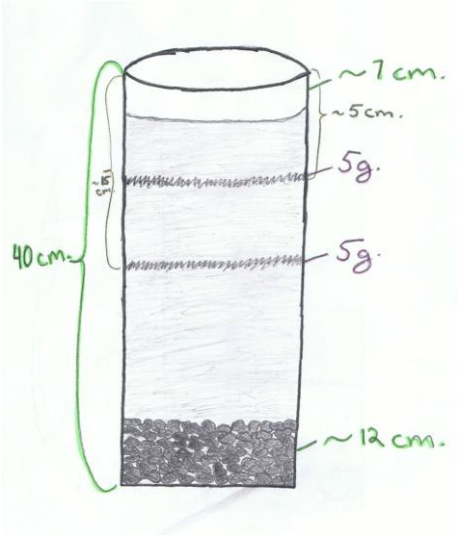

Figure 17: An outline of how the pots were prepared, with LECA pellets for water drainage and space filling in the bottom, and mycorrhiza inoculum placed as two layers further up.

Sowing

On March 8th, 64 pots placed randomly in four racks were placed in the greenhouse. All pots were fertilized first with 10 mL calcium followed by 10 mL of water, to mobilize the calcium further down and avoid precipitation due to contact with other nutrients. Then, 20 mL of the two mixed fertilizer solutions were applied to their respective pots, and all pots were refilled with some growth medium because soil compaction had lowered the soil surface during preparation. The different turfgrass seeds were distributed as evenly as possible by hand in their respective pots, and some growth medium was sprinkled over until the soil surface was ~0.5 cm from the pot edge. All pots were then carefully irrigated with 40 mL of water, to ensure wetting of seeds. The control pots were sown in the same manner as the experimental pots. To avoid drying of the growth medium surface during germination, collars were taped around the pot edge and covered with a plastic film until the seeds had germinated (Figure 18). Pots were humidified daily until the seeds began to sprout, at which stage the plastic film and collars were removed.

Figure 18: The 64 pots in the greenhouse at the start of the experiment, randomly placed in four racks, and covered with collars and plastic film after sowing. Photo: Tina E. Andersen.

2.2.4 Pot maintenance

Irrigation

During the first half of the experiment, all pots were frequently watered (from every day to every other day) with relatively small volumes of 10 – 20 mL, to ensure a moist environment around the fragile seeds and seedlings at all times. When the grass had become more established and the different species started showing their characteristic properties, water was only given as often as necessary to prevent visual drying of the soil surface. At the same time, larger volumes were applied to ensure wetting of the deeper growing roots, and these volumes varied with how dry the pots were. An important reason to stop water less frequently was that the risk of pathogen infection increased when the surface was damp at all times. Moreover, by exposing the plants for some drought stress, deep root growth was stimulated. The field capacity of each pot was measured by adding water until this began running out through the bottom, and then recording the weight of the saturated cylinder. During the last three weeks of the experiment, the pots were regularly weighed and watered to this level if more than 200 mL had been lost to evapotranspiration. Throughout the whole experiment, water was also given as required after fertilizing to wash the nutrients further down. Dressing that was applied to the pots before and right after seed germination was also moistened, to protect the seedlings from drying out.

Mowing

Most seeds germinated already during the week after sowing, and the grass grew rapidly in height. With the intention to let the young, fragile plants grow a little stronger, and also to ensure that sufficient carbohydrate for the mycorrhizal fungi was produced in the start phase, mowing was performed for the first time by the end of experimental week 3. By then, some of the grass had reached up to 10 centimeters in height (Figure 19). The grass was cut to between 10 and 15 mm the first time (Figure 20), and it was cut with scissors at regular intervals of two times a week after this. The cutting height was gradually reduced to between 5 and 7 mm.

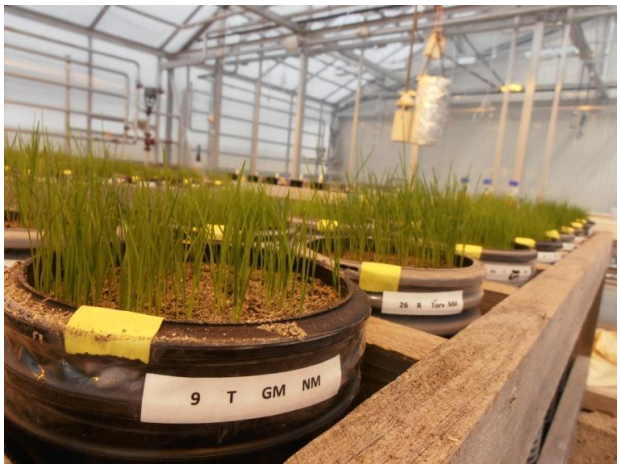

Figure 19: The grass had reached a height varying from 4 to 10 cm before mowing was implemented by the end of the third experimental week. Photo: Tina E. Andersen.

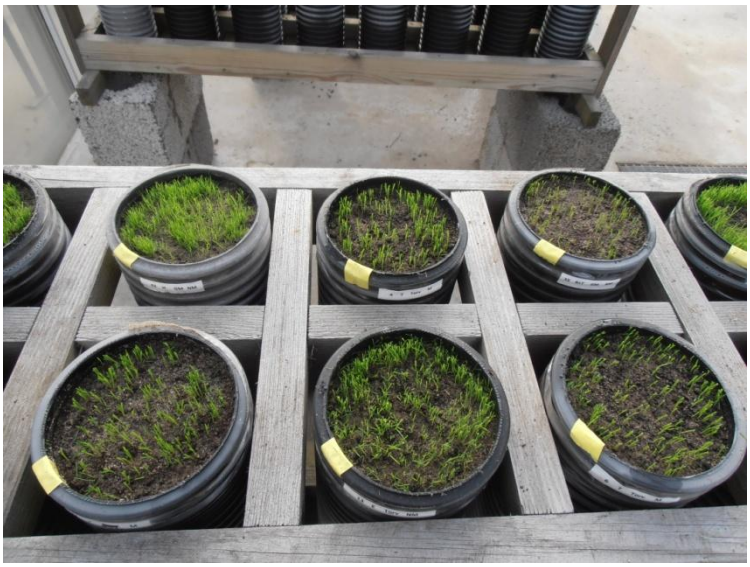

Figure 20: At first mowing, the grass was cut to between 10 and 15 mm. Photo: Tina E. Andersen.

Fertilization

Fertilizer was applied at the day of sowing (experimental week 1), and then in week 2, 3, 4, 6, 8, 10 and 12. The Green Mix – and peat solutions both consisted of the nutrients NH_4NO_3, KCl, $MgSO_4$ and Rexolin APN, while the calcium solution was made with Rexolin Ca. The total amount of N, P, K, Mg, S, Ca, Fe, Mn, Cu, Zn, Mo and B applied during the 13 week experimental period was 10 (20 for peat), 0, 6.5, 0.8, 0.8, 0.6, 0.07, 0.04, 0.001, 0.006, 0.001 and 0.020 g/m^2, respectively. Calcium was always applied first, followed by 10 mL of water, to reduce mixing with the next fertilizer type and thereby prevent chemical precipitation. The five control pots were roughly fertilized with the same amounts despite the lower soil volume.

Dressing

Because of irrigation and other disturbances, the growth media were constantly compacted, and the soil surface was sinking in. A 1 – 2 mm layer of pure USGA – sand was applied to the surface whenever a cylinder had been lowered so much that it was apparent that the distance from the surface and up to the pot edge had become more than 1 cm.

Fungicide treatment

In the seventh week of the experiment, symptoms of fungal pathogen attack were discovered in all pots with colonnial bentgrass, apparent as dry, brown grass that looked dead (Figure 21A). Shortly after, the velvet bentgrass also showed degraded quality, and fungal mycelium appeared in several of these pots. Although precautions were taken when dealing with the infected grass, like cleaning the scissor between mowing and not touching the infected grass directly, this was not sufficient to prevent more infection. Neither was lowering the relative humidity, and the disease became more severe

(Figure 21B). All pots with velvet bentgrass were placed together in an additional rack, and the colonial bentgrass pots were randomly placed at some distance from the other species, in an attempt to limit contamination of healthy plants. Red fescue and annual bluegrass showed a higher resistance, and were not affected by the fungus. By the tenth experimental week, it was decided to treat the infected grass with fungicide. Investigation of spores and hyphae found on some sick plant material, performed by the plant pathologist Maria Herrero at the Bioforsk division "Plant health", indicated that the disease was due to *Fusarium spp*. According to HSE rules and user instructions on the packaging, all bentgrass pots were then equally sprayed with the contact agent 'Octave WP, Prokloraz 005" (0,1 %), (Grøn Plantebeskyttelse ApS)'. Since the product was not systemic, mycorrhizal fungi living in the soil was not likely to be affected by fungicide saturation of the leaves and soil surface. AMF is also not a target organism to this fungicide, so the treatment was regarded as safe. Spraying was repeated two more times, at one week intervals. While the colonial bentgrass was sprayed every time, the velvet bentgrass recovered rather quickly once treated, and was not sprayed the last time. Healthy pots, which were not to be treated, were shielded using a plastic tray as a shield while spraying one and one of the other pots through a carved hole in the center of the tray.

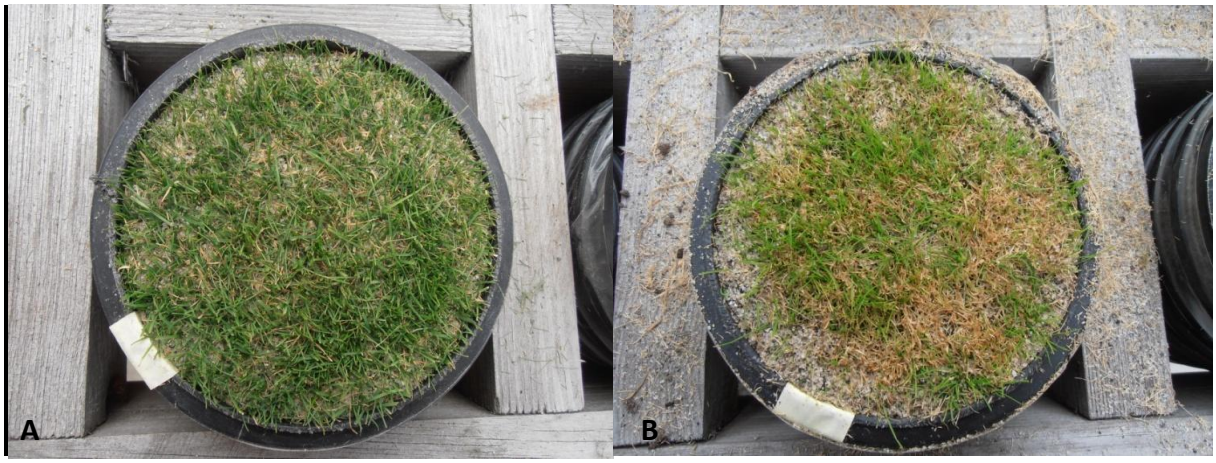

Figure 21: A: The colonnial bentgrass, showing symptoms of disease. Some pots had grass that was quickly dying. B: Especially the velvet bentgrass, which was also infected, was quickly withered after infection. Photo: Tina E. Andersen

2.2.5 Collection of data

Collection of clippings

At every fourth mowing from week 8, clippings were collected and dried to constant weight in a drying oven, at 37 °C. This was done by brushing off any loose sand and old clippings before moisturizing the pot surface, holding the pot horizontally over a table, and carefully cutting the grass to the correct height while letting the clippings fall down on a big sheet. Clippings were put in marked paper bags, dried, and weighed. Registered values from the total of four samples collected per pot were added, and the total dry matter collected during the period was converted to total dry matter of clippings in g/m^2.

Visual data collection

Percent turf coverage in pots was visually estimated for the first time in experimental week 10, on the day before second collection of clippings (Figure 22). Total grass coverage was registered, as well as diseased grass only, and net coverage (percentage of pot covered by healthy grass) was calculated. Such registrations were made every other week, always the day before collecting clippings. At each registration, the pots were also photographed. Mean values based on a total of three registrations were calculated for both total coverage, diseased grass, and healthy grass.

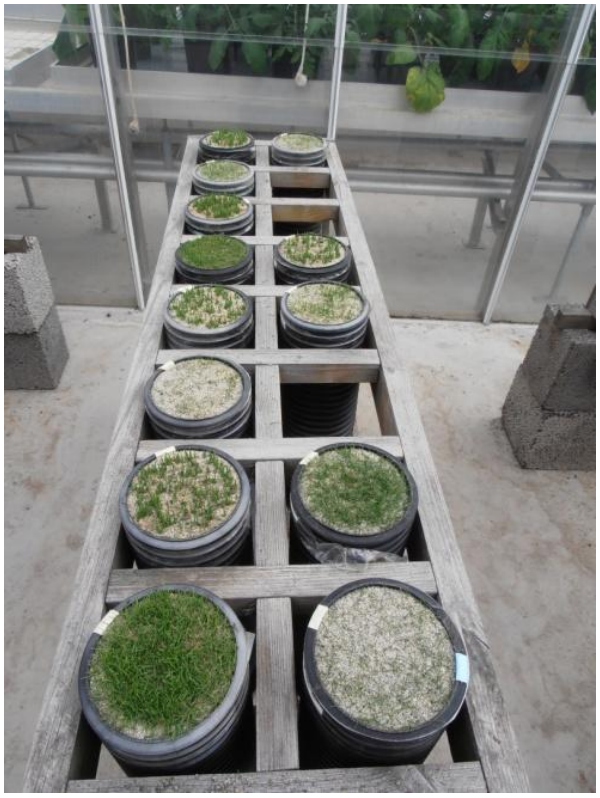

Figure 22: Percent coverage of both total and diseased grass was visually estimated in each of the 64 pots. Here for the first time out of three, at the day before second collection of clippings. Total coverage, proportion of disease and sward density varied considerably between species. Photo: Tina E. Andersen.

Harvesting the experiment

On June 5th, at the beginning of experimental week 14, all pots were harvested. The nylon web was removed from the bottom of the pots, and the LECA pellets were collected in bags for later reuse. Approximately 1 cm of the cylinders was pushed out through the pot from below, and the above ground plant material was cut off as a whole piece, directly below the plant crowns. These 'discs' were carefully rinsed free from sand and other organic material, and the clean grass was dried, weighed and converted to dry matter of crowns in g/m^2. The remaining root system was washed free from sand and organic matter, and underwent the same treatment as the samples from the field trials: Each root system was divided into three parts, where the first part was weighed and stored for later DNA analysis, the second part was weighed, stained and analyzed for mycorrhizal colonization, and the rest was weighed and dried to constant weight at 37°C before it was weighed again. The dry weight of the total root system was calculated, and converted to dry matter of roots in g/m^2. The same methods as in the field studies were then followed for estimating the percentage of mycorrhiza colonizing the roots (including controls).

2.2.6 Statistical analysis

Statistical analysis were performed using the SAS software package, version 9.2 (SAS institute, Cary, NC, USA). Significant differences among treatments were identified by Fisher's protected LSD test at the 0.05 probability level. Some effects within the 0.05 – 1.0 probability levels were expressed as tendencies. ANOVA was performed on data recorded at different dates and on mean values for plant coverage percentage, disease coverage percentage, and plant coverage percentage of healthy grass. Similarly, ANOVA was performed on individual dates for dry weight of clippings, total dry weight of clippings per m^2, raw weight of roots per m^2, dry weight of roots per m^2, dry weight of crowns per m^2, and mean percentage of AM colonization. Effects of species, growth media and inoculation on grass quality and AM colonization were thus tested according to a completely randomized design, using the PROC ANOVA procedure.

3 Results

3.1 Study 1: The Niblick experimental green

Soil analyses

Of all parameters analyzed with the soil samples taken on October 23rd 2012, the results for pH showed significant effects of N amount. The new analyses of P–Al with the samples taken on April 24th 2013, showed significant effect of P treatment. These results are shown in Table 7. The lowest N level led to a less acidic soil, and fertilizing with P naturally gave a significantly higher P content. Moreover, there was an indication of a lower P–content in inoculated plots compared to plots without any form for P treatment, although this was not significant. Generally, the pH had increased, and the P-Al content decreased, since the first soil analyses were performed prior to seeding in August 2010.

Table 7: Effects of N amounts and P treatments on soil pH and available phosphorus, in putting greens with red fescue as predominant species in October 2012 and April 2013.

		2012		2013	
Factor		**pH**†		**P-AL**‡	
				mg/100g	
N, kg/100 m²	0.5	6.42	a	1.27	
	1	6.31	b	1.25	
	1.5	6.30	b	1.25	
P, kg/100 m²	0	6.33		1.15	b
	0 + myc.	6.39		1.03	b
	0.18	6.31		1.61	a
		ANOVA			
	N	**		NS§	
	P	NS		***	
	NxP	NS		NS	

** Significant at 0.01 probability level.

*** Significant at 0.001 probability level.

† Measured by H_2O extraction.

‡ Measured spectrophotometrically by the molybden-blue method.

§ NS, not significant.

Turf quality

Because of generally low quality scores and little difference between treatments in the spring (Figure 23), data collected on general impression and take – all fungus from June to October 2012 was averaged and analyzed for effects of the experimental factors. The data were more consistent throughout the summer and fall. Their results are shown in Table 8, together with the diameter development and coverage of the annual bluegrass sample that was planted in August 2011 and measured last time on the 11th of October 2012.

General impression was significantly better with the higher mowing height, and increased significantly with N-level (Figure 24 and 25). No effects of P treatment could be observed. Of the species combinations, there was a tendency ($p < 0.1$) towards a lower impression of plots with pure red fescue. A species x N interaction also occurred on general impression (Figure 26). At the middle and the highest N–level, pure red fescue had a lower general impression than the other species combinations, but at the lowest N–level, colonial bentgrass combined with red fescue had the lowest values.

Take all–patch: A significant difference in disease ocurrence was found between pure red fescue and the other species combinations. Significantly more patches were observed with the lowest N level, and there was also a trend towards more disease at the middle level versus the highest level. The fungus became more prevalent in the fall, as shown in Figure 25. Regarding the P treatments, there were no significant effects. It was showed a significant species x N interaction for take all patch (Figure 27), and the p-value for this interaction was < 0.10 when the undiseased plots of pure red fescue was excluded from the analysis. The tendency pointed towards more disease in the velvet bentgrass combination when applying 1.0 kg N/100m^2.

Competition against annual bluegrass: The last measurement in October 2012 showed that only species combination had a significant effect on diameter development of the cylinder, which increased significantly in pure red fescue and decreased in the other combinations. There were also tendencies ($p < 0.1$) to less annual bluegrass when plots were subjected to high mowing and the lowest N–level. Furthermore, P fertilizing tended to increase the annual bluegrass extension, while no P treatment had the opposite effect. Inoculation with AMF seemed to help maintain the original sample size, leaving its diameter virtually unchanged. The coverage percentage was significantly lower at the lowest N–level, while the cover seemed to increase also with the next respective levels.

Table 8: Effects of management practices on turf quality and competition against annual bluegrass, on putting greens with red fescue as predominant species in 2012.

						Annual bluegrass, 1.2 year old sample†			
Factor		**General impression**		**Take all-patch**		**Diameter development**		**Coverage**	
		1-9, 9 is best quality		**% of plot area**		**% increase/decrease in sample**		**% of sample area**	
Species combination	Red fescue (Rf)	4.6		0.0	a	6.3	a	65.8	
	Rf + Velvet bent	5.6		4.2	b	-3.8	b	70.6	
	Rf + Colonial bent	5.4		3.9	b	-2.8	b	74.6	
Mowing height, mm	4.0	4.9	a	2.9		2.0		73.1	
	5.5	5.5	b	2.5		-2.2		67.6	
N, kg/100 m^2	0.5	2.9	a	4.6	a	-4.0		57.7	a
	1.0	5.7	b	2.2	b	1.2		74.4	b
	1.5	7.0	c	1.2	b	2.5		79.1	b
P, kg/100m^2	0	5.1		2.7		-2.9		69.5	
	0 + myc.	5.2		3.1		-0.3		68.5	
	0.18	5.2		2.3		2.9		73.1	
		ANOVA							
	Species	(*)		*		**		NS‡	
	Mowing	**		NS		(*)		NS	
	N	***		***		(*)		***	
	P	NS		NS		(*)		NS	
	Species x N	***		***		NS		NS	
N x P, Species x P, Mowing x N, Mowing x P, Species x mowing, Species x N x P, Mowing x N x P, Species x mowing x N, Species x mowing x P, Species x mowing x N x P						NS			

* Significant at 0.05 probability level.

** Significant at 0.01 probability level.

*** Significant at 0.001 probability level.

(*) Significant at 0.1 probability level.

†The sample was established on the 18th of August in 2011. Analyzed data are from the last recording, on 11th of October 2012.

‡NS, not significant.

Figure 23: The Niblick (study 1) green on March 30th, 2012. The field impression was generally low right after the ice and snow had disappeared, and except for nitrogen level, there was little difference between treatments in the spring. Photo: Tatsiana Espevig.

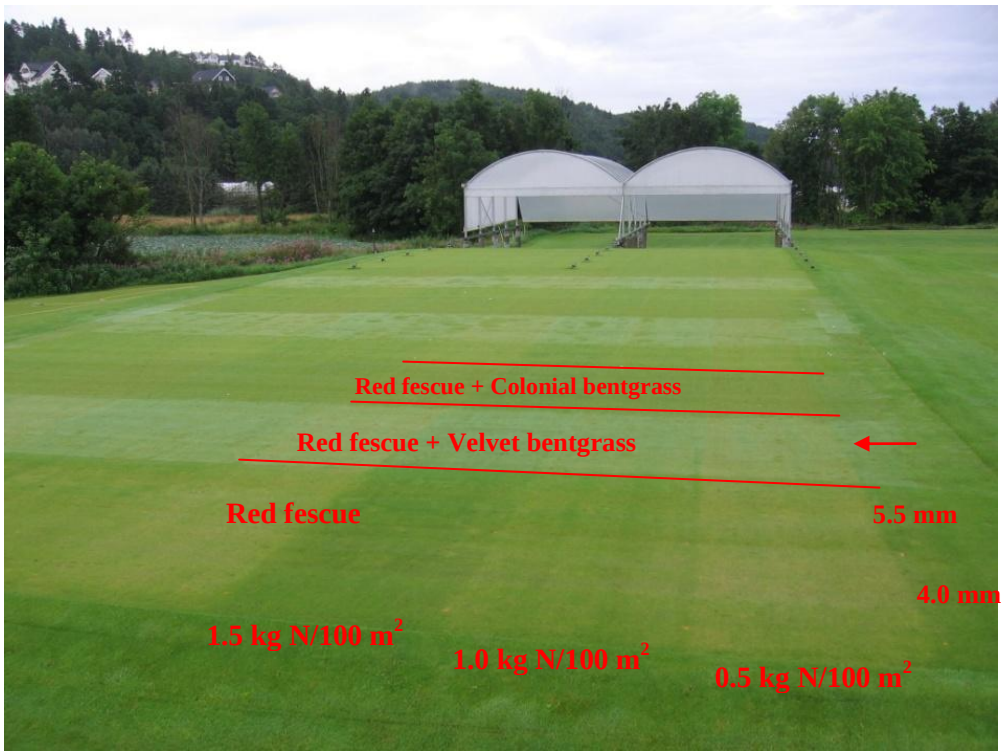

Figure 24: Overview of the Niblick green (study 1) in the first week of august 2012, showing clear quality differences between the three N – levels and the two mowing heights. Plots sown with red fescue and velvet bentgrass (arrow) are covered with morning dew. Photo: Tatsiana Espevig.

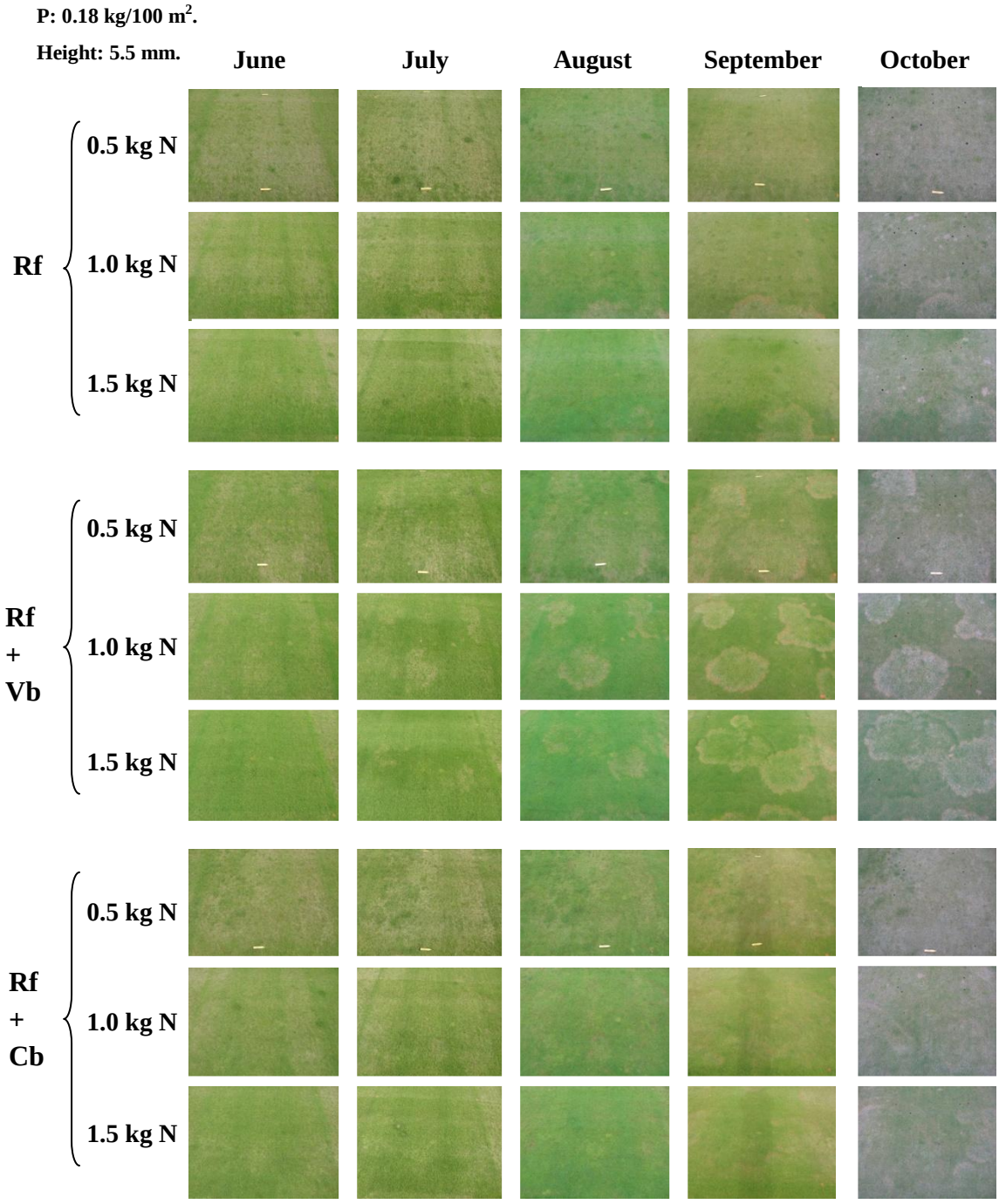

Figure 25: A comparison of turf quality on block 3, through the summer and fall of 2012. Rf = red fescue, Vb = velvet bentgrass, Cb = colonial bentgrass. It was decided to use plots that had received P fertilizer for illustrating the effects of N, because these had similar conditions to a conventional green. In addition, there were no significant differences between P treatments on neither general impression nor take-all patch. Plots mowed to 5.5 mm were chosen because of the greater chance of detecting differences between treatments where the grass experienced minimal stress. General impression was best in the late summer, and the parameter increased with increasing N–levels throughout the period. The take–all patches started to show in late June/ early July, and got more extensive during the summer. The lowest N–level had sigificantly more dead patches compared to the intermediate level, especially for the velvet bent combination, which also showed a tendency of being more affected by fungi than the colonial bent. The trend towards further reduced disease prevalence with the highest N-level can also be seen. Red fescue is resistant to take–all fungus, and patches seen in pure red fescue belongs to neighboring plots. Photo: Tatsiana Espevig.

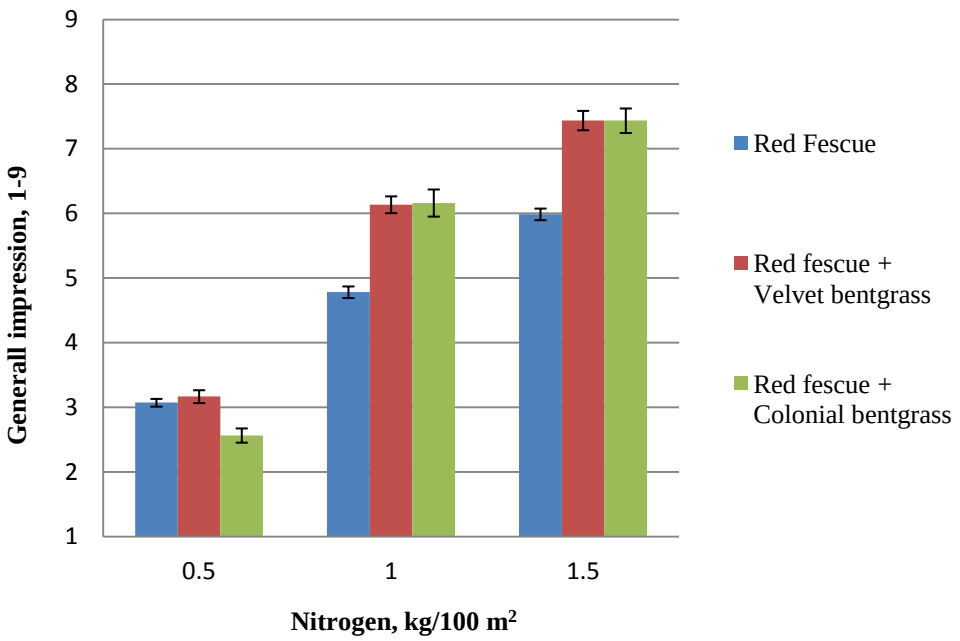

Figure 26: Combined effect of nitrogen level and species combination on general turf impression from June to October 2012. 1 is lowest and 9 is the highest quality. $P_{interaction} < 0.0001$*. Error bars indicate +/- 1SE (standard error of the mean).*

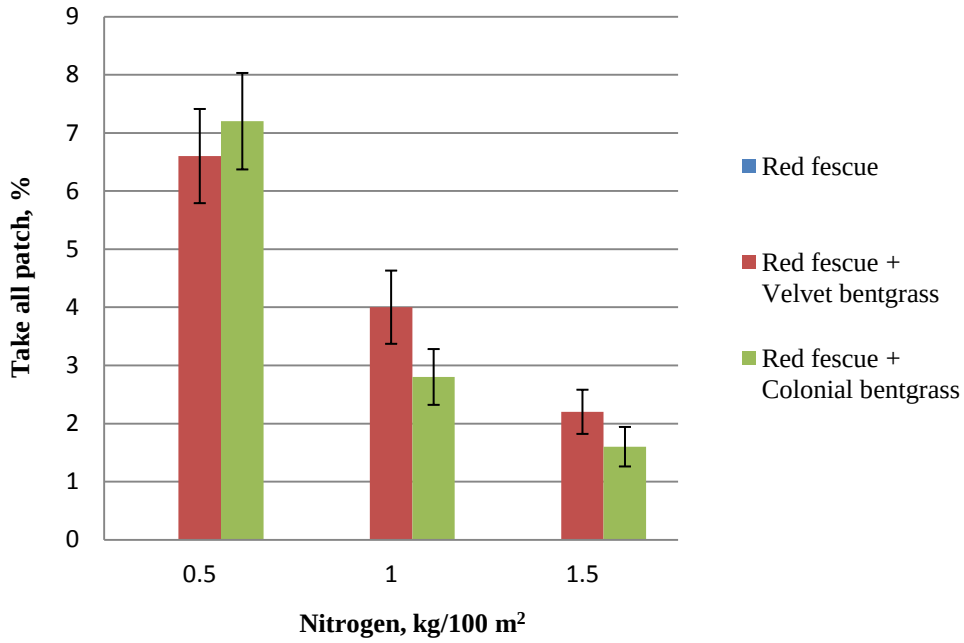

Figure 27: Combined effect of nitrogen level and species combinations on % of plot infected by take-all fungus from June to October 2012. Red fescue was not subjected to disease. $P_{interaction} < 0.0001$ *(0.0766 when the analysis is performed without pure red fescue). Error bars indicate +/- 1SE (standard error of the mean).*

Root analysis

No significant effects of species, N level or P treatment were observed on the estimated amount of roots per plot that was colonized by AMF in September/October 2012 (Table 9), but there was still a slightly lower mean for plots fertilized with P. The level of colonization was generally high, ranging between 50 and 80 % in all sampled plots, and a tendency ($p < 0.1$) pointed towards a higher colonization rate with decreasing N amounts.

The root weight was significantly higher with the red fescue/velvet bent combination than with the other combinations. Although not significant, red fescue alone further seemed to have slightly more root mass than the red fescue/colonial bent combination. Fertilizing with P seemed to cause a reduced root weight when compared to mycorrhizal inoculation or no P treatment at all.

Table 9: Effects of species combination, N amount and P treatment on AM colonization and root mass of putting greens with red fescue as predominant species, in September/October 2012.

Factor		Mycorrhiza	Roots	
		% roots colonized	DW†, g/m²	
Species combination	Red fescue	68.0	400.9	b
	Red fescue + Velvet bent	63.2	481.0	a
	Red fescue + Colonial bent	64.7	367.7	b
N, kg/100 m²	0.5	67.9	397.9	
	1	64.4	439.3	
	1.5	63.5	412.4	
P, kg/100m²	0	65.8	422.7	
	0 + myc.	65.8	425.8	
	0.18	64.2	401.1	
		ANOVA		
	Species	NS‡	*	
	N	(*)	NS	
P, N x P, Species x N, Species x P, N x P x Species		NS		

* Significant at 0.05 probability level.

(*) Significant at 0.1 probability level.

†DW, dry weight.

‡NS, not significant.

3.2 Study 2: The red fescue experimental green

Turf quality

Data collected on general impression through the whole growth season of 2012 were averaged before statistical analysis was performed, as were the three recordings of annual bluegrass from May to July 2012. Results are shown in Table 10.

General impression increased significantly with the use of Green mix both in the root zone and in the topdressing material (Figure 28). The effects of root zone and topdressing combinations on general impression throughout the growing season are shown more closely in Figure 29. The effect of Green Mix as topdressing started to show up already about one month after first application.

Competition against annual bluegrass: Root zones amended with peat had significantly decreased the annual bluegrass coverage.

Nutrient uptake

N, P and K removal in clippings through the whole growth season of 2012 were averaged and converted to g/m^2 before statistical analysis was performed. Results are shown in Table 10. Nutrient uptake was significantly higher when the root zone contained Green Mix. There waere also tendencies, significant at a 0.1 probability level, to a greater P and K uptake when plots were dressed with Green Mix.

Root analysis

Root mass: Analysis of root weight was performed on data from samples collected on October 18th 2012, and the root mass was significantly greater in root zones amended with peat (Table 11). The root weight also seemed to be slightly higher in plots dressed with sand, although not significant.

Mycorrhiza colonization: AM colonization was studied on three occacions; October 18th 2011, June 5th 2012 and October 18th 2012 (Table 11). At the first two samplings, AM colonization was studied independently in annual bluegrass and red fescue, but at the last sampling, no annual bluegrass was left in the plots. Results from the first two samplings showed that the colonization was very limited, and there were no significant effects of either rooivt zone or topdressing. However, on June 5th there was significantly ($p = 0.013$) more colonization in the annual bluegrass (1.3%) than in red fescue (0.8 %, data not shown in table). The estimated AM colonization in red fescue on October 18th 2012 varied from approximately 10 to 70 %, and was more than twice as extensive in roots from plots with Green Mix versus peat (Table 11).

Table 10: Effects of root zone and top dressing on turf quality, nutrient uptake and root development of a red fescue green in 2012.

Factor		General impression	Annual bluegrass	Nutrient removal in clippings		
				N	P	K
		1-9, 9 is best quality	% of plot area		g/m^2	
Root zone	Peat	3.8	10.3	8.3	1.1	5.2
	GM	5.5	17.0	12.6	1.7	7.5
Top dressing	Pure sand	4.3	13.8	10.3	1.3	6.1
	GM	5.0	13.5	10.7	1.4	6.6
				ANOVA		
	Root zone	***	***	***	***	***
	Top dressing	***	NS†	NS	(*)	(*)
	Root zone x top dr.	NS	NS	NS	NS	NS

*** Significant at 0.001 probability level.

(*) Significant at 0.1 probability level.

†NS, not significant.

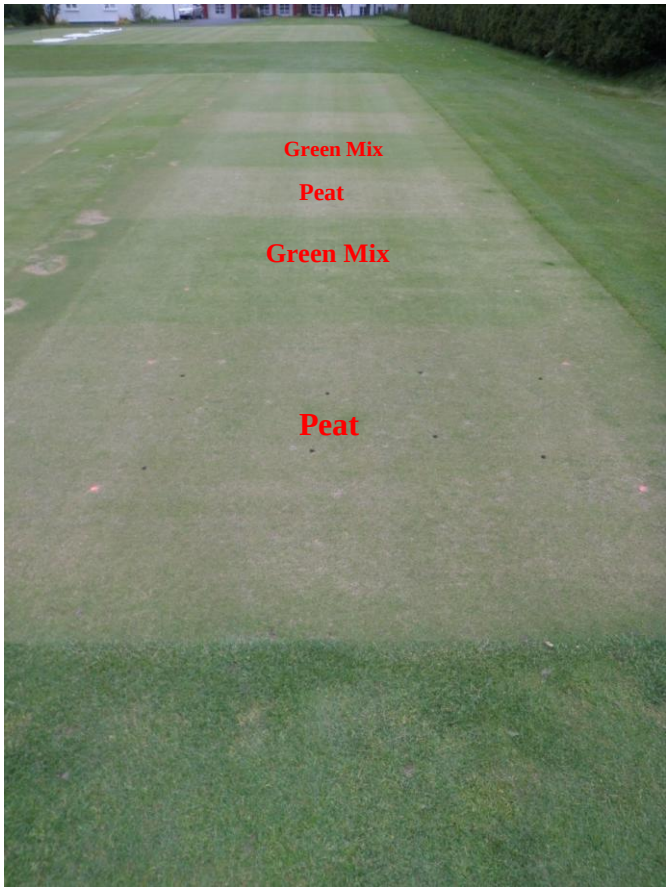

Figure 28: Replicate 4 of the red fescue green on October 8th 2012, showing a clear difference in general impression between plots of different root zone construction. Photo: Trygve S. Aamlid.

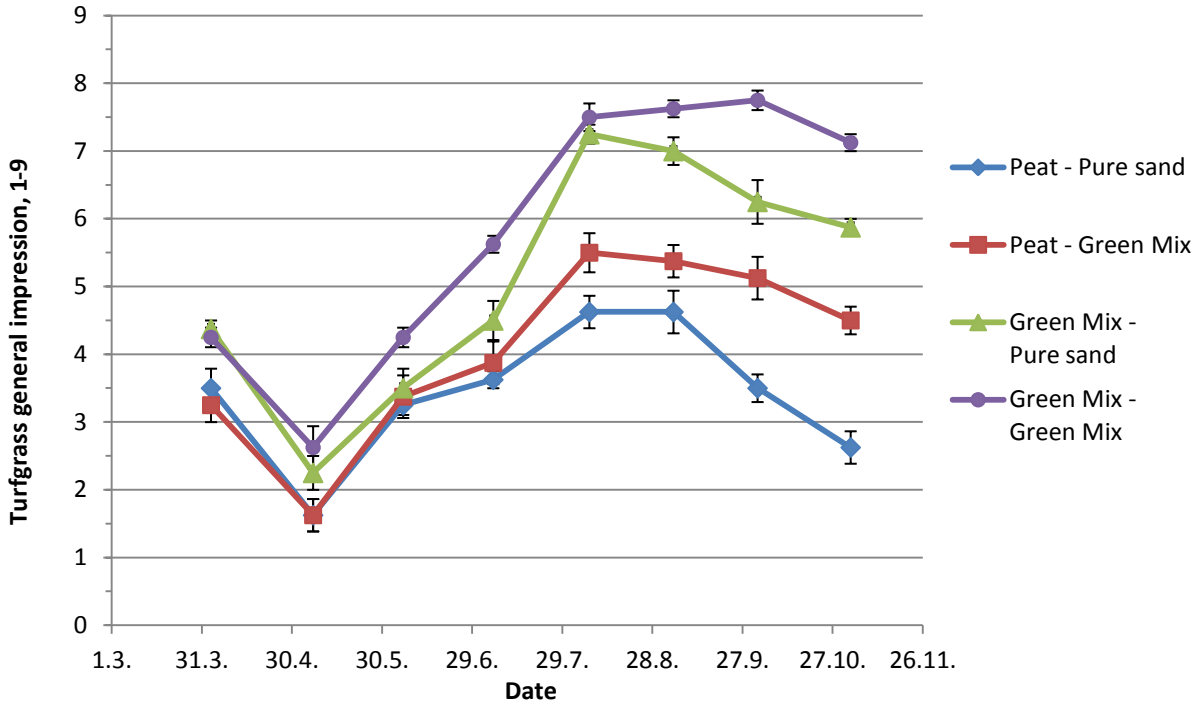

Figure 29: General impression (on a scale of 1-9) of a red fescue green with different root zone/top dressing combinations, through the whole growth season of 2012.

Table 11: Effects of root zone and topdressing on AM colonization of a red fescue green, in 2011/2012.

		Mycorrhiza			Roots
Factor		**18.10.2011**	**05.06.2012**	**18.10.2012**	**18.10.2012**
		% roots colonized			**DW†, g/m²**
Root zone	Peat	2.1	0.9	21.6	294.5
	GM	1.9	1.2	58.7	187.1
Top dressing	Pure sand	x	1.0	40.3	258.8
	GM	x	1.1	40.0	222.9
		ANOVA			
	Root zone	NS‡	NS	***	**
	Top dressing	NS	NS	NS	NS
	Root zone x top dr.	NS	NS	NS	NS

** Significant at 0.01 probability level.

*** Significant at 0.001 probability level.

† DW, dry weight.

‡NS, not significant.

3.3 Study 3: Greenhouse experiment

Turf quality: coverage and disease

Statistical analysis was performed on mean values based on a total of three coverage - and disease registrations. Results are shown in Table 12.

In all parameters involving plant cover, the bentgrasess differed significantly from each other, and from the other species. Velvet bentgrass had the best coverage, which was almost 100% before disease (*Fusarium spp*) was subtracted. Colonial bentgrass had the second best coverage, but the species also had most disease, which led to a net coverage that was almost 20 percent units lower than for the velvet bentgrass. No disease occurred in red fescue and annual bluegrass, and their coverage was not significantly different. Growth medium and inoculation did not have significant effects on coverage or disease, but Green Mix (combined with the lower input of N) tended to produce a slightly lower coverage while disease occurrence increased by almost 2 percent units. This resulted in a tendency ($p < 0.1$) towards lower net coverage on Green Mix compared to peat root zones. Inoculation of mycorrhiza had no effect on either coverage or disease.

The differences in coverage among the various species during the last experimental week are illustrated in Figure 30. Figures 31 to 33 further compare effects of growth medium and inoculation on coverage. A significant species x medium interaction for disease percentage was shown (Figure 34). For velvet bentgrass, the fungal infection was most severe in pots with peat (combined with the higher input of N), while the opposite was true for the colonial bentgrass. There was also an interaction between species and medium for net coverage; colonial bentgrass being favoured by peat (Figure 35).

Dry weight of clippings

Total dry matter collected during the four clipping collections was converted to total dry matter of clippings in g/m^2, before statistical analysis was performed. Results are shown in Table 12.

Total dry weight of clippings was significantly higher in annual bluegrass than for both the bentgrasses and the red fescue combinations, the latter being significantly lower than the bentgrasses as well. Growth medium also had a significant effect on clippings, in that the use of peat led to greater dry weight. For inoculation, there was no significant difference between the treatments. An interaction occurred between species and growth medium for weight of clippings, where peat led to the greatest dry weight in both annual bluegrass and the bentgrasses, while red fescue combinations were unaffected (Figure 36).

Dry weight of crowns

Weight of crowns by the end of the experiment was significantly greater in velvet bentgrass than in colonial bentgrass, which again had significantly greater weight than annual bluegrass and red fescue (Table 12). There were no effects of growth medium and inoculation. An interaction between species and growth medium occurred, as velvet bentgrass was favoured by Green Mix (Figure 37).

Root analysis

By the end of the experiment, velvet bentgrass had a significantly greater root weight than colonial bentgrass, which in turn had a significantly greater root weight than annual bluegrass and red fescue (Table 12). The significantly lowest root weight was found in annual bluegrass. Green Mix gave a significantly higher root weight compared to peat, while the effect of inoculation was not significant. A species x medium interaction for dry weight of roots showed that there was a strong increase of root weight in colonial bentgrass, and to some extent in velvet bentgrass, when Green Mix was used (Figure 38). The other species also showed small but insignificant preferences for Green Mix regarding root weight. An interaction between growth medium and inoculation treatment showed that although there was no significant difference in root weight between inoculation and no inoculation, trends pointed in opposite directions for the two media (Figure 39). Inoculation seemed to cause an increase in root weight when applied to Green Mix, while the opposite was true for peat. A three factor interaction also occurred between species, growth medium and inoculation, in that velvet bentgrass in peat had a greater root weight when not inoculated, while both bentgrasses tended to have a greater root weight in Green Mix when inoculated (Figure 40).

No significant effects were observed on the estimates of AM colonization (Table 12). If the lack of significance is not taken into account, the estimated colonization percentage increased in the order: red fescue < annual bluegrass < velvet bentgrass < colonial bentgrass. Pots with heat – killed inoculum seemed to have slightly lower colonization than pots with healthy inoculum, although not significant. The control pots with terranean clover had no colonization in either the pot without inoculum or the pot with heat – killed inoculum. Of the three positive controls, their estimated colonization varied from 26 – 36 %, and the average colonization percentage was 31.3 % (data not shown in table).

Table 12: Effects of species, growth medium and inoculation on grass quality, above - and underground plant mass, and mycorrhizal colonization.

Factor		Coverage	Disease	Net coverage	Clippings	Crown	Roots	Mycorrhiza
		% of pot area		% of pot area with healthy grass		DW†, g/m²		% roots colonized
Species	Annual bluegrass	31.1 c	0.0 c	31.1 c	45.9 a	111.4 c	57.2 d	2.2
	Colonial bentgrass	79.9 b	28.8 a	51.2 b	37.8 b	311.5 b	227.1 b	2.8
	Velvet bentgrass	94.8 a	21.0 b	73.9 a	36.9 b	585.2 a	326.6 a	2.5
	Red fescue	27.2 c	0.0 c	27.2 c	21.4 c	148.4 c	94.8 dc	1.0
	Red fescue + Ann. Bluegr.	29.4 c	0.0 c	29.4 c	24.7 c	162.5 c	97.6 c	1.5
Medium	Peat	53.4	9.1	44.3	38.4 a	223.9	126.5 b	2.0
	GM	51.6	10.8	40.8	28.3 b	303.7	194.8 a	2.0
Inoculation	No mycorrhiza	53.3	10.6	42.7	34.1	252.0	155.8	1.7
	Mycorrhiza inoculated	51.7	9.3	42.4	32.6	275.6	165.5	2.3
					ANOVA			
	Species	***	***	***	***	***	***	NS‡
	Medium	NS	NS	(*)	***	NS	***	NS
	Inoculum	NS	NS	NS	NS	NS	NS	NS
	Species x medium	NS	***	*	***	*	**	NS
	Species x inoculum.	NS	NS	NS	NS	NS	NS	NS
	Medium x inoculum.	NS	NS	NS	NS	NS	*	NS
	Species x medium x inoc.	NS	NS	NS	NS	NS	*	NS

* Significant at 0.05 probability level.

** Significant at 0.01 probability level.

*** Significant at 0.001 probability level.

(*) Significant at 0.1 probability level.

†DW, dry weight.

‡NS, not significant.

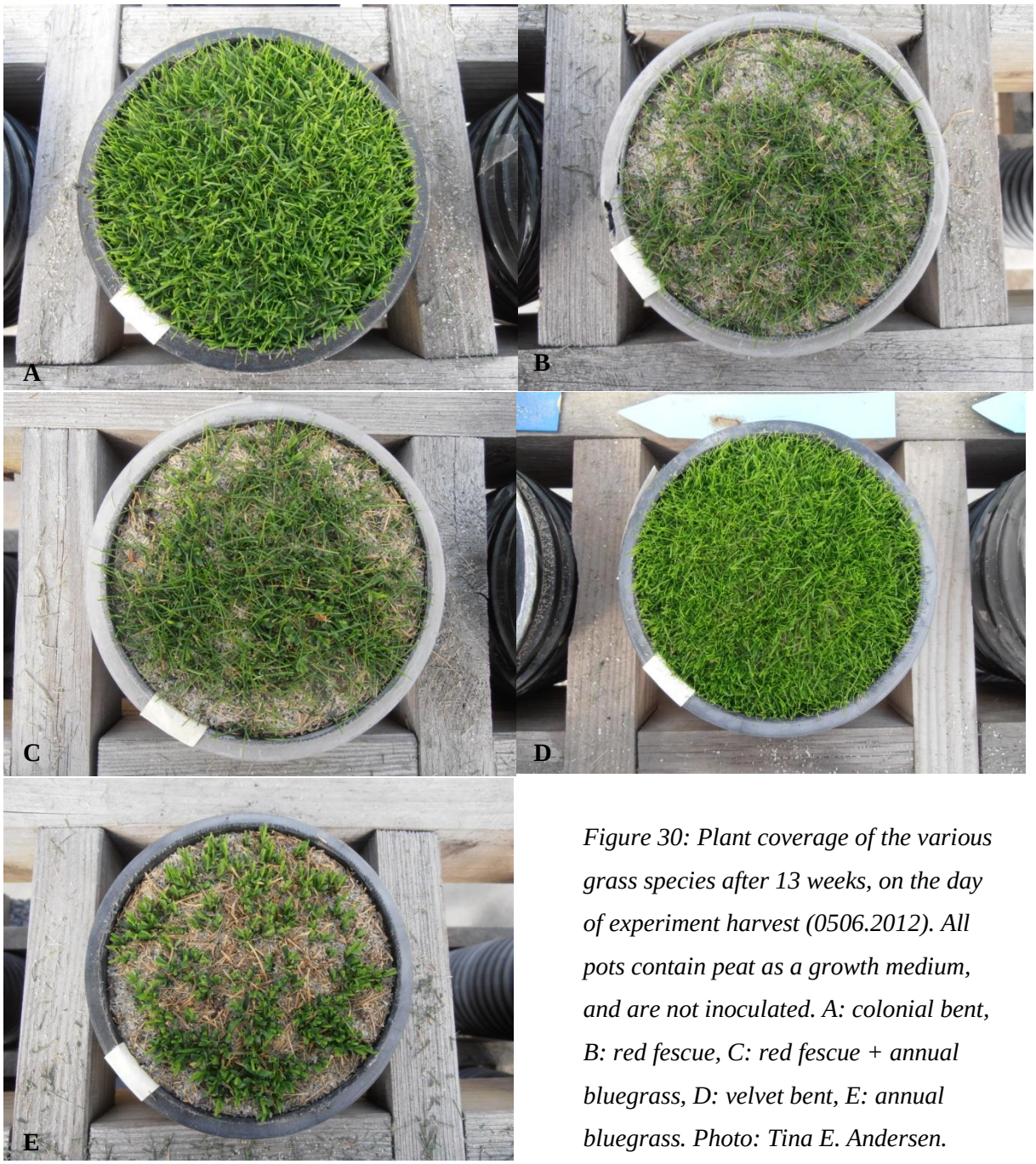

Figure 30: Plant coverage of the various grass species after 13 weeks, on the day of experiment harvest (0506.2012). All pots contain peat as a growth medium, and are not inoculated. A: colonial bent, B: red fescue, C: red fescue + annual bluegrass, D: velvet bent, E: annual bluegrass. Photo: Tina E. Andersen.

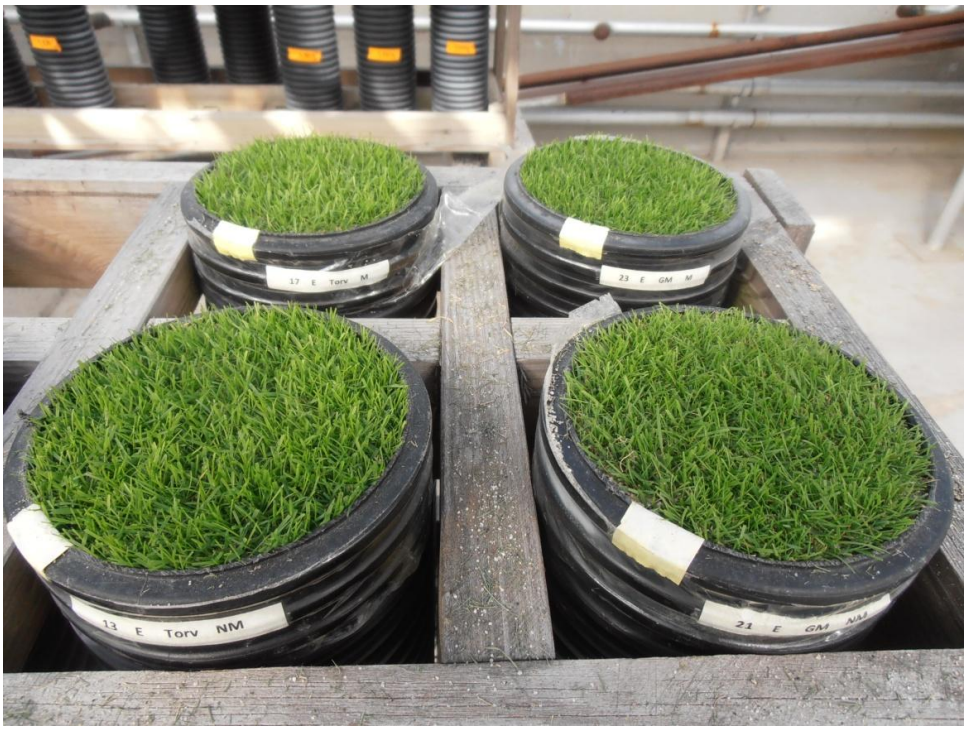

Figure 31: Colonial bentgrass in peat (left) and in Green Mix (right) before experiment harvest. The pots in the front are not inoculated, while the pots in the background have been supplied with AMF. The quality difference between treatments is unnoticeable. Photo: Tina E. Andersen

Figure 32: Velvet bentgrass in peat (left) and in Green Mix (right) at the day of experiment harvesting. The pots in the front have been inoculated, while the pots in the background have not. The quality difference between treatments is unnoticeable. Photo: Tina E. Andersen

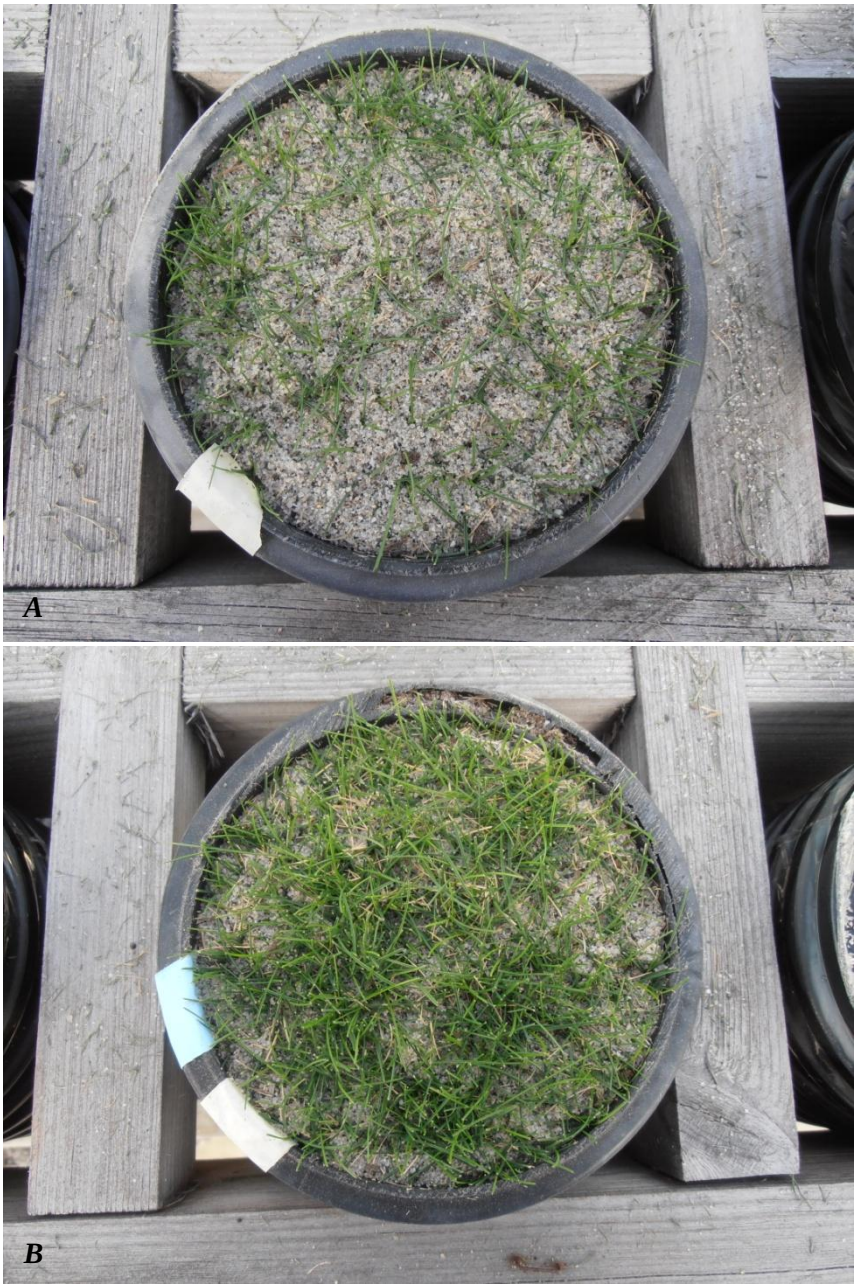

Figure 33: Red fescue in Green Mix (A) and peat (B) right before harvesting the experiment. Both pots are inoculated. Photo: Tina E. Andersen.

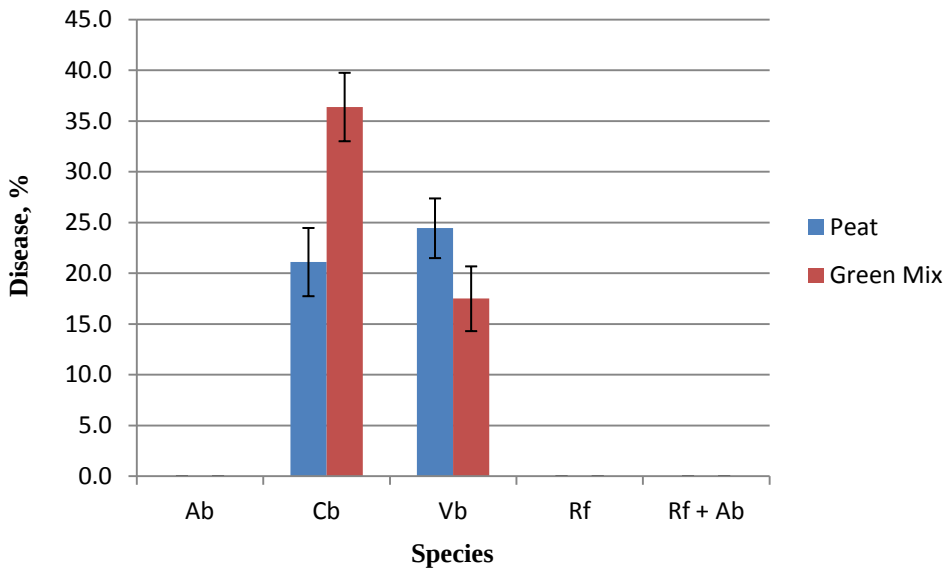

Figure 34: Combined effect of turfgrass species and growth medium on % of pot covered with diseased grass. Ab = annual bluegrass, Cb = colonial bentgrass, Vb = Velvet bentgrass, Rf = red fescue. $P_{interaction} < 0.0001$*. Error bars indicate +/- 1SE (standard error of the mean).*

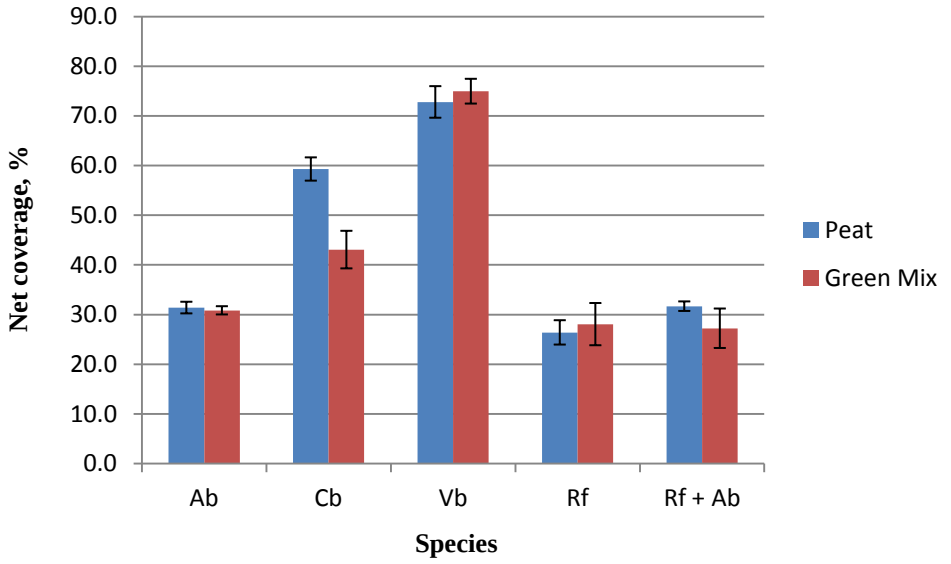

Figure 35: Combined effect of turfgrass species and growth medium on % of pot covered with healthy grass. Ab = annual bluegrass, Cb = colonial bentgrass, Vb = Velvet bentgrass, Rf = red fescue. $P_{interaction} = 0.0140$*. Error bars indicate +/- 1SE (standard error of the mean).*

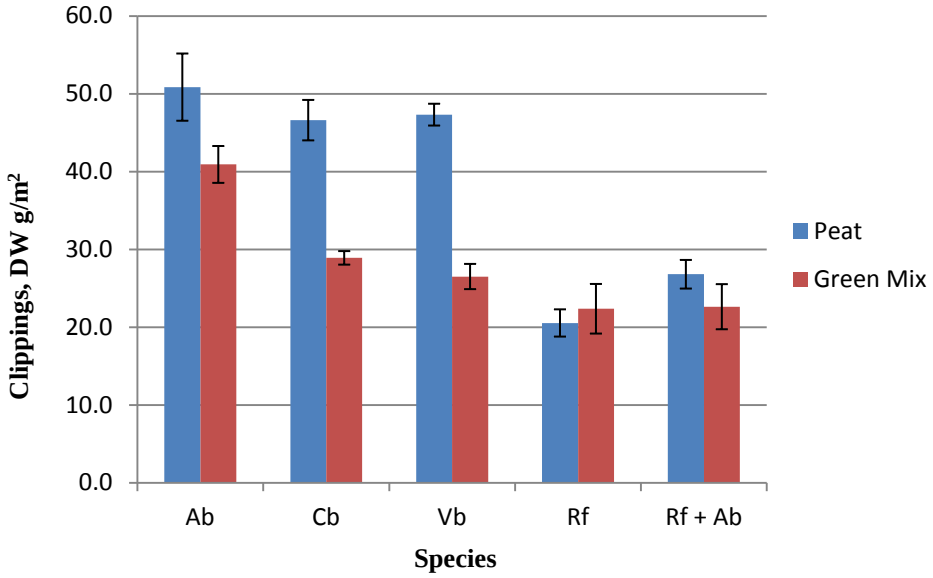

Figure 36: Combined effect of turfgrass species and growth medium on dry weight of clippings. Ab = annual bluegrass, Cb = colonial bentgrass, Vb = Velvet bentgrass, Rf = red fescue. $P_{interaction}$ *= 0.0003. Error bars indicate +/- 1SE (standard error of the mean).*

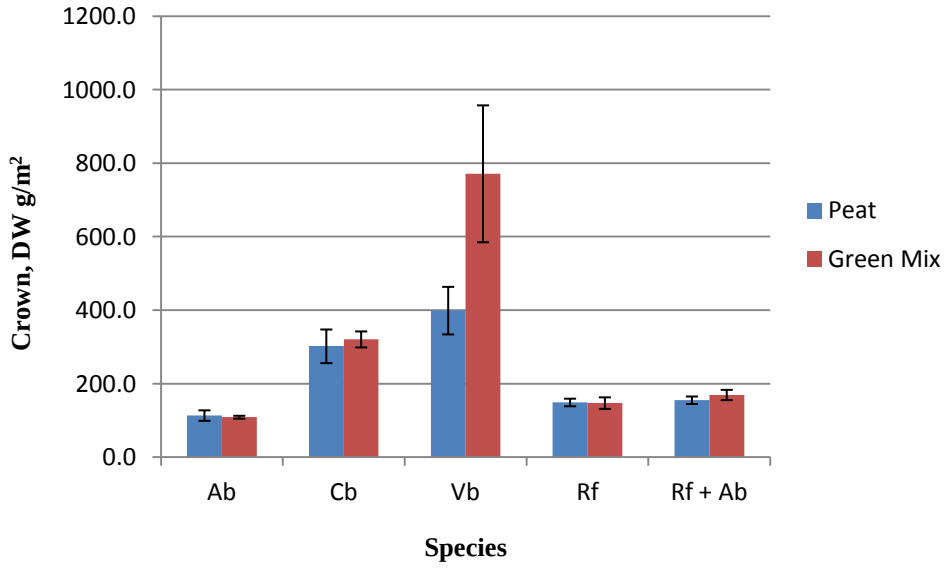

Figure 37: Combined effect of turfgrass species and growth medium on dry weight of above ground plant material cut right below the plant crowns. Ab = annual bluegrass, Cb = colonial bentgrass, Vb = Velvet bentgrass, Rf = red fescue. $P_{interaction}$ *= 0.0279. Error bars indicate +/- 1SE (standard error of the mean).*

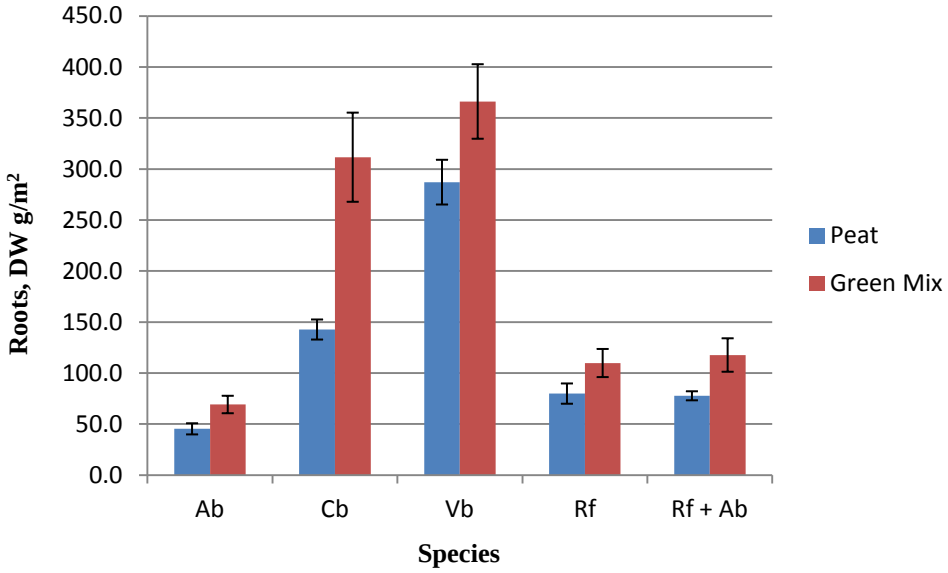

Figure 38: Combined effect of turfgrass species and growth medium on dry weight of roots. Ab = annual bluegrass, Cb = colonial bentgrass, Vb = Velvet bentgrass, Rf = red fescue. $P_{interaction} = 0.0024$. *Error bars indicate +/- 1SE (standard error of the mean).*

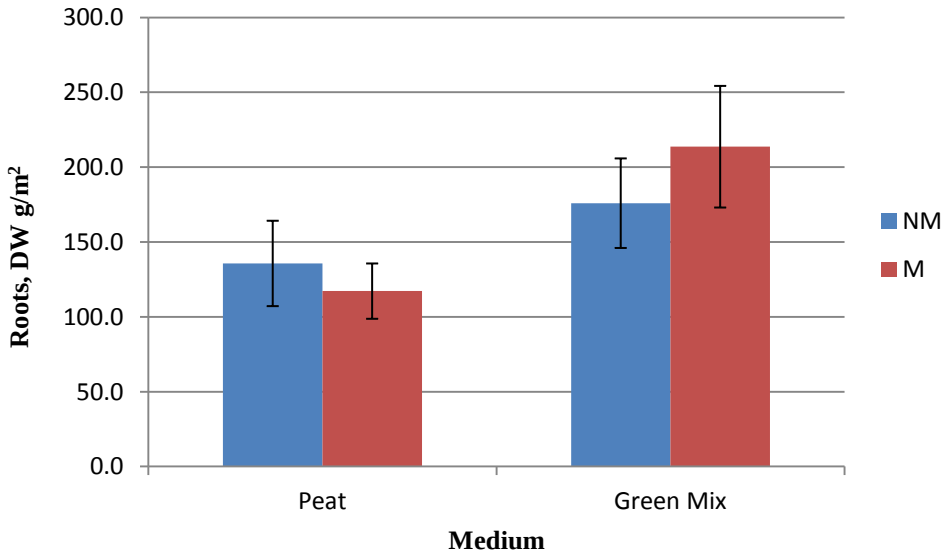

Figure 39: Combined effect of growth medium and mycorrhizal inoculation on dry weight of roots. NM = no mycorrhiza, M = mycorrhiza inoculated. Pinteraction = 0.0251. Error bars indicate +/- 1SE (standard error of the mean).

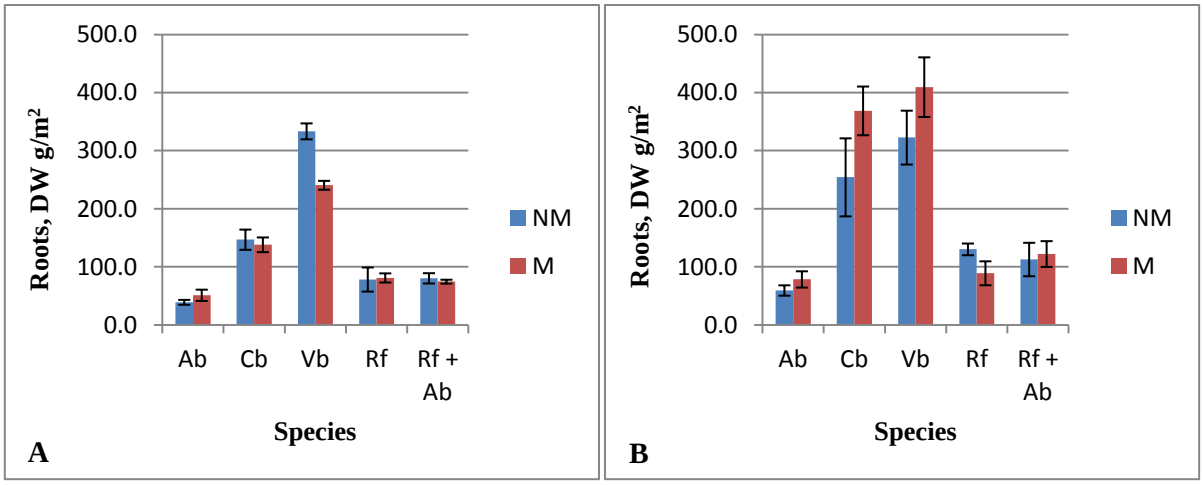

Figure 40: Combined effect of turfgrass species and mycorrhizal inoculation on dry weight of roots in A) Peat and B) Green mix. Ab = annual bluegrass, Cb = colonial bentgrass, Vb = Velvet bentgrass, Rf = red fescue, NM = no mycorrhiza, M = mycorrhiza inoculated. The 3-factorial interaction P value = 0.0337. Error bars indicate +/- 1SE (standard error of the mean).

3.4 Mycorrhizal diversity

Diversity in field

Although study 2 had a generally lower colonization percentage, the same pattern was found in both experiments; The AM diversity was high, and it was dominated by natural populations. Fungal structures observed, like hyphae, arbuscules, vesicles and auxiliary cells, indicated presence of different species belonging to *Glomus*, *Scutellospora*, and *Gigaspora.* There were also lightly stained structures that resembled those in *Paraglomus,* and some special spore formation structures typical for *Archaeospora*. The chance for additional genera being present in the many unrecognizable spores and other structures that were found must also be taken into account. Thus; a significant number of families within the *Glomeromycota* were represented at the location. Some of the observed structures, photographed through the microscope, shown in the Figures 41 to 47.

The plots that had been inoculated with SYMBIVIT® in study 1, had roots that were seemingly colonized by *Glomus* species found in the product. Structures typical for *Glomus spp.* were observed in many of these roots, as was the case in all other plots as well, but the hyphae were often thicker and more 'coarse', and the vesicles large when compared to those typical for Scandinavian *Glomus* populations (Ruissen, pers. comm.) This indicated presence of foreign strains. Despite the fact that spores from the inoculum obviously were able to germinate and form symbiotic structures within the grass roots, all samples were also colonized by naturally present species, of which *Scutellospora spp*. seemed to be the most frequently encountered. *Scutellospora* was the dominating genus in study 2 as well, regardless of root zone composition.

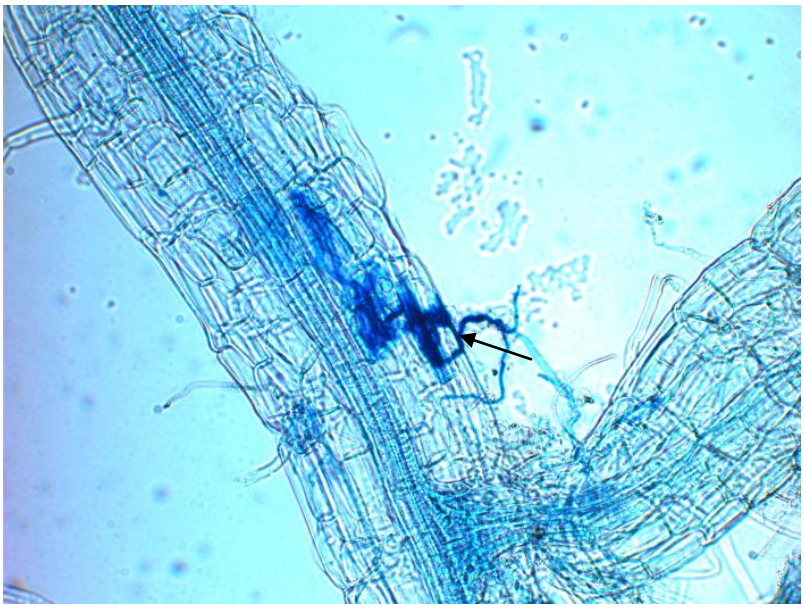

Figure 41: A typical infection structure, of an undefined AMF species. The fungal hypha has formed a hyphopodium (arrow) on the root epidermis when encountering its host, and penetrated into the cortex through a pre-penetration apparatus (PPA). Photo: Theo Ruissen.

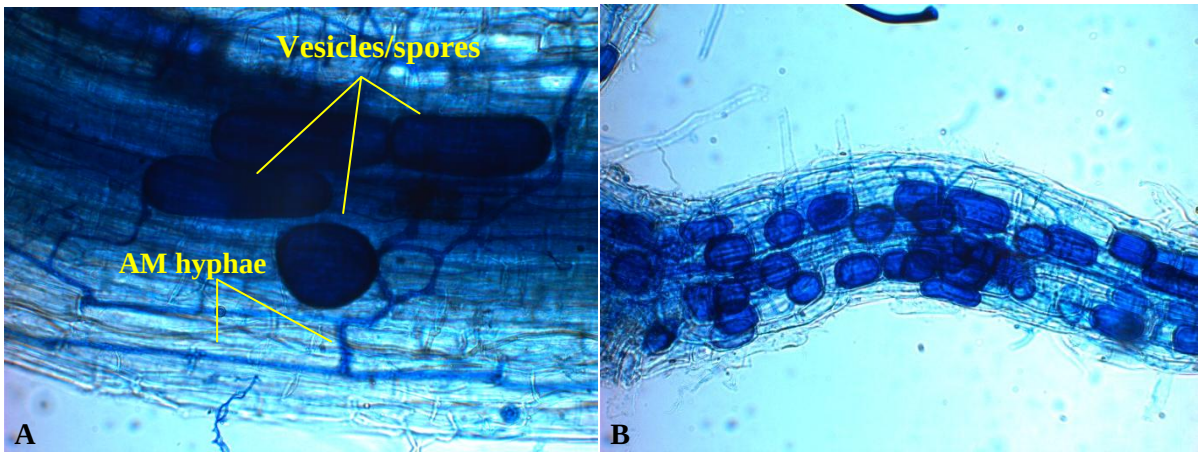

Figure 42: A: Intraradical vesicles and/or spores attached to characteristic AMF hyphae, which are branching and growing laterally along the root axis. The vesicles have round to oval shapes, depending on how much free space there is in the apoplast. B: The many vesicles and spores, seen from a lower magnification. The structures resemble those of Glomus spp. Photo: Theo Ruissen.

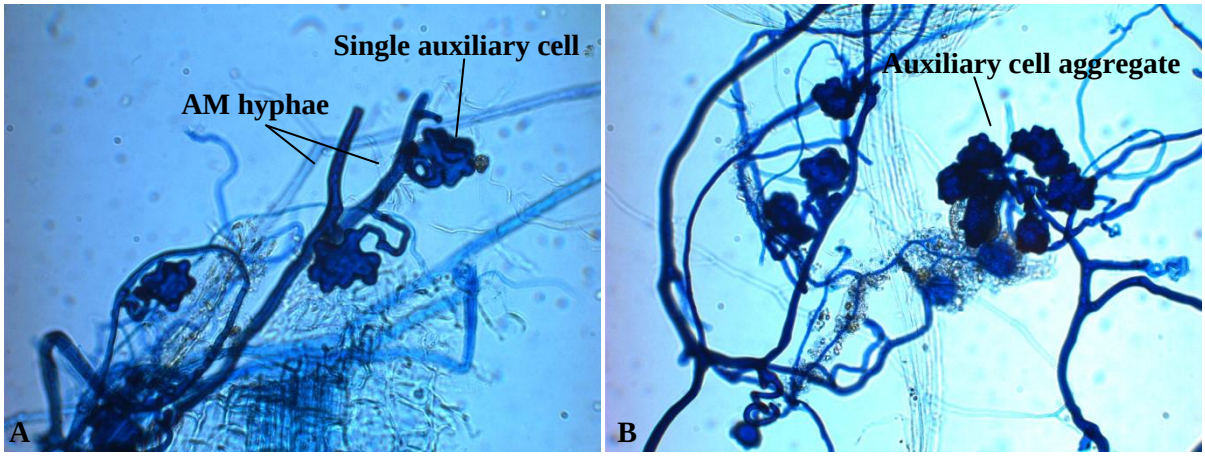

Figure 43: Fungal hyphae with auxiliary cells; single (A) and in aggregates (B), which are typical for both Scutellospora and Gigaspora. The latter usually have narrow projections on the cell surface, but these are hard to spot without a micoroscope of higher resolution. Photo: Theo ruissen.

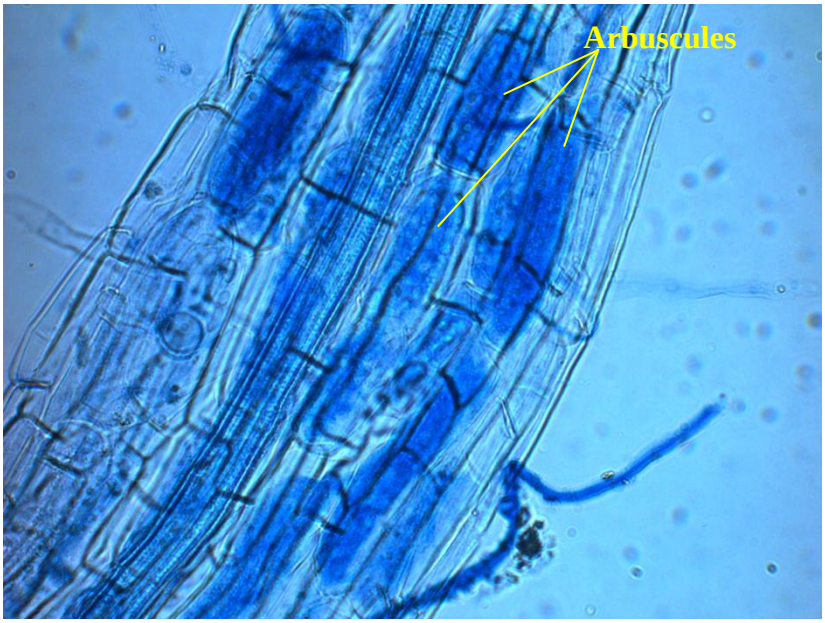

Figure 44: Root colonized by an unidentified AMF species. Fungal hyphae have entered the cortical cells through a PPA – like structure, and branched to form many arbuscules. Photo: Theo Ruissen.

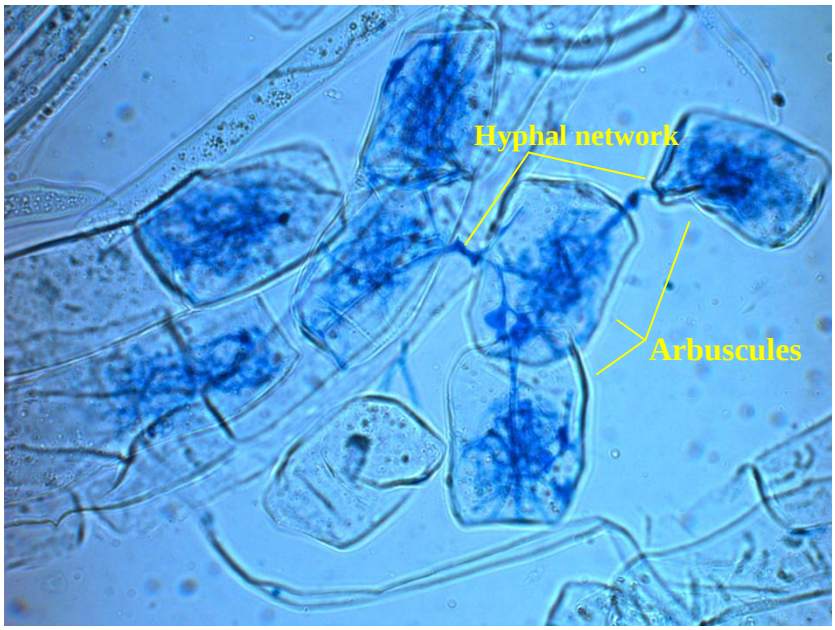

Figure 45: Close – up of a hyphal network forming arbuscules inside root cortex cells. The root was crushed in advance, to reveal distinct structures. Photo: Theo Ruissen.

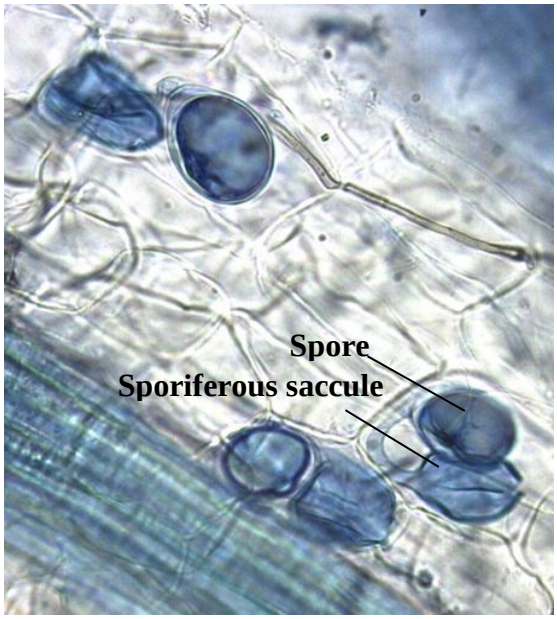

Figure 46: Spores of Archaeospora sp. with sporiferous saccule in the root cortex cells. Photo: Theo Ruissen.

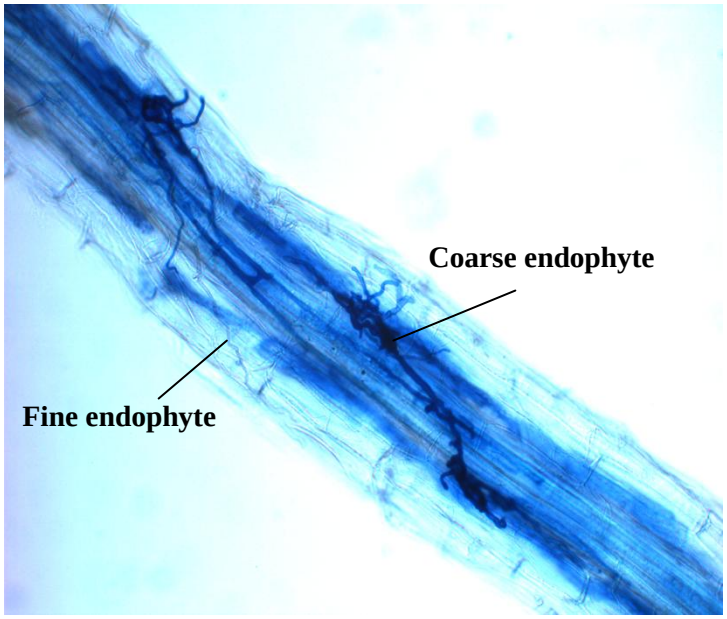

Figure 47: A typical 'coarse' AM fungus and a fine endophyte, colonizing the same host. Photo: Theo Ruissen.

The greenhouse study

In the few roots that had been scored as colonized, AM structures resembling those of *Glomus spp*. were found. Mostly hyphae and vesicles were observed, in addition to some unclear structures that could be deformed arbuscules. Much of the hyphal network was also deformed. Thus; it was hard to tell which structures belonged to strains from the inoculum and which did not. Similar examinations were made in the controls. Here, mycorrhiza was only detected in the pots with living inoculum. The results were not reported, but the colonized root estimate for the negative and positive controls were 0 and 30%, respectively. Some of the observed structures, photographed through the binocular lupe, are shown in the figures 48 to 50.

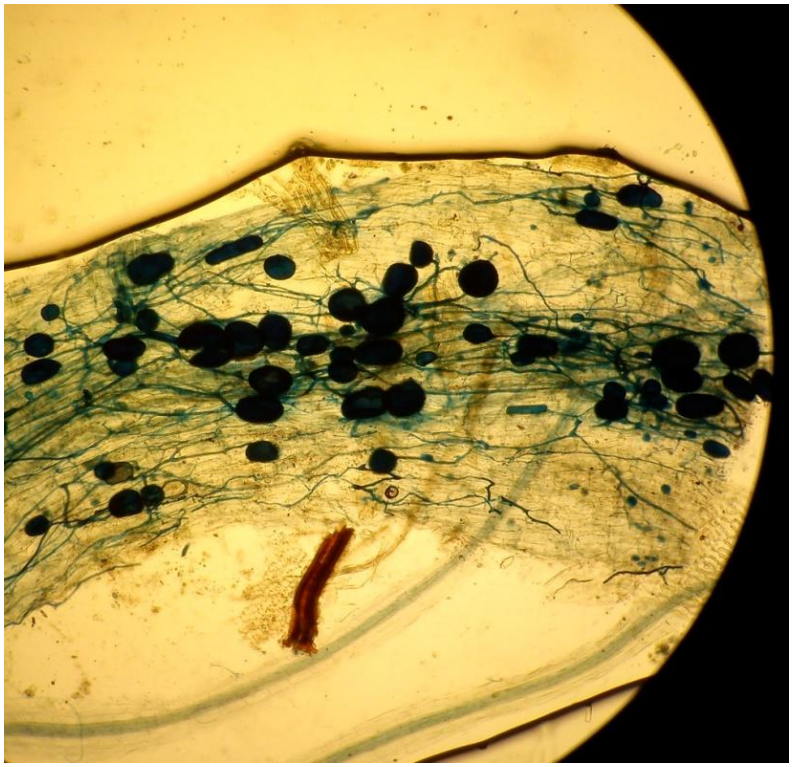

Figure 48: A heavily colonized root, showing an extensive hyphal network with vesicles/spores attached. The sample was taken from an inoculated Green Mix pot sown with red fescue, and the symbiotic fungi are probably Glomus spp. Photo: Tina E. Andersen

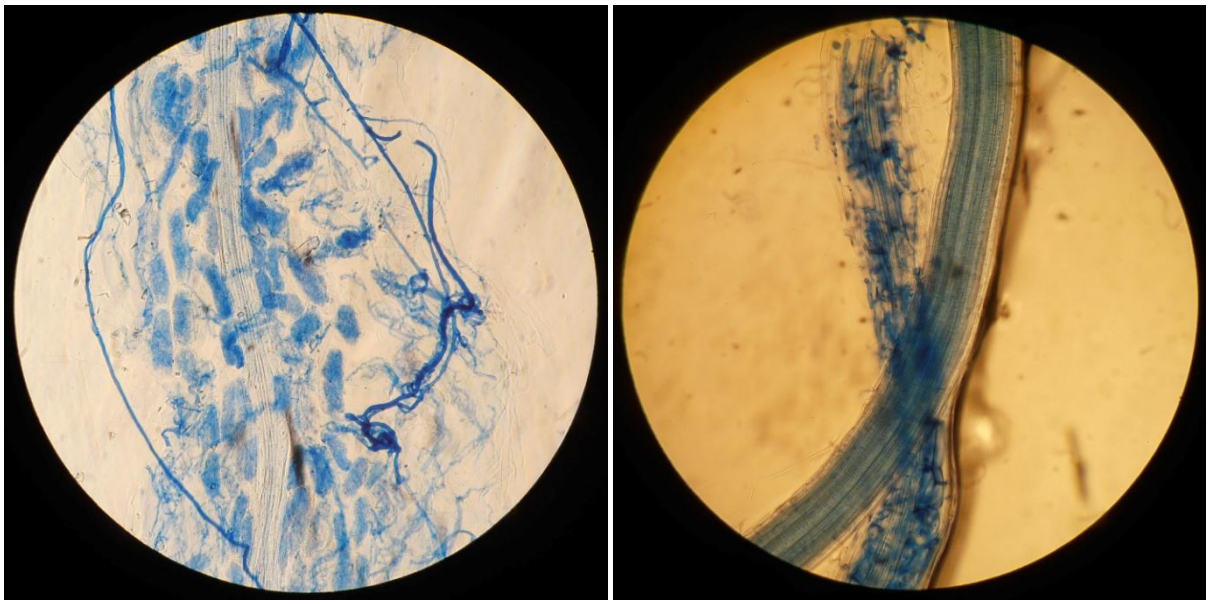

Figure 49: A: A somewhat broken root, with detached hyphae running along its sides, and cells filled with an indeterminate mass that could be deformed arbuscules. The sample was taken from a not inoculated pot containing annual bluegrass and peat. B: Colonized versus not colonized root, from a mixture of annual bluegrass and red fescue in inoculated peat medium. Photo: Tina E. Andersen.

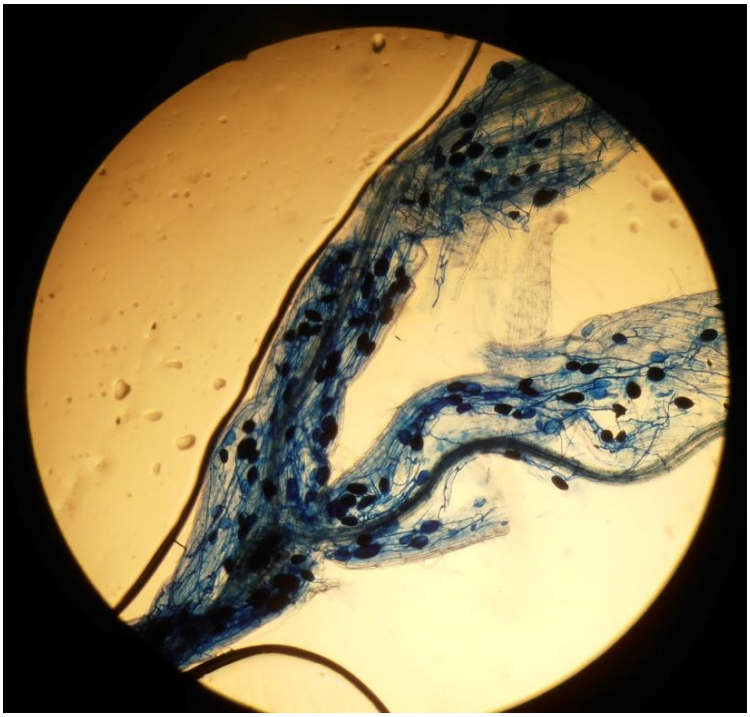

Figure 50: Colonization, seen as hyphae and vesicles/spores, by what is probably Glomus spp. The sample was taken from one of the inoculated control pots, which were sown with sub clover and had Green Mix as a growth medium. Photo: Tina E. Andersen

4 Discussion

4.1 Mycorrhiza and nutrient availability

N/P rates can be essential to AM prevalence and identity

Surprisingly, the different P treatments in study 1 had no effects on either turfgrass quality or AM colonization. According to Smith & Read (2008); the general assumption that mycorrhiza is negatively affected by increasing P levels should be treated with care. Elevated P concentrations may directly suppress colonization by reducing the growth of hyphal germ tubes in the soil, or the symbiosis development may simply be 'delayed' because of a reduced carbon allocation to the roots, and fewer initiation signals and Myc factors being excreted from the plant and the fungi, respectively (Abbott et al. 1984; Jasper et al. 1979; Olsson et al. 2010; Tawaraya et al. 1998). If irradiation is low or defoliation is high, the rate of colonization may be further limited due to the lower amounts of photosynthates being translocated to the roots (Daft & Elgiahmi 1978; Hayman 1974). Defoliation was, indeed, high in all studies of this thesis. However, to what extent the colonization is reduced always depends on both the host and the fungal species involved in the symbiosis, and whether the main reason to less efficient colonization is due to altered availability of soluble carbohydrates, or other mechanisms, remains to be elucidated (Smith & Read 2008). Mycorrhizal colonization may even increase slightly if P is applied to soils that are strongly deficient of this substance (Bolan et al. 1984; Treseder & Allen 2002), and it is thus not as simple as to say that elevated P levels always suppress AM symbiosis.

Increased root growth due to higher fertilization levels may give false indications of AM reduction, as root colonization is often expressed as "percentage of root length colonized" (Blanke et al. 2011; Bruce et al. 1994; Smith & Smith 2011). High P may also lower the frequency of arbuscule appearance, which may be synonymous with a lower colonization if identifying a colonized root depends on the presence of arbuscules (Bruce et al. 1994; Dickson et al. 2007; Smith & Read 2008). In this thesis, the estimate of fungal abundance in each sample was expressed as the percentage of roots that were colonized, where the total number of root pieces containing vesicles, hyphae, arbuscules or other AM structures was a percentage of the total observations. Thus; colonization was not under – estimated, and the lack of significantly less AM symbiosis on plots fertilized with phosphorus is in line with studies like that of Nagy et al. (2009), suggesting that the MP, as followed by mycorrhizal colonization, is only suppressed to a noticeable level if P concentrations are exceptionally high (Smith & Smith 2011). The applied amount of P in study 1 may simply have been too low for observing any significant differences between fertilized and not fertilized plots, especially if the combinations of hosts and fungal species form symbioses that are relatively unaffected by elevated P levels (Smith & Read 2008). Grasses, which generally have extensively branched and fine roots with long, abundant

root hairs, are thought to form less mycorrhiza than plants with coarser and less branched root systems (Graham & Eissenstat 1994; Hill et al. 2010; Joner 2012). It is also a common phenomenon that mycorrhiza are less prevalent in cultivated plants exposed to strong fertilization, and that colonization rates of the same species might be higher in more natural situations (Gange et al. 1999b; Joner 2012; Koske et al. 1997b). If the turfgrasses, seen from an evolutionary perspective, are not as dependent on mycorrhiza for gaining nutrients and water as other strongly mycorrhizal species, their rate of AM development may be constantly low due to genetic programs that are limiting the amount of C used for supporting mycorrhizal growth through signalling pathways (Graham & Eissenstat 1994; Reinhardt 2007). Thus; significant effects of altered P levels on AM colonization will probably not be seen in the grasses.

What was interesting with the results related to nutrient availability was that the AM colonization rates tended to increase with decreasing N – levels. Little is known about how N affects AM development, but it is believed that underlying mechanisms are similar to those for P regulation (Corkidi et al. 2002; Johnson et al. 2003; Olsson et al. 2005). Research has suggested that it is actually the available N:P ratio in the soil, and not just N or P alone, which decides whether increased fertilization will reduce or increase the AM colonization rate: N supply reduces colonization only if the soil contains adequate amounts of P (Blanke et al. 2011; Corkidi et al. 2002; Treseder & Allen 2002), and similarly, it has also been shown that P addition reduce colonization only when plants are not deficient in N (Sylvia & Neal 1990). This suggests that plants form mycorrhiza to enhance both N - and P uptake. Findings indicating that elevated N–levels also can cause neutral or even positive alterations of colonization in roots have been reported (Corkidi et al. 2002; Sylvia & Neal 1990), and can be justified on the basis of the nutritional status of the individual plants. N is the most important nutrient governing plant growth (Ericsson et al. 2012; Vitousek & Howarth 1991), and if the P concentration in plant tissues are increased, a ‘concentration effect’ makes the need for N to support dry matter production even higher (Sylvia & Neal 1990). Likewise, elevated N levels will reinforce the need for uptake of P and other nutrients in the plant. Since AM colonization is reduced as a consequence of nutrient supply only if both N and P is already sufficient in the soil and plant tissue, N applied to soil lacking P, or conversely, may maintain or increase root colonization in the plant because it needs to obtain large amounts of the substance that is limiting its growth. This theory has been supported by Johnson et al (2003), among others, who showed that N enrichment reduces allocation of C to AM structures for nutrient exchange in grasslands with high P concentrations in the soil, but that allocation increase with N enrichment in grasslands with P-deficient soils.

In addition to the plant's nutritional status and which plant/fungus species are involved in the symbiosis (Corkidi et al. 2002; Smith & Read 2008), the rate and extent of AM colonization is also governed by environmental factors like temperature and pH (Smith & Read 2008), and the fungal responses to nutrient availability have also been shown to be important to take into consideration when predicting colonization patterns . According to Treseeder & Allen (2002), mycorrhizal growth should

increase in response to fertilization if both the plant and fungi are nutrient limited, and it should decrease if only the plant is nutrient limited (as is often the case), due to a reduced allocation of C by plants to the fungi. The researchers also found that different genera of AMF displayed various responses to soil fertility that that was due to either direct influences of N or P availability, or plants controlling the fungal abundance and community composition through selection of the most beneficial symbionts. In their study, *Glomus* was very abundant in fertile soils, and less prevalent when N was limiting. *Scutellospora*, on the other hand, was more abundant in P fertilized soils than in N fertilized soils. In this thesis, lack of molecular methods as well as experience in determining mycorrhizal species/genera limited how much of the AMF community structure that could be solved. Community composition was further not the main focus in the thesis, but the brief examination of mycorrhizal diversity in the field showed high abundance of both *Glomus* and *Scutellospora*. Methods used did not allow a close comparison of the fungal diversity in plots subjected to different nutrient treatments, but *Scutellospora* roughly seemed to be dominating both greens. These findings can be explained by the relatively large amounts of fertilizer that were applied, making the soils rich in both N and P and favoring growth of both fungal genera. Several plots in study 1 were not fertilized with P during the experimental periods, but significant quantities may still have been left in the soil from grow-in fertilization, because little P is normally lost as leachate or run–off from greens (Linde et al. 1994). Because of time pressure, not all plots in this field could be sampled. It was decided to focus on plots with the higher mowing height because the grass experienced less stress and grew better here. Better growth increased the chance of finding any differences between treatments. Moreover, it was not expected to see any effects of mowing height on AM colonization because defoliation have shown to not significantly reduce mycorrhizal colonization despite the prevalent theory that defoliation and shoot removal of plants may reduce the extent of symbiosis due to less photosynthates being allocated to roots (Barto & Rillig 2010). Moderate levels of rabbit grazing did, in fact, lead to greater AM colonization in a study of Wearn & Gange (2007), and a possible explanation was that moderate grazing stimulated some of the mechanisms underlying photosynthesis, leading to more allocation of C to roots and mycorrhizal development. However, high defoliation may affect the AMF diversity, and it is speculated that fields dominated by AMF possessing auxiliary cells, like in the field study of this thesis, is a typical phenomenon in areas that are exposed to intensive grazing or other forms of high defoliation (Ruissen, pers.comm). Thus; other management factors than just fertilization may be involved in determining the AMF community composition of a green or other habitat.

Since the AM colonization levels tended to increase with decreasing N–levels in study 1, N appears to be a major controller of the AM colonization extent in greens, which are usually rich in both N and P. This emphasizes the fact that plants depend on large N amounts for growth enhancement (Vitousek & Howarth 1991) However, the N effect could also be an interaction with pH, since the pH was significantly higher at the lowest N–level. It has earlier been shown that nitrogen source has an effect on colonization levels due to pH modifications. (Thompson 1986). In study 1, the

experimental N was applied in form of liquid ammonium nitrate (NH_4NO_3), and the suppression of AM colonization at high N–levels might have been due to NH_4^+ making the soil more acidic, as suggested by Chambers et al. (1980). Acidification of soils by means of NH_4^+ happens when plants take up N in this form, and when soil microbes convert NH_4^+ to NO_3^-. In both situations, H^+ ions are released into the soil. Whether N interacts with soil pH to regulate the extent of colonization or not, the fact that turf quality increased with increasing N–levels at the same time as the AM colonization decreased, further suggests that the turfgrasses are not strongly dependent on AM symbiosis for nutrient uptake, as previously discussed. Direct uptake of N applied through fertilizers, together with higher mowing, seems to be the primary cause of increased plant fitness in study 1, since neither the extent of AM colonization nor the turf quality differed significantly between the P treatments. Fertilization with P caused, indeed, a slightly lower mean colonization estimate at the same time as the root mass seemed to decrease, indicating that P fertilization makes the plant allocate less photosynthates for root development and mycorrhizal growth. The same trend was observed with the N fertilization, but the middle N–level seemed to be optimal when considering root development. The lowest N–level had the greatest extent of colonization and AM growth promotion, but total plant growth was limited due to insufficient N–amounts. Again, this shows that N is a substance that largely controls plant priorities, and it has earlier been suggested that plants growing in nutrient – rich soils tend to prioritize development of shoots and leaves instead of root growth and maintenance of AM relationships (Ericsson et al. 2012; Marschner et al. 1996) It should be borne in mind that plant MGRs are not always related to colonization percentages. Not all plant species benefit from nutrient uptake through mycorrhizas because MGRs varies with both host/fungus physiology, ecological conditions and environmental factors (Blanke et al. 2011; Corkidi et al. 2002; Smith & Smith 2011), as mentioned in the introduction. Additionally, hidden uptake of P (and possibly other nutrients) may mask the MP contributions to nutrient uptake because the DP is sometimes down-regulated to varying degrees (Smith & Smith 2011). Whether the AM symbioses inflicts costs or benefits to the turfgrasses that are not immediately obvious is not known, but if this is the case, quality differences between the various treatments may be more distinct in the absence of AMF. Furthermore, reduced nutrient availability could reveal positive or negative AM effects that are normally masked due to frequent fertilization.

The lack of significant difference in AM colonization extent on natural vs. inoculated plots without P application in Study 1, suggests that the mycorrhiza product was not viable. However, suspicions that some of the *Glomus* structures observed during examination of mycorrhizal diversity in the field belong to foreign strains that are not typical for Scandinavian AM populations (Ruissen, pers.comm.), indicated that the inoculum contained at least a small amount of viable propagules. A small colonization by *Glomus spp.* was also observed in both growth media in study 3. The media are expected to be free from AMF when not in contact with surrounding environments, because the processing of peat and compost considerably reduces microbial life. Thus; the presence of *Glomus* in

the pots of study 3 must have been due to germinating propagules from the inoculum. If the fungi in the product were effective and ensured a higher nutrient removal from the soil, this would lead to significantly less P in inoculated plots than in plots with no P treatment. Statistical analyses did, indeed, show that inoculated plots had a lower mean P content, but this result was not significant although the trend was shown in two out of three replicates. However, the strong indications that inoculated strains removed P more efficiently from the soil, also suggests that these fungi enhanced the P–uptake of their hosts to a greater extent than the original AMF populations. The increase in fitness, which would be expected to be observed in the plants due to the more frequent P assimilation, was probably masked by the already sufficient nutrient levels in the soil that were created during grow-in. As already mentioned; little P is normally lost as leachate or run–off from greens, and may remain in the soil for a long time (Linde et al. 1994). The mean overall impression of plots fertilized with P was barely higher than that of plots without any P treatment, suggesting that the plants already had access to the amount of P that they needed, and thus did not benefit significantly from either additional P fertilizer or enhanced P–uptake through AM symbiosis.

If the commercial AMF strains further had potential to increase host fitness in other ways than just through enhanced nutrient uptake, this has probably also been suppressed, due to the low percentage of product propagules that must have been able to colonize the plants. For example, it did not seem to be less symptoms of take–all fungus infection in inoculated plots, rather the opposite, if the lack of significance is not taken into account. Mycorrhizal turfs have, indeed, previously showed tendencies toward higher suscpeptibility to take-all fungus at high P-concentrations (Koske et al. 1995). However, the colonization levels in inoculated plots and plots without any P treatment were identical, and the impression of the fungal diversity was that it was quite similar in all plots. This means that the inoculated strains may have replaced only a small fraction of the total AMF community, probably because the natural populations have a very dominant role. Some of the species that were introduced through mycorrhizal application may already exist in the field, but the foreign strains are not adapted to the current ecosystem and may have other preferences to host and environmental conditions that make them less competitive against the already established strains in the AMF community. Regarding the found of Treseeder & Allen (2002) again; different species and genera of AMF can show various responses to nutrient availability. Moreover, strong fertilization reduces the species diversity of AMF communities (Corkidi et al. 2002) and may result in selection of fungi that are able to colonize plant roots despite the high nutrient content, which often makes the plants less susceptible to symbiosis (Sylvia & Neal 1990). When plants are fertilized to such an extent that less C are allocated to the roots, less root exudates will consequently be excreted (Corkidi et al. 2002; Johnson 1993; Yoneyama et al. 2007), and a strong selective pressure alters the AMF community composition in favor of the most aggressive and C dependent strains. These fungi may be less beneficial mutualists because they are able to acquire C from the host despite low allocation, but tend to produce fewer hyphae and arbuscules which are important for nutrient exchange, as shown in

grasses by Johnson (1993). Johnson also suggested that more stable and mutualistic AM associations will always evolve over time in undisturbed soils, and that the negative or neutral MGRs that can often be observed in cultivated plants are due to human activities disrupting this dynamic system. If all this is true, the lack of a significant AM contribution to turfgrass quality in study 1 might be an indication of man–made imbalance within the AMF community that has obscured the mechanisms that normally coordinate and fine tune the interaction between hosts and their fungal symbionts.

Type of soil organic matter affects both field quality and AMF abundance

The comparison of the two different growth media in study 2 showed that the use of sand amended with Green Mix in the root zone is a good way to increase both turfgrass quality and mycorrhizal colonization. The general impression of the field was even significantly increased when sand and Green Mix was used as topdressing. The C/N and C/P ratio is lower for composted garden waste than for peat (Aamlid et al. 2009), and Green Mix consequently release nutrients more easily that can be directly absorbed by plants. This was confirmed by the significantly larger amounts of N, P and K that were removed from root zones containing Green Mix compared to peat, and the tendencies towards a larger removal of P and K with Green mix in the topdressing. Whether the increased nutrient uptake in the plants is due to the larger AM colonization or a direct effect of readily available nutrients to the roots can be discussed. According to the findings in study 1, increased N and P availability in soils that are already rich in both substances should reduce AM colonization through mechanisms involving less allocation of C for root development and mycorrhizal growth, among others. The root mass was, indeed, significantly larger in plots containing peat in the root zone, suggesting that more nutrients was invested in growth of shoots and leaves in the plots with Green Mix, despite the fact that root growth promotion by humic acids from organic residues have showed to be more efficient in composted than non – composted materials (Jindo et al. 2012). Since the AM colonization had gotten more than twice as extensive in Green Mix plots by the autumn of 2012, this shows that symbiosis development somehow has been strongly facilitated in this growth medium. A higher propagule density in Green Mix contributing to greater colonization is unlikely because the composting process subjects the plant material to temperatures near 70°C, killing virtually all present microbes including AMF. Likewise, the anaerobic environment where peat is formed suggests that there initially will be no microbial life in this material when establishing a green, and that all fungi and bacteria that eventually can be found in the soil have immigrated from the surrounding environment. The exception is various saprotrophic fungi that may have occurred at the time after compost completion or peat harvest (Green Mix producer, pers.comm). Presence of such fungi and/or fungal pathogens may have led to slight over–estimations of AM colonization percentages if they were mistakenly recorded as AMF, but this is not likely to significantly have obscured the thesis results.

No studies have previously been performed on mycorrhizal colonization of plants grown in Green Mix, but a possible explanation to the high level of AM colonization observed in Green Mix

compared to peat is that increasing nutrient availability through amendment with organic substances like compost may act differently on AM colonization than application of artificial fertilizers. Although the potential for acquiring nutrients is greater in root zones containing Green Mix than in those containing peat, it may well be that the plants makes use of AM colonization to harvest nutritional ingredients from this organic material even faster than they are able to do on their own. Plants may also be in an optimal state for developing symbiotic associations when they are growing stems and leaves, but can afford to allocate some photosynthates to production of root exudates at the same time. When regarding the results from the examination of AMF diversity, the composition of the AMF communities roughly seemed to be the same in both fields, regardless of plot treatments. Thus, a field dominated by potentially less mutualistic AMF, well adapted to the current environmental conditions, is likely to be the case also in study 2. As previously discussed, mycorrhizal colonization percentages often do not correlate with observed MGRs, and although the plants in Green Mix plots had more AM colonization than those in peat plots, this does not necessarily mean that the fungi contribute significantly to the increased nutrient uptake and the enhanced quality that was observed here.

It has been shown that AMF associates preferentially with organic matter in the soil and that their hyphal growth, by an unknown mechanism, can be stimulated by amendment with additional, organic substances (Gaur & Adholeya 2000; Johnson 1998; Joner & Jakobsen 1995; Stjohn et al. 1983), despite the fact that no active orientation (tropism) of hyphae towards such particles has been observed (Stjohn et al. 1983). As known, the only way for AMF to acquire C is thought to be from their hosts, but it has recently been discovered that the fungi support much of their own fitness through N obtained from organic material that is mineralized by other soil microbes, which they possibly interaction with to increase the decomposition rate (Hodge & Fitter 2010; Joner & Jakobsen 1995; Leigh et al. 2011). At the same time, various organic growth substrates have in some studies also shown to inhibit AMF and their colonization processes (Avio & Giovannetti 1988; Calvet et al. 1993), and it is suggested that chemical composition determines whether the fungi will respond negatively or positively to different organic materials (Medina et al. 2011). Little is known about which components that may cause the various responses that AMF have shown to different organic amendment. Medina et al (2011) suggested that ferulic acid from sugar beet waste inhibits hyphal growth, but that fermentation of this organic material by *Aspergillus niger* can lead to elimination the of hyphal growth reduction, possibly by means of compounds exudated by *A. niger* or release of special mineralized compounds. In a study of Hrselova et al. (1999), hyphal growth correlated positively with soil respiration rates, while no effects were seen of neither total soil C, oxidizable C (organic matter), N and available P. This indicated to the researchers that AMF prefer to associate with labile C fractions that easily release organic compounds, as well as CO_2, because of fast degradation by saprophytic microbes. CO_2 produced during decomposition of organic matter may stimulate (Becard & Piche 1989) or inhibit (Letacon et al. 1983) AMF development, probably depending on the atmospheric CO_2 concentration (Medina et al. 2011). The AMF may also be inhibited by soil bacteria, like

actinomycetes, which they compete for products from organic particle degradation with (Leigh et al. 2011; Stjohn et al. 1983).

Obviously, Green Mix has physiological and biochemical characteristics that make AMF abundant in the soil. For example; the soil was alkaline in Green Mix plots, but acidic in plots with peat, and pH is an important factor that affect the presence of various microbes. Some of the possibly stimulating substances in this organic material may be humic acids that are similar to those in composted municipal waste, which was found to effectively promote root growth in plants (Jindo et al. 2012). Green Mix may also contain few bacteria that are able to inhabit AMF development compared to other organic growth substrates, and its low C/N or C/P ratio could positively be affecting the nutrient uptake supporting fungal growth as well as that supporting plant growth. Thus, the most probable explanation to the high AM colonization levels observed in plots amended with Green Mix is a combination of plants being stimulated to develop more symbiotic associations, and the fact that Green Mix stimulates the growth of AMF. However, since the observed fungal composition was similar to that in study 1, where no positive MGRs were detected, the improved turfgrass quality could be mainly due to direct nutrient effects on plant growth also in study 2. This is further supported by the fact that the positive effects of Green Mix started to show shortly after grow–in, before the AM colonization levels had reached a significant level. The primary quality controller must be N, based on previously discussed literature and the lack of significance between P treatments in study 1, which indeed showed significant N-effects. This was also shown by the results from study 3, where no P was given to any of the pots. Sand amended with peat was fertilized with twice as much N as for Green Mix to follow what was done during the grow–in of study 2, explaining why the pots containing peat showed significantly lower root weight and a better turfgrass quality than pots containing Green Mix. The heavy application of N to peat has probably more than evened out the difference in nutrient availability between the two growth media, and this has consequently led to peat plants allocating more C to grow leaves and shoots while Green Mix plants have allocated more C to processes in the roots, namely the opposite of what was the case in study 2. There was a medium x roots interaction in study 3 that suggested a higher root weight for plants that were inoculated when grown in Green Mix pots, while the opposite was true for plants grown in peat pots. A three factor interaction also occurred between species, growth medium and inoculation, in that velvet bentgrass in peat had a greater root weight when not inoculated, while both bentgrasses tended to have a greater root weight in Green Mix when inoculated. This suggests that for peat, which was very rich in available N, plants achieved the most efficient root growth when directly absorbing nutrients, while for Green Mix, which was fertilized with less N, plant root development benefited the most from AMF-assisted nutrient uptake. However, one should be careful about drawing any conclusions that the mycorrhizal treatment affected the bentgrass root growth responses to nutrient availability in the two growth media, because there were no significant difference in colonization levels between the inoculated and the not inoculated pots, and colonization was generally very low. Furthermore, root weights did not differ significantly

between the inoculation treatments.

It should be mentioned that even though Green Mix gave the highest quality in study 2 in 2012, there was a tendency towards a denser turf on plots with peat during grow–in (not shown). Thus; the additional fertilizer given to peat in this period seems to have compensated for the differences in nutrient availability between the two growth media, as intended. In study 3, the differences were more than evened out by the double N-amount given the peat medium, and the positive characteristics of Green Mix became overshadowed by the better growth in pots containing peat. Why the difference between the two media changed so dramatically in the greenhouse study although the pots were fertilized in the same manner as that during grow–in of the field, cannot be fully explained. However, soil analyses showed that the loss on ignition was lower for both growth media in study 3, and the lower organic material content has probably reduced the rate of which Green Mix was able to release nutrients in the pots. Moreover, complete fertilizers were used in study 2, while the different nutrients were added separately during the preparation of fertilizer solutions in study 3. Type of fertilizer may have influenced the achievement of balance in nutrient availability between the two growth media differently in the two studies. Leaching of nutrients may also have led to less accumulation of N in the peat root zone, especially during the first two months of grow–in, where the field location experienced much higher rainfall than normal. The pots in study 3 were rarely irrigated to such an extent that fertilizers were washed out with water running through the pots, and accumulation of more N in the pots than in the field may thus be an additional explanation to why study 3 experienced a greater masking of the Green Mix effects when extra N was given to root zones containing peat. Anyway, since sand amended with peat to a greater or lesser extent became the better growth medium when additional fertilizer was applied to even out the difference in nutrient availability between the two growth media, the ability of Green Mix to release nutrients over short time intervals must be slightly overrated. If the extensive AM colonization had an impact on the increased nutrient uptake in Green Mix in study 2, the low colonization percentages observed in study 3 may be an additional reason to why the plants were not able to benefit more from Green Mix in this study. The low AMF abundance and the lack of significantly more colonization in Green Mix compared to peat in study 3 support the suggestion of study 1 that SYMBIVIT® is not very efficient at establishing AM populations, at least not in turfgrasses grown in their respective environment. Furthermore, there were no significant mycorrhizal effects on any of the measured parameters, indicating that the commercial strains were not noteworthy beneficial to the plants when growing without competition from the species living in the field either. Regarding the indications of increased P – removal from inoculated plots in study 1, and the tendency towards a better root development in the least nutrient-containing growth medium when the pots were inoculated in study 3, the possible mycorrhizal effects might have been more evident if the colonization levels of the strains were higher, and the access to nutrients lower.

Again, it is shown that the growth and quality of turfgrasses is mainly controlled by direct uptake of nutrients, especially N. AMF growth and colonization can obviously be enhanced in the

presence of specific organic growth substances like Green Mix, despite ample nutrients available to the plant roots, but it is uncertain to what extent the fungi contribute to the increased nutrient uptake that is observed in plants with this growth medium. If translocation of nutrients to the host via symbiotic interfaces is ineffective, AMF may indirectly enhance the nutrient uptake in that they help the plant to decompose organic material. This could be particularly true for organic materials that stimulate fungal development. As mentioned, it has been suggested that AMF interact with special soil microbes to increase decomposition rates (Hodge & Fitter 2010; Joner & Jakobsen 1995; Leigh et al. 2011), and it is currently being discussed if the fungi can help the plant excrete more degradation enzymes, or even excrete some of the enzymes themselves (Ruissen 2012a; Smith & Smith 2011).

Regardless of how and to what extent AMF affected nutrient accumulation in the turfgrasses, a higher abundance of these fungi will always lead to increased removal of nutrients from the soil, due to hyphal uptake and storage. AM colonization have shown to significantly decrease P leaching into the groundwater from soil under low P conditions, through mechanisms that included AM-enhanced plant P removal followed by increased plant growth (Asghari et al. 2005). Unfortunately, the researchers in this study were not able to clarify whether P storage in AMF hyphae directly affected the leaching. Examinating the water collected in the lysimeter tanks in study 2 could, according to the findings of Ashgari et al., be a good way of solving whether AM colonization contribute significantly to increased nutrient removal by the turfgrasses in the field, because if this is the case, the amount of leachates in tanks belonging to Green Mix plots will be expected to be lower than those in tanks belonging to peat plots, where the AM colonzaiton was less extensive. However, the researchers also found that high P levels reduced leaching more efficiently than AM colonization, by the same mechanisms as with AM in the low P soil. A potentially smaller amount of leachates in the runoff water from Green Mix plots could thus not be used as an indication of significant AM contribution to nutrient accumulation in the turfgrasses in study 2, because the soil was well fertilized, and quite rich in both N and P. Enhanced nutrient removal by the plants could be due to both mycorrhizal effects and a direct effect of increased nutrient availability, the latter being the most likely according to previous arguments.

4.2 Turfgrass species, colonization levels and competition

Turfgrasses form abundant AM symbioses

Although relatively little research has been performed on the extent of mycorrhizal colonization in various turfgrass species, old statements that turfgrasses do not support significant AM colonization have recently been contradicted by the discovery of numerous AMF species, as well as high spore abundances, in the root zones of different turfgrasses in the field (Koske et al. 1997a; Koske et al. 1997b). Selected bent - and bluegrasses have received the most attention when investigating AMF/turfgrass interactions, and high levels of AM colonization have been found in species from both genera (Frank 1984; Gange et al. 1999b; Hartin et al. 2005). In this thesis, general field colonization levels ranged from approximately 10 to 80%, varying with type of organic material amendment and the age of the greens. The estimated root colonization percentages may have been slightly over – estimated because some non – mycorrhizal endophytes were probably mistaken by AMF when scoring the roots. Especially in study 1, where many of the plots had significant amounts of take – all fungus, the lack of recognition of this pathogen in the plant roots may have led to an over – estimation of AM colonization. However, the values are certainly not erroneous to such an extent that any of the conclusions made in the thesis has to be reconsidered, and the finding supports previous suggestions that turfgrasses actually may form extensive levels of AM symbiosis (Gange et al. 1999b; Gemma et al. 1997b; Koske et al. 1995; Koske et al. 1997b). Indeed, Koske & Gemma (2005) stated that the creeping bentgrass cultivar 'Penncross' normally has colonization levels of 70 % or more when grown in mature sand/peat greens. Whether the plant/fungus interactions increase turfgrass fitness or not is a complex issue, as discussed in previous paragraphs.

No significant differences in estimated AM colonization percentages were shown between the different turfgrass species in study 1 and 3. There was a relatively large spread in data, so this result could be partly due to the large variance. Disregarding the lack of significance, the estimated colonization percentages in study 3 increased in the order: red fescue < annual bluegrass < velvet bentgrass < colonial bentgrass. It is suggested that bentgrasses are strongly mycorrhizal, that is: they tend to form abundant AM symbioses, from which they benefit as long as the environmental factors allow it (Gange et al. 1999b; Gemma et al. 1997b; Koske et al. 1997a; Koske et al. 1997b). Annual bluegrass show varying colonization levels, indicating that this species is less mycorrhizal (Koske et al. 1997a). Furthermore, when its colonization percentage is high, this is often detrimental to the plants because annual bluegrass is negatively affected by certain AMF species that may be beneficial to other turfgrasses, like bentgrass (Blanke et al. 2011; Gange 1998; Gange et al. 1999b). Little is known about AM colonization in red fescue, but Gange et al. (1999) showed an increased AM colonization of *Festuca spp*. as a response to addition of a mycorrhizal inoculum, as long as the strongly mycorrhizal creeping bentgrass was not present. Red fescue is thus expected to be less mycorrhizal than the

bentgrasses, but more mycorrhizal than the annual bluegrass, which did not experience elevated colonization levels as a response to AMF inoculation. Because of this, it was somewhat surprising that annual bluegrass showed a higher mean colonization level than red fescue in study 3. On the other hand, the mycorrhiza samplings from June 5th in study 2 actually showed significantly more colonization in annual bluegrass than in red fescue, and three samplings taken from annual bluegrass growing at random places in the field at Landvik showed colonization levels ranging from 50 – 70 % (data were not presented). This indicates that annual bluegrass may be more mycorrhizal than previously believed, and this could possibly be due to its high growth potential which causes it to respond more strongly to fertilization than other turfgrass species (Kvalbein & Aamlid 2012; Vargas Jr & Turgeon 2004). Increased growth will also increase the need for nutrients, and this may be the reason to why the annual bluegrass forms AM symbioses so frequently. It is unlikely that all AMF species has an antagonistic effect on this plant, and high colonization could be beneficial if the right fungal symbionts are involved. At the same time, red fescue has a low growth potential (Beard 1998; Kvalbein & Aamlid 2012), and the following low fertilizer requirement of this species may be related to its lower AM colonization. Additionally, colonization levels are not always synonymous with negative or positive MGRs (Smith & Smith 2011), so it is fully possible that annual bluegrass benefit less from AM colonization than the red fescue does, despite their differences in colonization extent. The estimated colonization percentages in study 1 increased in the order: red fescue + velvet bentgrass < red fescue + colonial bentgrass < red fescue. Considering the results from study 2 and 3, it is unclear why pure red fescue suddenly showed the highest colonization levels in this green, but it must be born in mind that no means differed significantly, except those for red fescue and annual bluegrass in the samplings from June 5th in study 2. Thus, definitive conclusions cannot be drawn from the results, and it may be that red fescue has a potential to develop a more extensive AM symbiosis in response to specific conditions that are not known.

Individual plant characteristics are essential for competitive outcomes

It was difficult to study the competition between annual bluegrass and the other turfgrass species in study 1, because the seeded annual bluegrass was thinned out and eventually disappeared due to severe mechanical damage that was inflicted to the green after ice removal in the winter of 2010/2011, which greatly affected this species. Attempts to reseed annual bluegrass failed because by that time, the other grasses had become so well established that the annual bluegrass was not able to grow and compete for nutrients and space. Also, the annual bluegrass variety that was used was not adapted to the current environmental conditions, and this is probably an additional reason to why the species did not survive in this green. The pure annual bluegrass cylindrical core sample that for this reason was planted in each plot in August 2011 did not give any good measurement of competition with the surrounding species either, because their diameter was too small. The green management was custom to red fescue, and the annual bluegrass samples was again prevented from establishing properly in the new field.

However, some significant effects of the different experimental treatmens on diameter development were observed in the studied sample from 2011. The diameter increased significantly in pure red fescue plots, while it decreased with the bentgrass combinations. This is because red fescue does not grow as fast and dense as the other species, and the fact that it does not tolerate low mowing very well (Beard 1998), as illustrated by the tendency toward a better general impression of plots sown with fescue/bentgrass combinations. Thus, the species is unable to outperform the annual bluegrass, which have a larger growth potential and responds better to available nutrients, as previously mentioned. Velvet bentgrass is the most densely growing turfgrass species, and this is reflected in that the diameter mean had decreased with this species. There were also tendencies pointing towards a diameter decreasing with the highest mowing height and with decreasing N and P levels. Again, the high demand for nutrients in annual bluegrass to be able to achieve a vigorous growth is shown, and the increasing diameter with the lowest mowing height confirms that the species tolerate low mowing better than the other grasses in the study (Kvalbein & Aamlid 2012; Vargas Jr & Turgeon 2004).

Since the cylinder diameter tended to decrease slightly in inoculated plots, it might be that the small percentage of propagules from the product that managed to establish in the green have had a negative effect on the annual bluegrass. This is also suggested by the lower coverage that was observed in the same cylinders, although this was not significant. Indeed, SYMBIVIT® contains some of the same AMF species that Gange et al. (1999) found in the green where an antagonistic effect of AM on annual bluegrass was shown, namely *G.etunicatum*, *G.intraradises* and *G.mosseae*. Thus, the inoculum may have potential to favor turfgrasses other than the annual bluegrass, despite its poor efficiency regarding establishment efficiency and competition against native turfgrass species. However, the nutrient availability and individual plant species characteristics seems to be the main controller of competitive outcomes, and these factors also governs AM colonization to a large extent. Again, the importance of N is protruded, in that the coverage of annual bluegrass significantly decreased in cylinders subjected to the lower N – level. Species respond differently to nutrient availability after how great their growth potential is, but quality and competition is also affected by tiller density, degree of branching and disease susceptibility, as was clearly seen in study 3. Within the 13 weeks, a close sward had developed only in some of the pots. Red fescue and annual bluegrass showed a significantly lower coverage than the bentgrasses, and red fescue had the lowest mean of the two although this had been expected to be true for annual bluegrass, since the variety used for this species was coarse and produced few tillers. It is recommended to not cut red fescue to a mowing height of 5 mm or lower (Beard 1998), but this sometimes happened during pot maintenance, because the grass was cut by hand and it was hard to ensure that the whole sward was cut to exactly the same height. The red fescue was thus inflicted with stress, and showed poor growth. The mowing height in these pots was deliberately increased with a few millimeters during the last experimental weeks to enhance the growth of this species, but although the coverage got better at the end, annual bluegrass ended up with a slightly higher mean value. Velvet bentgrass had significantly higher coverage than

colonial bentgrass, confirming that this species produce the highest number of tillers and is the most densely growing of all turfgrass species (Christians 2007; Kvalbein & Aamlid 2012).

The bentgrasses were subjected to disease in both study 1 and 3. Red fescue was not infected in study 1 due to the fact that it is completely resistant to take – all fungus. Velvet bentgrass seemed to have slightly more patches than the colonial bentgrass, suggesting that it is genetically weaker and more suscpetible to this disease (Kvalbein & Aamlid 2012). There was also a species x N interaction for take all patch, where a tendency pointed towards more disease in the velvet bentgrass combination when applying 1 kg N/100m^2. When 0.5 kg/100m^2 was applied, the opposite seemed to be the case. This can be explained by the fact that colonial bentgrass has a higher growth potential than both velvet bentgrass and red fescue (Beard 1998; Kvalbein & Aamlid 2012), and its consequently high demand for nutrients causes poor growth followed by clearer symptoms of disease at low fertilization levels. This was further suggested by the species x N interaction for general impression, where the lower growth potential (and nutrient demands) of red fescue was also clear. The situation was similar in study 3. Here, colonial bentgrass had significantly more disease than velvet bentgrass, and a species x disease interaction showed that colonial bentgrass had much more disease when grown in Green Mix pots than in peat pots, which were given double amounts of N. Velvet bentgrass seemed to have slightly less disease in Green Mix, and this may be a coincidence indicating that nutrient levels are not of equal importance for the extent to which this species are able to reduce infection symptoms. All species further had a net coverage that was similar in both growth media except colonial bentgrass, which again grew better in the pots containing peat because these had higher N – levels, as shown in the ocurring species x net coverage interaction. It was not determined which fungus caused the disease in study 3, but annual bluegrass and red fescue were not affected by what was believed to be *Fusarium sp*. It seems likely that the species have higher resistance to this disease, and the infection of the bentgrasses might also have been accelerated by the high air humidity and frequent irrigation, creating favorable growth conditions within the dense grasses.

The plots were not treated with fungicides in study 1 because it was not desirable to introduce factors which could interfere with the biological treatment, and the grass seemed to be able to recover from the infections without any other help than the fertilizing. However, the infected pots in study 3 had grass that were quickly withering and not showing any signs of recovery, and had to be sprayed to survive. Since the fungicide was a contact agent and not a systemic, AMF was not likely to be affected by the treatment. Mycorrhizal fungi are also not target organisms of this fungicide, so the treatment was regarded as safe. There are many studies suggesting that application of various fungicides can reduce AM colonization levels of various plants, including turfgrasses (Gange et al. 1990; Rhodes & Larsen 1981; Venedikian et al. 1999; Wan et al. 1998) , but none of these studies were performed on established turfgrasses in golf greens. Bary et al. (2005) investigated how AM colonization levels were affected in 18 putting greens on four golf courses subjected to organic fungicides, and also performed a six month manipulative experiment which involved application of the same fungicides to a practice

green. Neither of the two studies gave indications that modern fungicides can reduce AM colonization in sports turf, and the suggested explanation was that "chemicals may be entrapped and degraded within the thatch layer before they reach the roots". There there was also asuggestion that AMF in greens develop resistance to fungicides over time. Furthermore, Frank (1984) suggested that fungicides may prevent AMF development in turfgrasses if they are applied shortly after seeding, before the fungi have established properly in the roots, but that accumulation of these agents in the soil surface will ensure that AM colonization are not significantly reduced. Thus; application of fungicides does not seem to be fatal to the extent of AM symbiosis in greens.

The diseased bentgrasses in study 3 recovered quickly after spraying. Some of the differences between the individual grass species in this study was probably reduced or masked by the disease, and the pots should have been treated much sooner after the disease was discovered. However, the difference in coverage between the species was about as expected, despite the disease, and the clipping yields in the individual species matched their growth potentials very well: Annual bluegrass produced the most above – ground biomass and differed significantly from the bentgrasses, in which colonial bentgrass had a slightly higher mean than velvet bentgrass. This was also expected, and the higher growth potential of colonial bentgrass would probably have been clearer if the fungal infection had not occurred. Red fescue produced the least above – ground biomass, and differed significantly from the other species. Its lower growth potential and nutrient demand was very well illustrated by the species x clippings interaction, which showed that all species except red fescue had a larger plant production in the ample N – peat medium.

Grasses prioritize growth of above - and underground material differently (Christians 2007) This was shown by their recorded root weights in study 3, which did not match their respective plant production in clippings. Annual bluegrass had significantly lower root weight than the other species, emphasizing its enormous growth potential leading to high biomass production above ground. Velvet bentgrass had significantly greater root weight than the other species, confirming that this species allocate high amounts of photosynthates to root growth. The same result was shown in study 1, where velvet bentgrass was the only species that differed significantly in root weight. Velvet bentgrass have, indeed, previously shown to have more root mass than other turfgrass species. However, the difference between colonial bentgrass and red fescue is somewhat unclear because the root mass of colonial bentgrass was only larger than that of one of the two red fescue varieties which were examined in the study (Aamlid, unpublished). This supports the lack of significance regarding the difference in root weight between colonial bentgrass and red fescue in study 1, while the significantly higher root weight in colonial bentgrass compared to red fescue in study 3 is somewhat surprising. The red fescue would be expected to produce a root mass similar to or even higher than colonial bentgrass, due to its low growth potential causing poor biomass production above ground. It is believed that the poor growth in red fescue that was caused by excessive cutting during the first part of the experiment also left its mark on the root development in this species. The 'crown' weights, which were actually the weight of the

whole sward cut off approximately below the formative region, generally followed the pattern that was seen with the net coverage of the various species. The only difference was that annual bluegrass had the lowest crown weight, despite the fact that it also seemed to have a slightly higher coverage than red fescue. Again, the poor growth in red fescue during the first experimental weeks had led to annual bluegrass having a higher mean value for net coverage, although it was shown that red fescue actually grew more dense than annual bluegrass when it recovered from the excessive cutting. The crown values also reflected the pattern in the root weight results, except that annual bluegrass did not differ significantly from red fescue. The crowns probably contained a small part of the root system, linking these results. This is supported by the occurring species x crown interaction, which showed that velvet bentgrass had a higher crown weight in Green Mix than in peat. There was, as already discussed, more roots in Green Mix compared to peat in study 3, explained by the lower N fertilization of this medium. This was particularly evident for the two bentgrasses, which significantly increased their root growth in response to Green Mix, according the occurring species x roots interaction. Again, the response was greatest for colonial bentgrass, confirming its high nutrient demand. If the crowns included parts of the root system for some or all species, the results from the root weight analyses must consequently have affected the crown weights.

Biological control of annual bluegrass

Gange et al. (1998, 1999) proposed that inoculation with AMF may be a good way of keeping the prevalence of annual bluegrass in golf greens at bay, while the costs and environmental challenges related to application of herbicides or other maintenance measures are also restricted. The background for why AMF are regarded as potential biological control agents of this annual, invasive weed is that the fungi have shown to facilitate the growth of the desirable perennial bentgrasses, which are also more mycorrhizal and form symbiotic associations with a higher diversity of AMF species than the annual bluegrass (Gange 1998; Gange et al. 1999b; Gemma et al. 1997a; Koske et al. 1997a). In addition to this competitive argument, AM colonization may also inhibit the annual bluegrass directly, in that some fungal species may have an antagonistic effect on this grass but not on other turfgrasses (Blanke et al. 2011; Gange 1998; Gange et al. 1999b). The mechanism behind this growth suppression is unknown, but it is thought to involve mycorrhizal carbon costs exceeding nutrient inflows to the plant (Gange & Ayres 1999), which has a high demand for water and nutrients. The negative MGR could also be due to a strong down – regulation of the DP in combination with ineffective symbiosis owed to the physiological characteristics of the plant, the fungus, or both. If the reduced activity of the DP is not compensated for by the MP, plants will experience a lack of vital nutrients, such as N and P, and consequently show negative MGRs (Smith & Smith 2011). Furthermore, AM colonization may have detrimental effects on some plant species if soil N/P levels are high (Smith & Read 2008), as is often the case in golf greens. If the high fertilization rates makes AMF affect annual bluegrass

negatively, despite the fact that this species has an enormous growth potential and normally respond well to increased N/P levels (Kvalbein & Aamlid 2012), this is promising to the quality of golf greens where annual bluegrass is undesirable. The observations are especially valuable in context with the fact that treatment with modern fungicides, as is often required on greens, have shown not to affect AM colonization significantly (Bary et al. 2005). However, if the nutrient availability is too high and the soil is rich in both N and P, this can lead to a reduced rate or extent of AM colonization (Blanke et al. 2011; Corkidi et al. 2002; Johnson et al. 2003; Smith & Read 2008; Sylvia & Neal 1990), as previously discussed. If the growth response in annual bluegrass is related to its colonization levels, AMF reduction caused by high fertilization rates may then thwart the restrictive effects that these fungi possibly have on their host. It is important to find a middle way, and fertilize neither too little nor too much, for biological control of annual bluegrass to be effective.

By applying inoculums containing AMF species that have antagonistic effects on annual bluegrass but not on the desirable turfgrass species, the fungal selection of the latter will be greatly enhanced, as proposed in the studies of Gange et al (1998,1999). If the growth responses of the various turfgrasses are related to AM abundance, the enhancement would also partly be due to a boost of the relatively low AM colonization levels that are often observed in greens compared to natural ecosystems (Gange et al. 1999b; Koske et al. 1997b). However, things are rarely as simple as the theory suggests, and experiments conducted in the greenhouse may not offer the same results in the field because of the disturbing factors and complex relationships that exist here. The inoculum tested in this thesis showed not to be very effective regarding AMF establishment or competition against native turfgrass species, but a small tendency pointed towards a reduction of the annual bluegrass diameters, as well as increased P removal from the soil, in inoculated plots from study 1. In addition, it was suggested that the mycorrhizal treatment affected root growth responses to nutrient availability in the two growth media, at least for the bentgrasses, which have large growth potentials and also invest much photosynthate in root development. This indicated that the inoculum have potential to help the other species out-compete annual bluegrass populations. However, study 3 did not show any remarkable growth reductions in annual bluegrass when the species was cultured alone in pots that were inoculated compared to those that were not, and the species grew generally well in this study due to the high nutrient availability. Furthermore, competition was also subject of study 2, where annual bluegrass formed a great part of the sward when the field was newly established. The grass was significantly more abundant in plots amended with Green Mix, which is logical because annual bluegrass has a high demand for nutrients, and grows best where these are readily available. During the next summer, the species was almost completely gone. Since the AM abundance was also greater in Green Mix plots, it might be that the natural AMF community living in the field also contained species that had an antagonistic effect on annual bluegrass without necessarily showing positive effects on the other turfgrass species, as already discussed. However, the greens was maintained by a regime that was customized for red fescue (higher mowing, less fertilizer and less irrigation), and it is

more likely that the annual bluegrass, which is highly susceptible to drought stress as well as nutrient deficiency, got outperformed in study 2 because the red fescue had superior growth in the summer, when the field was subjected to less rainfall. Also, like study 1; the annual bluegrass variety that was used was not adapted to the current environmental conditions, and this is probably an additional reason to why the species did not survive.

This thesis highlights the importance of considering individual species characteristics when predicting plant competitive outcomes on a green. Although biological control of annual bluegrass by means of AMF is promising to a future environmentally friendly and cost – effective green maintenance, management techniques must be developed to encourage and sustain high levels of these fungi for the method to be successful (Bary et al. 2005). However, turfgrass species respond differently to nutrient availability due to their various growth potentials, and they also tolerate mowing and other mechanical treatments to various degrees. This is important to take into account when developing management programs, so that the treatments favoring AMF does not counteract growth of the desired grass species at the same time. Furthermore, screening more plant species and varieties to reveal their mycorrhizal relationships in nature is necessary to know which AMF species they normally form symbioses with and to what degree, in addition to how these fungal species affect potential hosts (Wang & Qiu 2006). Many AMF are generalists and colonize a wide spectrum of plants, but can affect plants differently because environmental conditions and host/symbiont physiology also affect the outcome of such symbiotic associations (Smith & Read 2008; Smith & Smith 2011). If AMF are to successfully prevent annual bluegrass from establishing dominant populations in golf greens, it is necessary to know exactly which fungal species have an antagonistic effect on this grass in particular, without affecting the desired grasses in the same way. It is namely not as simple as to say that lower colonization in annual bluegrass than in, for example, velvet bentgrass, gives the latter a competitive advantage despite the fact that it is normally more mycorrhizal. Additionally, annual bluegrass exist as both annual and perennial bio-types, and it is important to find out whether these respond to mycorrhiza in the same way (Gange 1998). The mechanisms underlying the various effects that AMF have shown to have on their hosts must be revealed to be able to predict how environmental changes and various ecosystems may affect the different symbiotic associations, and it is also important to understand how different AMF species affect each other in competition for host carbon, and if some species compositions are more useful than others. In addition, plant communities that are composed of different species involves competition between these, and more field studies are definitely necessary in the future because many questions regarding mycorrhizal effects cannot be truly answered through experiments performed under controlled greenhouse environments. When the mystery of host/species combinations and the variety of effects that these may show under different ecological, environmental and nutritional conditions are solved, specially designed inoculums and management techniques can be made that favors the plant species of interest, under defined conditions (Koske et al. 1995). Molecular methods are indispensable tools along the

way, and are now providing unique opportunities to mycorrhizal biology and ecology which can ensure rapid aquisition of new and important knowledge (Gorzelak et al. 2012).

4.3 Establishing mycorrhizal populations

A variety of factors are involved in determining whether commercial inocula are efficient

Lately, people have become more aware of the problem that the ancient and beneficial mycorrhizal fungi, normally found in large quantities in natural soils, are in danger of being reduced or in the worst case eliminated because of the human intervention in nature when constructing, for example, greens, and managing these with the goal of a best possible quality by our criteria. Many old and perfectly adapted interactions between plants and AMF may be destroyed because intensive mowing and fertilizing, among others, reduce the species diversity of AMF communities and selects the most aggressive species, which can often cause neutral, or in worst case negative, growth responses in their hosts if the environment favors this . For these reasons, there are now a number of mycorrhiza products available on the market, with the hope that inoculation with selected species can re-introduce these ancient and beneficial associations so that culturing of variuos plants will become as efficient and environmentally friendly as possible (Amaranthus 2001; Corkidi et al. 2002; Gemma et al. 1997a; Hartin et al. 2005; Johnson 1993; Koske et al. 1995; Tarbell & Koske 2007).

Newly constructed greens are often devoid of most microbial life due to the type of growth materials constituting the root zone, and the processing which these have been subject to (Gemma et al. 1997a; Koske et al. 1997b). In this context, many studies have found that inoculation with AMF at seed or seedling stages may enhance the growth and establishment of various turfgrasses, mainly through a more efficient nutrient uptake, better resistance to drought and higher salinity tolerance (Gemma et al. 1997a; Gemma et al. 1997b; Hartin et al. 2005; Koske et al. 1995; Koske & Gemma 2005; Pelletier & Dionne 2004). Early playability on greens that show high resistance to many biotic and abiotic stresses may offer a financial advantage to green-keepers (Hartin et al. 2005), and mycorrhizal inoculation have shown to produce high quality seedlings for other culturing species as well (Ortas et al. 2011).

The product that was tested in this thesis showed no significant effects on either AM colonization levels, turfgrass quality or competition, although there were some tendencies described in previous sections. In the field study, inoculum was applied in study 1for the first time after grow-in was completed. If the product had potential to increase establishment rates or seedling quality of the turfgrasses used in this green, this would thus not have been detected because the product was not incorporated during green construction. Moreover, application was only performed once a year. To

establish healthy AM populations, it is recommended to incorporate inoculum in both spring and fall, for several years (Amaranthus 2001), and too long intervals between applications may have been an additional reason to why the commercial strains did not establish properly or cause any significant effects in study 1. However, there were no significant effects of the product in study 3 either, although this was a study of the same turfgrass species during establishment, and had the inoculum incorporated from the day before seeding. There is no doubt that the product contained viable propagules, as colonization was detected in both the inoculated pots and the positive controls. Suprisingly, some pots containing heat-killed inoculum were also colonized, although the growth media should initially be lacking AMF, as previously discussed, and despite the fact that no AM colonization was detected in non-mycorrhizal control pots. It is unlikely that the propagules had survived the heating treatment, and contamination during preparation or maintainance of the pots is also not likely because all precautions were followed. Moreover, AMF spores are so large that they are not easily dispersed through the air, like spores of other fungi. Thus, the AM colonization that was recorded in non-mycorrhizal pots was probably due to contamination of other fungal genera that were mistaken by AMF.

The fact that the subterranean clover in the control pots showed significantly higher colonization levels than the grasses indicates that this is a more mycorrhizal species, which has a physiology that makes it more dependent on AM symbiosis for nutrient acquisition (Graham & Eissenstat 1994; Hill et al. 2010). Moreover, although most AMF are generalists, the commercial strains might have been a better 'match' for the subterranean clover, resulting in faster symbiosis development. Mycorrhizal effects were not measured in the controls except for colonization levels, but it was noted that the inoculated plants actually recovered less from withering after a period of insufficient irrigation. The controls had received the same total amounts of fertilizer as the experimental pots, despite their lower soil volume. Thus; the deleterious effect of AM colonization that was observed in the subterranean clover plants during drought may have been due to the excessive fertilization making the symbiosis redundant, combined with a poorer ability to decrease the rate of colonization compared to that of the turfgrasses (Graham & Eissenstat 1994; Smith & Read 2008). No P was given to any of the pots in this study, but the Green Mix probably provided sufficient amounts.

Regardless of how the controls were affected, the inoculum was clearly ineffective with the turfgrasses, and had a poor ability to colonize the plant roots. Possible explanations that have already been mentioned are inadequate incorporation of the product, imperfect combinations of host/AMF species, and that the commercial strains are not adapted to the typical environmental conditions and the microbial flora in Scandinavian golf greens. There are several AMF inocula available on the market which have shown to be ineffective, in that they lead to poor root colonization or have insignificant effects on turfgrass germination and establishment when tested in USGA greens (Carey & Gunn 2000; Tarbell & Koske 2007). In addition to the explanations above, the ability of an inoculum to colonize plant roots are also determined by the abundance of infective propagules in the product (e.g. spores, hyphae, colonized root pieces) (Tarbell & Koske 2007). Intraradical vesicles have

previously been found to increase inoculum potentials because they showed high infectivity both when inoculated in the form of VAM-colonized root pieces, and when they were inoculated separate from the colonized roots (Biermann & Linderman 1983). However, intraradical vesicles are easy to confuse with spores, and the infectiveness of these structures is thus questionable (INVAM 2013). If an AMF inoculum contains vesicles that are actually non-infective, this will affect the ability of the product to efficiently colonize the plants of interest. Moreover, the viability of the propagules and the amount applied also affects colonization rates. This means that a high propagule density still can lead to low or total absence of colonization if the propagules are dead or dormant, for example due to the processing they have undergone during manufacturing of the product. Inocula may also have a lower propagule density than claimed by the manufacturer, and will thus not be efficient unless the inoculum is applied at higher product rates than recommended. Furthermore, type of growth medium may affect the rate or extent of which some AMF colonize their hosts (Tarbell & Koske 2007). Root zones amended with special types of peat have been found to reduce both the extent and effects of AM colonization (Linderman & Davis 2003), while in this thesis, it was shown that amendment with compost increased the colonization rates, and possibly also the advantage of, natural AMF populations in a green that had not been inoculated. A combination of organic biostimulants and AMF inoculation have further shown to be more beneficial than inoculation alone, probably because the biostimulants offer some of the same advantages to the plant as AMF does, while stimulating hyphal growth and colonization at the same time (Koske & Gemma 2005). Finally, timing of inoculation may also affect the result of such treatments. Hetrick et al. (1991) found that both cool – and warm season grasses had the highest mycorrhizal activity late in their respective growth season, and that mycorrhizal dependence must be greatest at the temperature that favors growth in the host.

Greens always get colonized eventually. Is it worth the wait?

Because much knowledge is still lacking about what are the positive and negative characteristics of the different AMF species, how they interact with different hosts under varying environmental and ecological conditions as well as which AMF species combinations are the most optimal, the importance of inoculating greens to increase the rate of turfgrass establishment and improving their quality has recently been questioned. AMF are found in almost all soils, and colonization of new roots is usually rapid. However, sand/peat media or other growth media that are low in microbial life are usually lacking AMF, and colonization of newly constructed USGA greens occurs when nearby AMF populations are spreading from native soils at the margins of the green (Koske et al. 1997b). AMF spores are poorly dispersed due to their large size and underground placement, and lateral spread of hyphae is very slow. Thus; natural establishment of AMF communities in greens takes time, and likewise, a colonization lag phase is often observed at first when inoculating AMF, if the product has a low propagule count (Koske et al. 1995; Koske et al. 1997b; Tarbell & Koske 2007).

Factors that often accelerate the natural incorporation of AMF in greens are management and other human activities, flooding, wind, soil invertebrates, birds and other factors that may carry soil containing fungal propagules from adjacent areas and onto the green (Koske et al. 1997b). Unfortunately, development of healthy AMF populations still occur at a realtively slow rate, but once the fungi are properly established, colonization levels may be just as high as in any inoculated green or other managed, cultivated or natural site (Koske et al. 1995; Koske et al. 1997b). This was confirmed through the field studies of this thesis, where the uninoculated green (study 2) did not show significant AM colonization until a year after sowing. The remarkable increase in AMF abundance from June to October in 2012 was very surprising. Person effects may have slightly affected the results because the first two samplings from this green were analyzed by a different person, but the colonization rate must still have been significantly higher these months. This is in line with the findings of Hetrick et al. (1991), that mycorrhizal dependence are greatest in the late growing season of respective hosts. By October 2012, the colonization levels in study 2 were almost as high as those for the one year older inoculated green in study 1. However, this was only true for plots that had a root zone amended with Green Mix, suggesting that the establishment of AMF in newly constructed greens can be accelerated by incorporating organic materials with certain characteristics into the growth medium.

Johnson (1998) found that increasing soil organic matter and avoiding heavy fertilization could enhance AM colonization of a late successional grass. The researcher further suggested that measures favouring mycotrophy could be more cost-effective than inoculating the soil with AMF during reclamation operations. There is no doubt that incorporation of AMF inocula during green construction is necessary to achieve early benefits (Gemma et al. 1997a; Gemma et al. 1997b; Hartin et al. 2005), but regarding the aforementioned challenges concerning maximal utilization of AM symbiosis through commercial products and the fact that greens always get colonized eventually, not inoculating may be more profitable in the longer term. Inoculation may furthermore reduce plant growth if application rates are too high and the AMF species involved have a high C-demand, so at least until more efficient inocula that are customized to favor plant species of interest under defined conditions are developed, together with optimal inoculation rates ensuring good plant response, natural AMF populations may benefit greens the most when they eventually establish (Koske et al. 1995; Pelletier & Dionne 2004). However, natural AM colonization in greens is also a challenge, because mycorrhizal benefits may be lost or replaced by deleterious effects with too low or too excessive fertilization (Koske et al. 1997b). Green management in general is typically obscuring the stable and balanced host/symbiont interactions that occur in a natural ecosystem, because the diversity of the AMF community is reduced through selection for aggressive and less mutualistic species that have a high demand for C. (Johnson 1993). Thus, it is important to develop management regimes that favor desirable AM interactions, as well as plant growth, also when the green is not inoculated.

5 Conclusions

The work of this thesis has shown that turfgrasses indeed form significant AM colonization, as opposed to some previous beliefs (Gange et al. 1999b). However, these grasses are probably less dependend on the symbiotic associations than many other mycorrhizal forming species, due to both physiological reasons and the special environmental conditions that they get exposed to when cultivated in greens (Graham & Eissenstat 1994; Koske et al. 1997b). The physiology of the hosts and their fungal symbionts, ecological conditions and management factors are all involved in determining the prevalence and identity of AM colonization, and whether the interactions are beneficial, neutral or negative to the plants (Smith & Smith 2011). On the basis of the results from the studies of this thesis, direct uptake of available N is probably the main controller of processes related to plant growth and quality as well as symbiosis development in greens, but an N/P interaction seems to be essential to whether fertilization or altered nutrient availability may reduce the extent of colonization (Blanke et al. 2011; Johnson et al. 2003). This deserves more attention in the future because previous research has primarily been focused on the familiar problem of P limitation, and how the availability of this element controls colonization development as well as mycorrhizal growth responses in plants. Elevated P levels may, indeed, lead to reduced colonization rates, but the reduction is only significant if the soil contains ample N as well (Sylvia & Neal 1990), making the symbiosis truly unprofitable. Likewise; high N availability may also suppress colonization, but only if the soil has sufficient P (Corkidi et al. 2002). However, it is important to also remember that colonization levels are not always related to the outcome of AM symbioses (Smith & Smith 2011).

Young greens are usually scarce of microbial life, as this thesis also confirmed, and inoculating with AMF during green construction may increase turfgrass establishment rates through enhanced plant quality and fitness (Gemma et al. 1997a; Hartin et al. 2005). Inoculation also has the potential to control the extent of annual bluegrass, because this is a water – and nutrient demanding species that is less mycorrhizal than the bentgrasses (Gange et al. 1999a; Vargas Jr & Turgeon 2004), as was also found in this thesis. The species may also be subject to antagonistic effects in symbiosis with certain AMF species that do not affect other turfgrasses in the same way (Gange 1998). However, the AMF inoculum that was testet in this thesis did not show any significant effects on either AM colonization levels, turfgrass quality or competition, illustrating the challenge of producing efficient commercial products (Tarbell & Koske 2007). In addition to developing better manufacturing methods, many questions need to be solved about what are the positive and negative characteristics of different AMF species, how they interact with different hosts under varying environmental and ecological conditions as well as which fungal species combinations are the most optimal. When the mechanisms underlying the complex interactions that occur between plants, fungal symbionts and their respective environments are solved, specially designed inocula and management techniques can be created that favors the plant species of interest under defined conditions, while reducing the large

amounts of energy, fertilizer and other chemicals that are usually investied in daily maintenance practices on greens (Koske et al. 1995). Manipulating plants to make nutrient uptake through the direct and the mycorrhizal pathyway additive could also be possible in the future (Smith & Smith 2011), and extended field trials and molecular work are both major tools along the way towards these goals. However, until better products are available on the market, implementing measures that accelerate establishment of natural AMF populations may be more cost-effective than inoculation (Johnson 1998).

Amendment with composted garden waste instead of peat in sand-based root zones seems to be a good way of enhancing natural AM colonization rates, according to results from this thesis, but excessive fertilization and other intence maintenance practices that are typically performed on greens may neutralize or turn the positive effects of AM symbioses into something that is harmful to the plant. This is because the plants are allocating less photosynthate to root growth and colonization development, which in turn selects for aggressive and less mutualistic species that are able to colonize hosts to gain sufficient C without returning equal amounts of nutrients (Johnson 1993). The AMF communities of the greens investigated in this thesis appeared to be dominated by *Scutellospora,* and *Glomus* was also very abundant. None of these naturally colonizing fungi seemed to have noticeable effects on the turfgrasses. Future studies should investigate which management regimes are optimal in favouring desirable AM interactions and plant growth at the same time, both for inoculated and uninoculated greens (Johnson 1993). Only then can utilization of the ancient mycorrhizal associations be considered as a good and sustainable solution to the many challenges that follows with operating a golf facility. As stated by Becking (1934): "Everything is everywhere, but, the nature selects".

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***Appendix 1**: Seed mixtures used on The Niblick green (study 1), sown on August 12th 2010:*

Pure red fescue (special mixture, Niblick)

- 39.6 % 'Cezanne'
- 19.8 % 'Calliope'
- 19.8 % 'Bargreen'
- 19.8 % 'Musica'
  = 99 % red fescue

- 1 % annual bluegrass (unspecified)

Red fescue + velvet bentgrass

- 36 % 'Cezanne'
- 18 % 'Calliope'
- 18 % 'Bargreen'
- 18 % 'Musica'
  = 90 % red fescue

- 1 % annual bluegrass (unspecified)
- 9 % velvet bentgrass ('Villa')

Red fescue + colonnial bentgrass

- 36 % 'Cezanne'
- 18 % 'Calliope'
- 18 % 'Bargreen'
- 18 % 'Musica'
  = 90 % red fescue

- 1 % annual bluegrass (unspecified)
- 4.5 % colonnial bentgrass ('Jorvik')
- 4.5 % colonial bentgrass ('Barking')

Amount of each mixture sown per m^2 = 2.5 kg.

***Appendix 2**: Total input of N, P, K and micronutrients (kg/100 m^2) to the Niblick green (study 1) in 2011 and 2012.*

	Fertilizing				**Experimental fertilizing**			
	KCl	**$MgSO_4$**	**Rexolin Ca**	**Rexolin APN**	**NH_4NO_3**			**Phosphoric acid, 85 %**
					0.5 kg N	**1.0 kg N**	**1.5 kg N**	
2011								
N					0.394	0.765	1.148	
P								0.131
K	0.747							
Mg		0.096						
S		0.128						
Ca			0.097					
Fe				0.015				
Mn				0.006				
Cu				0.001				
Zn				0.003				
Mo				0.001				
B				0.003				
Total fertilizer	1.50	1.00	1.00	0.25	1.13	2.19	3.28	0.49
2012								
N					0.500	1.000	1.500	
P								0.180
K	0.975							
Mg		0.120						
S		0.120						
Ca			0.090					
Fe				0.011				
Mn				0.0042				
Cu				0.0004				
Zn				0.0023				
Mo				0.0004				
B				0.0019				
Total fertilizer	1.96	1.24	0.93	0.18	1.49	2.97	4.46	0.70

***Appendix 3**: Total fertilizer inputs and amount of N, P and K given the red fescue green (study 2) at each application date during the experimental season March to October 2012.*

			kg/100m^2			
Week	**Date**	**Fertilizer type**	**Total**	**N**	**P**	**K**
12	mar.20	Arena Calcium	0.25	0.000	0.000	0.000
12	mar.20	Arena Crystal 19-2-15	0.05	0.010	0.001	0.008
13	mar.26	Greenmaster liquid NK 10-0-10	0.15	0.015	0.000	0.012
14	apr.04	Arena Crystal 19-2-15	0.11	0.020	0.002	0.016
15	apr.10	Greenmaster liquid NK 10-0-10	0.25	0.025	0.000	0.021
16	apr.17	Arena Crystal 19-2-15	0.16	0.030	0.003	0.024
17	apr.26	Greenmaster liquid NK 10-0-10	0.35	0.035	0.000	0.029
18	may 02	Arena Crystal 19-2-15	0.21	0.040	0.004	0.032
19	may 08	Greenmaster liquid NK 10-0-10	1.02	0.102	0.000	0.084
20	may 13	Arena Crystal 19-2-15	0.39	0.074	0.007	0.059
21	may 22	Greenmaster liquid NK 10-0-10	0.83	0.083	0.000	0.068
22	may 30	Greenmaster liquid NK 10-0-10	0.90	0.090	0.000	0.075
23	june 05	Arena Crystal 19-2-15	0.48	0.091	0.009	0.072
24	june 12	Arena Crystal 19-2-15	0.48	0.091	0.009	0.072
25	june 20	Greenmaster liquid NK 10-0-10	0.90	0.090	0.000	0.075
26	june 28	Greenmaster liquid NK 10-0-10	0.70	0.070	0.000	0.058
27	july 04	Arena Crystal 19-2-15	0.40	0.076	0.008	0.060
28	july 10	Arena Crystal 19-2-15	0.32	0.061	0.006	0.048
30	july 24	Greenmaster liquid NK 10-0-10	0.60	0.060	0.000	0.050
32	aug.07	Arena Crystal 19-2-15	0.32	0.061	0.006	0.048
34	aug.23	Greenmaster liquid NK 10-0-10	0.60	0.060	0.000	0.050
36	sep.05	Arena Crystal 19-2-15	0.26	0.049	0.005	0.039
36	sep.05	Arena Calcium	0.25	0.000	0.000	0.000
38	sep.18	Greenmaster liquid NK 10-0-10	0.40	0.040	0.000	0.033
40	oct. 02	Arena Crystal 19-2-15	0.15	0.029	0.003	0.023
42	oct. 17	Greenmaster liquid NK 10-0-10	0.20	0.020	0.000	0.017
44	oct. 31	Arena Crystal 19-2-15	0.05	0.010	0.001	0.008
Sum				**1.332**	**0.064**	**1.081**
Relative rate, N:P:K = 100:12:60 (ideally)				100.0	4.9	81.0