Master Thesis

How Plants and Mycorrhizal Fungi Contribute to Soil Aggregate Stability

Anja Eva Bader

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Supervised by

Referee: Dr. Thomas Niklaus Sieber
ETH Zurich Institute of Integrative Biology, IBZ

Co-Referee: Dr. Frank Graf
WSL Institute for Snow and Avalanche Research, SLF

Contents

Summary

Bioengineering measures to improve soil stability in landslide areas are widely investigated and have been improved over the years. But the measures are complex and it is a major challenge to perform adequate assessments of the measures. Besides technical measures, biological measures are also applied to protect the slopes against landslides. The main goal is to increase soil aggregate stability with a sustainable vegetation cover. Therefore, a knowledge about the soil and the vegetation is essential. In particular, mycorrhizal plant-fungus associations play a crucial role within bioengineering measures. Mycorrhizal fungi may increase plant growth which consequently influences soil aggregate stability. This is the research field of this Master's thesis.

The thesis is embedded in a research project of the WSL Institute for Snow and Avalanche Research (SLF) under the National Research Program (NRP) "Sustainable use of soil as a resource" and aims to quantify the effects of plants and mycorrhizal fungi on soil aggregate stability. Therefore, a pot experiment was designed using two different plant species. The soil material originated from the landslide area Schwandruebi which is situated in the municipality Dallenwil in the Swiss canton Nidwalden. The tree species *Alnus incana* (A) and the grass species *Poa pratensis* (P) were considered separately and in combination (AP), either inoculated with mycorrhizal fungi (M) or non-inoculated. Additionally, two control treatments inoculated (KM) or not (K) were added to the experiment. For each of the eight treatments 13 replicates were produced, resulting in a total number of 104 samples. After a growing period of 17 weeks, wet-sieving and soil aggregate stability tests were performed. During the wet-sieving, the pore water pressure was continuously measured using a high suction tensiometer. The soil was further analysed by an Ergosterol liquid phase extraction. After the staining of mycorrhized roots, the degree of mycorrhization was determined using a modified gridline intersect method. Further, all root systems were cleared of soil particles and the root length was measured with the root length scanning software WinRhizo. Statistical analysis was performed using the Kruskal-Wallis and pairwise Wilcoxon rank sum tests.

Obtained results of the tensiometer measurements showed in general a lower initial pore water pressure and a steeper curve during the wetting in mycorrhized treatments compared to non-mycorrhized treatments. With respect to the soil aggregate stability, planted treatments revealed significantly higher stabilities than the control treatments. Additionally, a significant negative effect of mycorrhized planted treatments on soil aggregate stability was observed compared to the corresponding non-mycorrhized treatments. Regarding the Ergosterol liquid phase extraction, no Ergosterol could be measured. Further, ectomycorrhizas were present on the roots of *Alnus incana* but no arbuscular mycorrhizas could be identified in the root systems of *Poa pratensis*. Considering the root analysis, treatments of *Alnus incana* (A, AM) yielded a significantly lower root length per soil volume compared to the treatments

of *Poa pratensis* (P, PM) and the combined treatments (AP, APM). Additionally, the root lengths of all mycorrhized treatments were lower compared to the corresponding non-mycorrhized treatments. Similar findings were observed with respect to the root dry weight. In contrast, aboveground plant dry weight resulted in significantly higher values in mycorrhized treatments which included *Alnus incana* compared to the corresponding non-mycorrhized treatments. The total amount of biomass was affected in the same way but differences were not significant in most cases.

In conclusion, soil aggregate stability was significantly higher in planted treatments compared to the control treatments but mycorrhized treatments did not positively affect the stability. In terms of the total amount of biomass, only mycorrhized treatments which included *Alnus incana* produced more biomass than the corresponding non-mycorrhized treatments. The effects on soil aggregate stability may be attributed to the lower initial water content in mycorrhized treatments compared to non-mycorrhized treatments. Additionally, the high soil dry unit weight might have hampered root growth and disabled a mycorrhization of *Poa pratensis*. Further, the increased aboveground biomass and decreased root growth may be the results of an excessive fertilizer application.

The investigations were slightly limited by the lack of mycorrhization within the treatments of *Poa pratensis*. Therefore, this plant-fungus association is questioned with respect to an application in bioengineering measures. However, the present thesis enhances the knowledge in terms of this new plant-fungus association. Additionally, the thesis makes noteworthy contributions to the understanding of pore water pressure measurements in small soil samples during wet-sieving using a high suction tensiometer. Nevertheless, further research regarding the pore water pressure and the plant-fungus association is strongly recommended. In particular, pore water pressure measurements using soil samples with similar initial water content values may result in meaningful observations.

1 Introduction

1.1 National Research Program 68

The Master's thesis is embedded in a research project of the WSL Institute for Snow and Avalanche Research (SLF) under the National Research Program (NRP) 68 (Graf et al., 2013) "Sustainable use of soil as a resource". The project "Soil stability and natural hazards: from knowledge to action" aims at (1) quantifying biological effects relevant to soil stability, (2) emerge appropriate indicators for superficial soil failure, and (3) developing a concept of sustainable soil use.

The Master's thesis particularly contributes to the first part of the project by providing reliable data and essential information with respect to a sound basis for calculating biological effects on slope stability. Therefore, it is of interest to quantify the effects of plants and mycorrhizal fungi on soil aggregate stability in particular as well as in relation to slope stability in general.

The approach pursued within the scope of the Master's thesis is a direct consequence of previous investigations related to the same topic. In a study of Frei (2009) soil aggregate stability of *Alnus incana* in pot experiments was investigated. Unplanted and differently compacted samples showed a good correlation between soil aggregate stability and dry unit weight. In contrary, soil aggregate stability did not differ considerably between compacted or non-planted soil samples and planted samples with or without mycorrhizas. Burri et al. (2009) analysed the soil aggregate stability over the course of time using space-for-time substitution. Three test sites with different developments were considered including a site with only technical stabilisation (raw soil with sparse pioneer vegetation), a revegetated site where 25 years before soil bioengineering measures were applied (*Alnus incana* and *Salix appendiculata*) and a target climax vegetation represented by a 150 year old fir-spruce-beech forest (*Abieti Fagetum*). The analyses of the natural soil samples included soil aggregate stability tests which were performed by a wet-sieving procedure, total root length measurements by the software WinRhizo© (WinRhizo, 2000), and the determination of morrhization degree (McGonigle et al., 1990; Vierheilig et al., 1998; Brundrett, 2008). A positive correlation was found between soil aggregate stability and the time course with significant differences among the three stages in development. Beglinger (2011) investigated the potential of *Salix appendiculata* combined with different mycorrhizal and endophytic fungi on soil aggregate stability with a laboratory approach. The soil material investigated was from the same landslide area as used for the samples representing the stage prior

to the application of soil bioengineering measures in the investigation of Burri et al. (2009). The highest soil aggregate stability was found in samples of *Salix appendiculata* inoculated with the ectomycorrhizal fungus *Hebeloma crustuliniforme*. Inoculation of *Salix appendiculata* with two endomycorrhizal fungi increased the root biomass but not the soil aggregate stability compared to the non-inoculated treatment. Beglinger (2011) used the same wet-sieving procedure to determine the soil aggregate stability as Frei (2009) and Burri et al. (2009). Additionally, the dry weight of shoot and root biomass was measured as well as the content of ergosterol using High-Performance Liquid Chromatography (HPLC) in order to determine the amount of fungal hyphae. In the study of Graf and Frei (2013), *Alnus incana* seedlings were inoculated with the cultivated ectomycorrhizal fungus *Melanogaster variegatus* s.l. which was also used in the present thesis. The authors found an increasing soil aggregate stability from soil samples without any vegetation cover to mycorrhized soil samples. Further, root length per soil volume revealed higher values in mycorrhized samples compared to non-mycorrhized samples. These findings resulted in a positive correlation between soil aggregate stability and root length per soil volume. Consequently, this plant-fungus association might be used for slope stabilization in eco-engineering projects (Graf and Frei, 2013).
In order to compare the results to these previous studies as well as for compatibility reasons with regard to the other investigations of the NRP 68 project, this Master's thesis analysed an association of two plant species. One tree *Alnus incana* and one grass species *Poa pratensis* were planted singly and in combination either inoculated or not with a commercial inoculum combining ecto- and endomycorrhizal fungi and additional cultivated ectomycorrhizal fungi. The sample preparation as well as the wet-sieving procedure and the soil aggregate stability measurements were conducted as described in Frei (2009) and Burri et al. (2009). Additionally the degree of mycorrhization, the Ergosterol content, the root length and root dry weight as well as the shoot dry weight were determined with slight modifications according to Beglinger (2011). New and important focus was the continuous measurement of the pore water pressure during the wet-sieving procedure of the soil aggregate stability analysis using a high suction tensiometer. Furthermore, this Master's thesis differs from previous studies by using different plant species.

The objective of this Master's thesis is to either confirm or reject the following hypotheses:

1. Planted soil samples mobilise higher aggregate stability compared to bare soil samples.
2. Planted soil samples inoculated with mycorrhizal fungi mobilise higher aggregate stability than non-inoculated samples.
3. Planted soil samples inoculated with mycorrhizal fungi produce a higher amount of biomass than non-inoculated samples.

1.2 Mycorrhiza - Fungus-Root

This paragraph briefly introduces the plant-fungus association, also known as mycorrhiza. Fungi are placed into an own eucaryotic kingdom. They are heterotrophic organisms which require organic carbon for their nutrition. Due to the fact that they can not produce the organic carbon (C) by themselves, they are dependent on an external organic C source. In contrary, plants are autotrophic and produce C via photosynthesis. To profit of this organic C source, fungi often live in a mycorrhizal symbiosis with a host plant (Egli and Brunner, 2011). Most of these symbioses are mutualistic symbiotic partnerships which indicates that both partners benefit from the effects of their association (Smith and Read, 2008). "Mycorrhiza" derives from the Greek words "mukes" which can be translated as fungus and "rhiza" which means root. Therefore a mycorrhiza is a "fungus-root" which means that the plant root is infected by a mycorrhizal fungus (Frank, 1885).

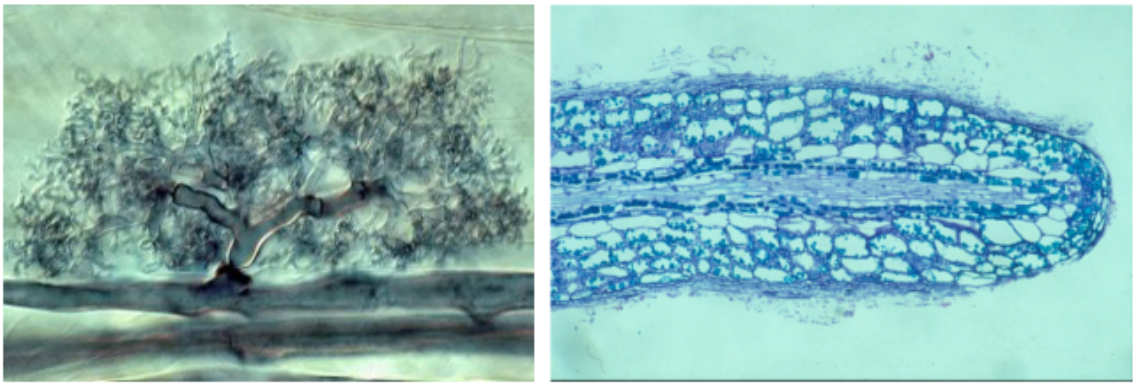


Figure 1.1: Left: Arbuscules of the arbuscular mycorrhizas growing inside a living plant root cell. Photo Brundrett M.: http://mycorrhizas.info/resource.html. Right: Ectomycorrhizal fungal mantle around the root and the hyphal network "Hartig net" between the plant root cells. Photo Graf F. 1995.

Depending on the fungal association seven different kinds of mycorrhizas can be distinguished: Arbuscular, ecto-, ectendo-, arbutoid, monotropoid, ericoid and orchid mycorrhiza. The present thesis focused on arbuscular and ectomycorrhiza. Arbuscular mycorrhizas are aseptated fungi from the fungal taxa Glomeromycota which associate with the plant taxa Bryophyta, Pteridophyta, Gymnospermae and Angiospermae. Arbuscular mycorrhizas infiltrate the living plant cells and form arbuscules and (or) vesicles. Ectomycorrhizas are septated fungi from the taxa Basidiomycota, Ascomycota and some Glomeromycota. Their association is concentrated on Gymnospermae and Angiospermae. Ectomycorrhizal fungi form a fungal mantle around the plant root and also penetrate the plant between the plant root cells with a fungal mycelium network which is called the Hartig net (Figure 1.1). Across the Hartig net the transfer of C and nutrients occurs (Smith and Read, 2008). The plant provides the C produced via photosynthesis

and the fungus in return ensures a more efficient water and nutrient uptake whereas major nutrients are nitrogen (N) and phosphor (P) (Brunner, 2001). The exchange may be intracellular using arbuscules or vesicular structures of arbuscular mycorrhizas or intracellular where the fungal mycelium of the ectomycorrhizas grows between the plant root cells (Smith and Read, 2008). Arbuscular mycorrhizas are found in a species rich environment, whereas ectomycorrhizas are predominant in forest ecosystems. Many mycorrhizas are edible fungi as for example *Boletus*, *Cantharellales* or *Tuber* but there are as well toxic mycorrhizal fungi (Egli and Brunner, 2011). It is likely that one plant root system is colonized by several mycorrhizal fungi. The presence of mycorrhizas influences plant root growth and formation (Smith and Read, 2008; Egli and Brunner, 2011). Root hair formation is suppressed by the mycorrhizal fungus. Instead the fungal mycelium expands into the surrounding soil. Due to the highly dense mycelial network and a hyphal diameter of 2 - 5 μm, the absorption surface is much higher compared to the plant root. Hence, mobilisation and absorbation of water and nutrients is much more effective (Brunner, 2001) compared to plant roots with a diameter between 15 - 20 μm (Graf and Gerber, 1997). Further influences of mycorrhizas are an increased tolerance against toxic metal concentrations or other pollutants in the soil (Leyval et al., 1997) and they may even improve disease resistance (Caron, 1989).

1.3 Landslide Areas Hexenruebi and Schwandruebi

Several studies were conducted in the landslide areas Hexenruebi or Schwandruebi or with the soil material from these areas (Frei, 2009; Burri et al., 2009; Beglinger, 2011; Graf and Frei, 2013). Both landslide areas are situated in a larger catchment of the Flueligraben which is located near the municipality Dallenwil in the canton of Nidwalden (NW) in Switzerland. The soil material of the present thesis originates from within the catchment area of the Flueligragen and more precisely from the upper part of the red circled Schwandruebi area as illustrated in figure 1.2 (Estimated coordinates [m] CH1903+/CV95: 2'671'012, 1'196'649). The area of Hexenruebi is situated around 1120 to 1260 m above sea level and Schwandruebi around 1040 to 1140 m above sea level. Both landslide areas are mainly oriented to North and East.
The corresponding geology can be characterized as a penninic carbonic formation with ultrahelvetic flysch. The soil is a moraine which forms the loose ground. Due to the glaciation this loose ground was highly compacted which resulted in a steep surface. Through weathering, surface erosion and superficial landslides occured several times in the past (Gerber et al., 2009). The loose ground prohibits the formation of a constant vegetation cover which directly links to the present thesis.

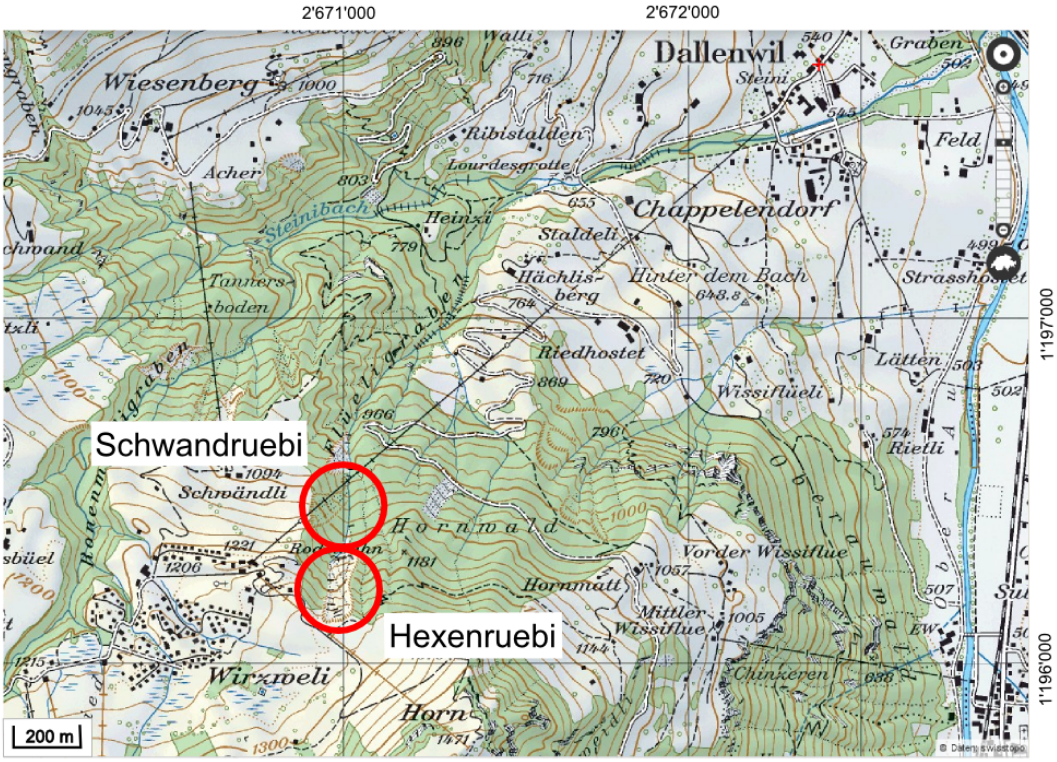


Figure 1.2: This map displays the location of the landslide areas Hexenruebi and Schwandruebi near the municipality Dallenwil in the Swiss canton Nidwalden. Bundesamt fuer Landestopographie geodata ©swisstopo (www.map.geo.admin.ch).

The central research question is which initial plant-fungus combination should be planted to reach a sustainable stabilization of the landslide area. Besides the technical measures, biological measures were applied in both landslide areas using two initial plant species. *Alnus incana* and *Salix purpurea* were planted to conduct the initial planting which has proven to be successful (Figure 1.3). Due to a symbiosis with the actinomycetes *Frankia*, *Alnus incana* has the ability to fix nitrogen. Additionally, numerous mycorrhizal fungi are known to form a symbiosis with *Alnus incana*. No chemical fertilizers were added because they are suspected to have negative influences on bioengineering measures. A chemical fertilization increases the aboveground plant biomass but also decreases root growth which would negatively affect soil aggregate stability (Cernusca, 1986; Graf and Gerber, 1997). Further, chemical fertilizers could encourage non-native and non-competitive plants during the initial growth phase and therefore suppress native ones. Additionally, the short-term excessive nutrient availability might strongly influence soil organisms as for example mycorrhizal fungi which might be suppressed or even eliminated due to these fertilizers. But mycorrhizal symbioses are essential for the survival in a sparse environment. To ensure an effective plant-fungus symbiosis in eco-engineering, it is therefore essential to add suitable organisms. Mycorrhizal inoculum was added in the beginning of the planting which increased the efficiency of nutrient uptake and stimulated root growth (Graf and

Gerber, 1997).
After the initial forest stand consisting of *Alnus incana* and *Salix purpurea*, site-specific tree species according to NaiS ("Nachhaltigkeit und Erfolgskontrolle im Schutzwald") should evolve (Frehner et al., 2005). With respect to the Hexenruebi and Schwandruebi area, the location type is a fir-beech forest of the superior montane zone in transition between 18 w *Adenostylo glabrae Abieti-Fagetum calamagrostietosum variae* ("Typischer Buntreitgras-Tannen-Buchenwald") and 18 v *Adenostylo glabrae Abieti-Fagetum calamagrostietosum variae, with Carex ferruginea* ("Buntreitgras-Tannen-Buchenwald mit Rostsegge") (Gerber et al., 2009; Frehner et al., 2005).

Figure 1.3: Landslide area Hexenruebi near the municipality Dallenwil in the Swiss canton Nidwalden. Photo Graf F. 2009.

1.4 Soil Aggregate Stability

One important focus within the present thesis is on the soil aggregate stability which is briefly introduced in this paragraph. Aggregate formation is a result of soil development (weathering, erosion, aggregation, etc.). A good soil has enough aggregates with a particle size between 1 and 10 mm. Further, a successful germination is dependent on a sufficient amount of macropores (>250 μm) to provide an efficient aeration and micropores (<250 μm) to retain water and nutrient resources (Tisdall and Oades, 1982; Graf and Gerber, 1997). According to Frei (2009) the quantity of pores with a diameter between 0.2 and 60 μm determines the available water resources for the plants. Water is hold in these pores due to capillary action. If the pore diameter is larger, water is drained freely under gravity. Within smaller pore diameters water is

retained by the pores and therefore inaccessible for the plant (Frei, 2009).
According to Tisdall and Oades (1982) the water stability of aggregates is dependent on organic materials. Three different organic binding agents were mentioned by the authors as (a) polysaccharides (b) roots and fungal hyphae and (c) resistant aromatic components in association with metal cations as for example aluminium oxides or iron oxides. Macro aggregates are stabilized by roots and fungal hyphae. Consequently, macro aggregates are controlled by soil management. In contrary, water stability of micro aggregates is dependent on the mentioned organic binding agents which was therefore characterized as soil specific (Tisdall and Oades, 1982). Further, bacteria and mycorrhizal fungi can highly influence soil aggregate stability at the macro aggregate level (Rillig and Mummey, 2006). In the study of Rillig and Mummey (2006) the influence of mycorrhizas on soil aggregation was characterized in three different levels namely by (a) the plant community, (b) the plant root and (c) and the fungal mycelium in the soil. With respect to the plant community (a), the composition of plant communities is affected by mycorrhizal fungi. Net primary production results in carbon deposition as roots or litter in the soil which consequently affects soil aggregate stability. Concerning the plant root level (b), Rillig and Mummey (2006) characterized five processes which might affect soil aggregate stability. Mycorrhizal fungi may change root architecture, increase stomatal conductance and therefore change the soil water regime, influence the release of root exudates (Rhizodeposition) or the decomposition of roots and entangle soil particles. The fungal effects (c) were subdivided by the authors into biochemical, biological and biophysical fungal effects. Biochemical effects may be attributed to Glomalin (a fungal protein which sticks the soil aggregates together) but also the effects of polysaccharides, other extracellular compounds, mucilages, hydrophobins and related proteins are discussed. The mycorrhizal symbiosis can further be affected by the microbial communities in the soil, which were characterised as biological effects. It is for example expected that bacteria and archaea have a high influence on microaggregates. Additionally, fungi might have an effect on microarthropodes due to the soil food web. Fungal hyphae may enmesh or align primary soil particles which were classified as biophysical effects (Rillig and Mummey, 2006).

1.5 Ergosterol: Chemical Compound

The following paragraph briefly introduces Ergosterol to provide a general impression of its characteristics and the occurrence with respect to plants and mycorrhzial fungi. Ergosterol, or Ergosterin, is a chemical compound in the group of the sterols. In most fungal groups sterols were detected as a constant part of the fungal biomass with an amount between 5 and 15 mg g^{-1} (Weete and Gandhi, 1996). In the exposure of UV radiation, Ergosterol forms into vitamin

D_2 and two different sterols (Newell et al., 1988; Beyer and Walter, 1988; De Vries, 2000). Ergosterol does not occur in plants but in the fungal cell membranes (Daood et al., 2008). Therefore it is believed to be a good indicator for living fungal biomass (Wallander and Nylund, 1992; Ekbald and Wallander, 1998). De Vries (2000) assumed that arbuscular mycorrhizas contain a smaller amount of Ergosterol within the fungal tissue compared to ectomycorrhizas and therefore Ergosterol is no biomass indicator to detect arbuscular mycorrhizal fungi (Kjoller and Rosendahl, 2000; Olsson et al., 2003). Althought Frey et al. (1992) could identify Ergosterol in roots which were colonized by arbuscular mycorrhizas several authors suggest that these findings might have resulted because of fungal contamination due to lack of *in vitro* conditions. It is more likely that arbuscular mycorrhizas contain different sterols than Ergosterol (Schuessler et al., 2001; Olsson et al., 2003). Consequently in most previous studies Ergosterol extraction is recommended to determine ectomycorrhizal infections in roots (Salmanowicz and Nylund, 1988; Ekbald and Wallander, 1998) or soil (Ekbald et al., 1995).

Another approach to determine fungal biomass are chitin measurements (Matcham et al., 1985). Chitin is the major component of fungal cell walls (Lezica and Quesada-Allué, 1990). Due to the fact that chitin analyses have the disadvantage of also measuring the dead fungal material (Wallander and Nylund, 1992) Ekbald and Wallander (1998) suggest, that it should be an indicator for total fungal biomass - active or not.

By comparing Ergosterol and chitin measurements Seitz et al. (1979) concluded that Ergosterol analyses were more sensitive, faster and easier than chitin analyses. Nevertheless, Ekbald and Wallander (1998) suggest a combination of both analyses to estimate the ratio of the living fungal biomass compared to the total amount of fungal biomass.

2 Material and Methods

2.1 Experimental Design

The treatments consisted of the two-levelled factor A (Mycorrhiza) and the four-levelled factor B (Vegetation) resulting in eight treatment combinations as presented in table 2.1.

Table 2.1: Treatments of the experimental design consisted of the two-levelled factor A (Mycorrhiza) and the four-levelled factor B (Vegetation) resulting in eight treatment combinations.

B (Vegetation)	**A1 (Mycorrhized)**	**A2 (Non-mycorrhized)**
B1 (*Alnus incana*)	A1 B1	A2 B1
B2 (*Poa pratensis*)	A1 B2	A2 B2
B3 (*Alnus incana* and *Poa pratensis*)	A1 B3	A2 B3
B4 (Unplanted control)	A1 B4	A2 B4

The experimental design was based on Graf and Frei (2013). Modifications were conducted with respect to an additional plant species (*Poa pratensis*) and therefore more treatment combinations. The two plant species *Alnus incana* and *Poa pratensis* were considered separately and in combination, either inoculated with mycorrhizal fungi or non-inoculated. For each of the eight treatments 13 replicates were produced, resulting in a total number of 104 samples.

2.2 Soil Analysis

Bare soil samples without vegetation were collected from the larger catchment Flueligraben (Burri et al., 2009). Due to the fact, that the landslide areas Hexenruebi and Schwandruebi are situated in this larger catchment, soil data and classification of Schwandruebi in the studies of Frei (2009) and Graf and Frei (2013) can be equaled to Hexenruebi soil. After collection, the soil was autoclaved once (20 minutes at 121 $^{\circ}$C) for the purpose of sterilizing and sieved to a grain size less than 10 mm (Beglinger, 2011). The exact soil classification, including the coarse

grains up to 63 mm, was performed by Frei (2009) and Graf and Frei (2013) (Figure 3.1). It must be noted, that further soil classification was conducted using the soil with a grain fraction smaller than 10 mm. To classify the soil, the Unified Soil Classification System (USCS) was used (ASTM D2487-06, 2006). The coefficient of curvature (C_c) and the coefficient of uniformity (C_u) were calculated according to the formulas 2.1 and 2.2. The values of the particle size diameters D_{10}, D_{30} and D_{60} were identified in the figure of the grain size distribution curve (Figure 3.2), which was performed according to ASTM D 422-63 (1998).

$$C_u = \frac{D_{60}}{D_{10}} \tag{2.1}$$

$$C_c = \frac{(D_{30})^2}{D_{10} \cdot D_{60}} \tag{2.2}$$

Further soil tests were conducted concerning the liquid limit, plastic limit, also called "Atterberg Limits", and plasticity index using ASTM D 4318-00 (2000). The liquid limit (LL) is defined as a specific water content of a soil, which produces a standard groove closure of half an inch within 25 blows using the Casagrande cup. Therefore a specific soil sample was mixed with water and filled into the round-bottomed Casagrande cup. A groove was then cut into the soil with a spatula. Following this, the crank was rotated, which lifted the cup up and struck it down as many times until the groove closure was half an inch wide (Bowles, 1992). Several soil experiments were conducted, in which the blow count (N) ranged from 13 to 30. The mass of each moist sample was determined and then oven dried (24 h at 105 ° C). To calculate the water content, the dryed soil sample was weighed again (Table 3.2). The blow count was then plotted on a semilogarithmic scale versus the water content and the best fitting curve was added. At the crossing point of the curve and the abscissa at 25 blows, the water content of the corresponding ordinate axis equaled the liquid limit (Figure 3.3). The plastic limit (PL) was identified by rolling down a soil thread to a diameter of 3 mm. At a specific water content the soil just crumbled when rolled it to this diameter, which is defined as plastic limit (Bowles, 1992). After rolling the soil thread was weighed, oven dried and weighed again to determine the average water content which was equal to the plastic limit (Table 3.3). The plasticity index (PI) is the difference between the liquid limit and the plastic limit calculated according to the following formula 2.3 (Bowles, 1992).

$$PI = LL - PL \tag{2.3}$$

2.3 Plants and Fungi

The seeds of *Alnus incana* (L.) Moench were of regional provenance and obtained from the WSL-nursery. The collection took place in Wettswil/Fischbach in the canton of Zurich at 500-530 m above sea level in 2010. *Alnus incana* is from the family of the Betulaceae and grows from the foothill zone to the montane (-subalpine) zone (Lauber and Wagner, 1996). Seeds of *Poa pratensis* L. were provided by the alpine garden center "Schutz Filisur" (Schutz Filisur; Alpin Gartencenter, 2014). *Poa pratensis* is from the family of the Poaceae and best adapted to a temperature range between 15-24 ° C (Baker and Jung, 1968; Antunes et al., 2011). It grows from the foothill zone to the subalpine (-alpine) zone (Lauber and Wagner, 1996). Ectomycorrhizal fungi *Melanogaster variegatus s.l.* (Ainsworth, 2005) and *Paxillus involutus*, derived from the WSL mycothec, were cultivated on Melin Norkrans (MMN) agar plates (Marx and Bryan, 1975).

2.4 Sample Preparation

The soil samples were prepared on the 4th of July 2013 following the procedure of Frei (2009). First, a PVC-plastic tube (diameter: 70 mm; height: 140 mm) was placed on a petri dish and a filter paper. To identify the types of treatment, PVC tubes were labelled with colored stickers (*Alnus incana* (A) - green; *Poa pratensis* (P) - yellow; *Alnus incana* and *Poa pratensis* (AP) - blue; Control group (K) - pink; Mycorrhized samples (M) - orange). A plastic film was placed at the inner surface of the PVC tube in order to facilitate the removal of the specimens after the growing period for further processing. In a next step the tare weight was determined and, subsequent, the autoclaved soil (20 minutes at 121 ° C) with a grain size less than 10 mm was filled and tamped into the plastic tube. Further preparation varied between mycorrhized and non-mycorrhized treatments as described in the following paragraphs 2.4.1 and 2.4.2.

2.4.1 Mycorrhized Treatments

The sample preparation of the mycorrhized *Alnus incana* (AM) treatment was based on Graf and Frei (2013). After filling the Hexenruebi soil into the plastic tube, a 40 ml volume of the commercial inoculum "Forst" (INOQ Forst, 2013) was applied (Appendix II.I). In a next step, ten inoculum cubes (side length: ca. 5 mm) from Melin Norkrans (MMN) agar plates (Marx and Bryan, 1975) of the cultivated ectomycorrhizal fungi *Paxillus involutus* (37.08; WSL mycothec) and *Melanogaster variegatus s.l.* (Graf and Frei, 2013), were added and covered with an additional soil layer. On top, seeds of *Alnus incana* (ca. 15-20 seeds) were applied.

Subsequently, seeds were covered with a 15-17 ml volume of sieved soil from less than 1 mm grain size (Figure 2.1). Finally a plastic tube (length: 50 mm; diameter: 6 mm) with a wooden stake (length: 60 mm; diameter: 5 mm) was placed in the centre of the cylinder as a tensiometer placeholder (Figure 2.2). To determine the net weight, the soil sample was then weighed again (Appendix I.I). As a final step, a 10 ml volume of liquid fertilizer (Maag Wuxal, NPK 10107.5: 2 ml l^{-1}) was added to each specimen.

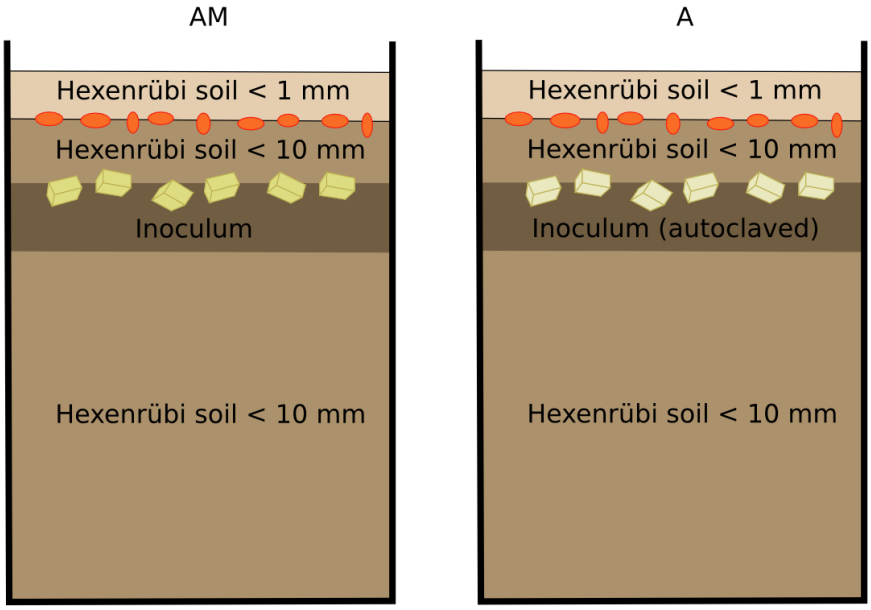


Figure 2.1: Illustration of the sample preparation. Left: Mycorrhized treatment of *Alnus incana* (AM); Right: Non-mycorrhized treatment of *Alnus incana* (A). The cubes are representing the MMN agar cubes with or without fungal inoculum (Marx and Bryan, 1975), the red oval shapes are displaying the seeds of *Alnus incana*. Drawing Bader A. 2014 based on Beglinger (2011).

Figure 2.2: Sample preparation of the mycorrhized treatment of *Alnus incana* (AM) with the commercial inoculum "Forst", the cultivated inoculum on MMN agar cubes (Marx and Bryan, 1975), the seeds of *Alnus incana* and the soil layer (grain size < 1 mm). Photos Bader A. 2013.

The treatments of *Poa pratensis* with inoculum (PM) were prepared slightly different compared to the AM treatments. A 40 ml volume of the commercial inoculum "Forst" was applied

and ten pure MMN agar cubes (Marx and Bryan, 1975). Seeds of *Poa pratensis* were sown 13 days after the seed dispersal of *Alnus incana*. The combined mycorrhized treatment of *Alnus incana* and *Poa pratensis* (APM) was prepared according to the AM treatment with seed dispersal of *Poa pratensis* following the PM treatment. The soil of the control treatment with mycorrhizal inoculum (KM) was prepared similar to the PM treatment without adding any seeds.

2.4.2 Non-mycorrhized Treatments

To ensure consistency, the non-mycorrhized treatments (A, P, AP and K) were prepared slightly different compared to the corresponding mycorrhized treatments. Autoclaved inoculum "Forst" (20 minutes at 121 ° C) was added to all treatments as well as ten pure MMN agar cubes (side length: ca. 5 mm) (Marx and Bryan, 1975).

2.5 Growing Period

The eight treatments were arranged side by side in the greenhouse. Air humidity was controlled by an automated vaporizer, temperature varied between 15 and 25 ° C. For every treatment scheme the arrangement of the samples was completely randomized. Eight days after the sample preparation and arrangement in the greenhouse, first germinations of *Alnus incana* were observed. Five days later, seeds of *Poa pratensis* were added to the treatments P, PM, AP and APM. In order to increase the germination process, 5 ml of liquid fertilizer were applied to all samples. The germination of *Alnus incana* was not successful in every sample. Some seedlings (e.g. in the sample AM10) were brown or already dead. Therefore, seedlings from other samples of the same treatment, where the germination was considered satisfactory, were transferred.
Germination of *Poa pratensis* started after three weeks. At the same time seedlings of *Alnus incana* were first reduced to a number of six individuals to enable a transfer where germination was not successful. The thinning was conducted with a pair of tweezers. To avoid contamination, the tweezers were sterilized with Ethanol (EtOH 60%) and flamed after each sample. A second thinning took place four weeks after the sample preparation and continued weekly until the final numbers of five individuals of *Poa pratensis* and three individuals of *Alnus incana* were stable following the methodology of Graf et al. (2009). Six and nine weeks after the sample preparation a 10 ml and 5 ml volume of liquid fertilizer was added to the samples, respectively. All 104 samples remained in the greenhouse for ten weeks. Due to decreasing temperature in the greenhouse, all samples were transferred into a climate chamber (Date: 12th of September 2013) with a duration of the day from 05:00 to 20:00 hrs, a constant air humidity between 55 and 70 %, a temperature of 24 ° by day and 17 ° by night. After transferring, some *Alnus incana*

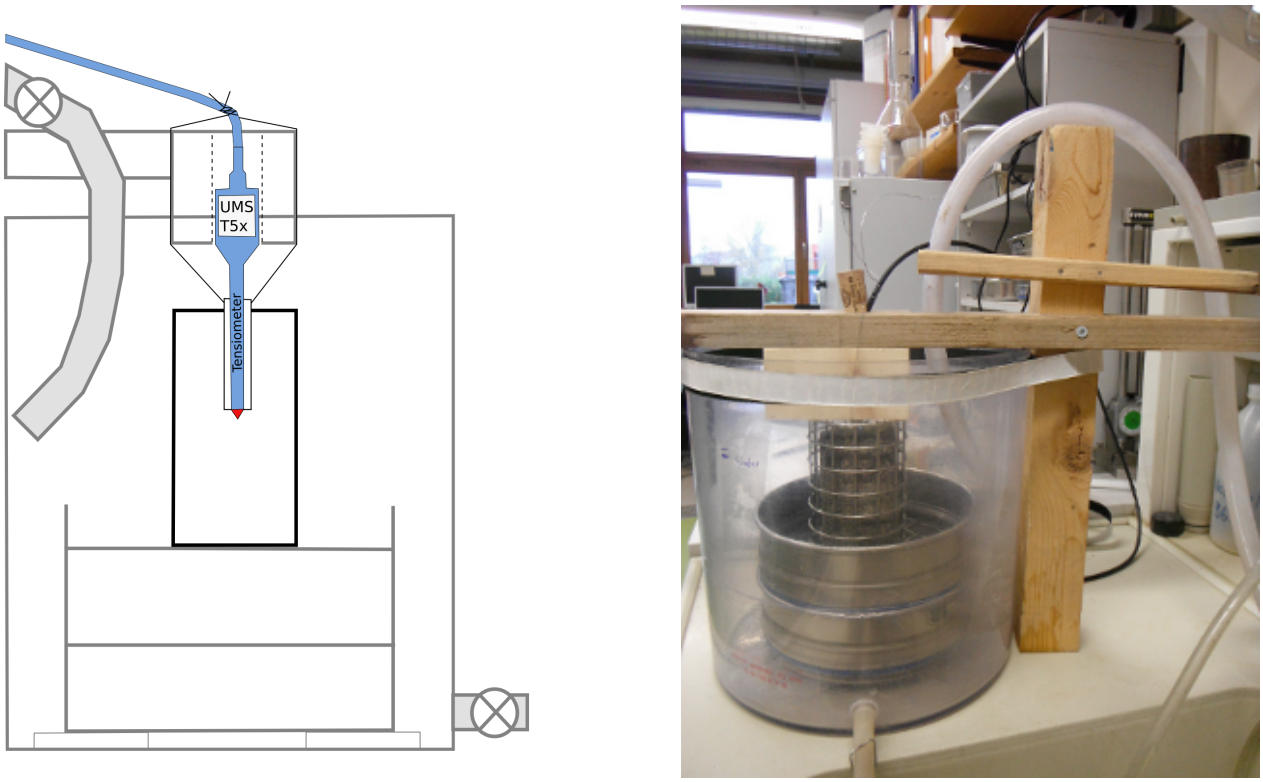
seedlings of the combined treatment of *Alnus incana* and *Poa pratensis* (AP) died (Figure 3.23). All samples remained in the climate chamber for another seven weeks, which resulted in a total growing period of 17 weeks. To increase the soil aggregate stability, the samples were not watered for five to seven days before the wet-sieving procedure (Beglinger, 2011).

2.6 Wet-Sieving

2.6.1 Test Setup

The wet-sieving test setup was based on Frei (2009) and Beglinger (2011) with some adjustments due to the additional pore water pressure measurements by a Tensiometer. Two mesh wire sieves with a mesh opening of 20 mm (Prüfsieb; W 20; Norm: DIN 4188; Rohrer-Technik CH-8706 Feldmeilen) on top and a mesh opening of 10 mm (Prüfsieb; W 10; Norm: DIN 4188; Rohrer-Technik CH-8706 Feldmeilen) at the bottom were placed on three petri dishes, spaced evenly next to each other to provide an adequate spacing, in a Plexiglas© pot (diameter: 300 mm; height: 300 mm). A wooden construction was attached with a hook-and-loop tape to the pot. The drain valve and the tensiometer T5x (National Instruments, 2009) were fixed on the wooden construction as illustrated in figure 2.3.
Before starting the wet-sieving procedure, the software packages LabVIEW Run-Time Engine 2011 and NI ELVISmx 4.5 2008 which includes NI-DAQmx 9.7.5© (National Instruments, 2009) were installed on a Windows 7 computer. Tensiometer data acquisition was carried out using the application software LogAI produced by Andreas Moser from SLF electronics division. After installation, the tensiometer was connected with the NI myDAQ© driver device (National Instruments, 2009) and 10.6 voltage was applied. To check the offset the tensiometer can be placed into a cup which is filled with deionized water up to the center of the sensor body (Appendix III.I; Figure 6.2). After the voltage record is stable the pore water pressure measurements should be between +3.0 and -3.0 kPa (National Instruments, 2009) which equals +30.0 and -30.0 hPa. Voltage data (V) was recorded in a textfile at intervals of one second and converted into hectopascals (hPa) according to the formula 2.4. A vertical installation of the tensiometer resulted in the largest deviation of the pore water pressure (National Instruments, 2009). Therefore the offset value is shifted to -5.0 hPa for the 5 cm long shaft of the tensiometer (Appendix III.I).

$$y(x)[hPa] = \frac{10'000[hPa]}{[V]} \cdot x[V] - 5[hPa] \tag{2.4}$$



(a) (b)

Figure 2.3: (a): Schematic illustration of the test setup. Drawing Bader A. 2014.
(b): Test setup at WSL Birmensdorf. Photo Bader A. 2013.

2.6.2 Test Procedure

The wet-sieving test procedure was based on Frei (2009) and Beglinger (2011).The selection of the sample was conducted considering the sample number not the treatment number according to Beglinger (2011), beginning with the number 13. Before starting the wet-sieving procedure, the subsequent parameters of each soil sample were accurately documented. After the removal of the aboveground biomass, the gross weight of the sample was determined with a Mettler PM 34 balance. Once the PVC plastic tube and the transparency film were removed, the net weight (m_0) and height (h_0) were measured. The volume (V_0), gravimetric water content (w_0) (Fredlund and Xing, 1994), bulk (γ) and dry unit weight (γ_d) were calculated using formulas 2.5, 2.6, 2.7 and 2.8 (Frei, 2009), in which m_d represents the oven dried soil after the wet-sieving procedure. The water content is defined as the amount of water within the pores of a soil (Fredlund and Xing, 1994). It has to be noted that most water content values ranged between 0.02 and 0.05 g g^{-1} and only one yielded 0.095 g g^{-1}. Consequently, the latter was considered as an outlier and not used for further analysis.

$$V_0[cm^3] = \left(\frac{d}{2}\right)^2 \cdot \pi \cdot h_0 \tag{2.5}$$

$$w_0[-] = \frac{m_0 - m_d}{m_d} \tag{2.6}$$

$$\gamma[kNm^{-3}] = \frac{m_0 \cdot g}{V_0} \tag{2.7}$$

$$\gamma_d[kNm^{-3}] = \frac{\gamma}{1 + w} \tag{2.8}$$

To stabilize the samples during wet-sieving, the soil was put into a cylindrical mesh wire with a mesh opening of 20 mm (Beglinger, 2011). After putting the sample into the cylinder, two tiny holes were pinched into the central plastic tube and a wire was attached to each hole (Figure 2.3). Additionally, a drop of silt (fraction < 63 μm) was added into the plastic tube to increase the conductivity between the coarse-grained soil and the ceramic cup of the tensiometer which was then placed vertically in the wooden construction and fixed with the wires and a cork. This technique garanteed the precise fitting of the acrylic glass shaft (length: 50 mm) of the tensiometer in the central plastic tube and that the high grade porous ceramic cup was in direct contact with the soil, in order to measure the pore water pressure (Appendix III.I, Figure 6.2). Time measurement started simultaneously with the flooding. Within a mean value of 171 seconds the Plexiglas pot was filled with water until the soil sample was completely flooded. After a flooding time of 5 minutes, the water was drained passing a sieve with a mesh opening of 0.5 mm (Prüfsieb; W 0.5; Norm: DIN 4188; Rohrer-Technik CH-8706 Feldmeilen) within an average time span of 115 seconds. The soil remaining on the upper sieve was transferred separately into a plastic bucket (diameter: 180 mm). For further analyses, the roots were completely cleaned from soil particles and stored in a glass filled with Ethanol (EtOH 50%) according to Beglinger (2011). Additionally, a soil sample for ergosterol assay was taken of each mycorrhized sample (AM, PM, APM and KM), immediately stored in a black box to prevent a conversion of ergosterol and air dried. Subsequent, the soil was rinsed from the upper sieve with a plastic wash bottle, oven dried (24 h at 105 $^\circ$C) and then weighed. The soil part that passed the sieve with a mesh opening of 20 mm was rinsed into another plastic bucket (diameter: 250 mm). To calculate the sedimentation velocity of soil particles, the formula of Stokes was used (Formulas 2.9, 2.10 and 2.11). The specific gravity (G_s) values of sand and silty sand ranged from 2.63 to 2.67 and from 2.67 to 2.70, respectively. As a result, the sedimentation velocity of sand was calculated as 28 seconds, that of silt as 7.5 hours. To facilitate the sedimentation of soil particles with sand and silt fraction, the plastic bucket remained in place for at least four hours. After sedimentation the excess water was sucked-off from the plastic bucket using a water jet vacuum pump (Frei, 2009). The remaining soil was then oven dried and weighed. All data was

recorded in a wet-sieving protocol (Appendix III.III.1, III.III.2, III.III.3).

$$\rho_f = G_s \cdot \rho_W \tag{2.9}$$

$$u[ms^{-1}] = \frac{2 \cdot (\rho_f - \rho_W) \cdot g \cdot r^2}{9 \cdot \eta} \tag{2.10}$$

$$t[s] = \frac{x}{u} \tag{2.11}$$

G_s	[-]	Specific gravity
ρ_f	$[kgm^{-3}]$	Soil density
ρ_W	$[kgm^{-3}]$	Water density
g	$[ms^{-2}]$	Gravity
r	[m]	Radius of soil particle
η	$[kgm^{-1}s^{-1}]$	Dynamic viscosity
t	[s]	Seconds
x	[m]	Water level height

2.6.3 Calculation of Soil Aggregate Stability

After oven drying the soil samples, the soil aggregate stability (stab) was calculated according to the formula 2.12 (Frei, 2009; Beglinger, 2011). The soil remaining on the sieve with a mesh opening of 20 mm (m_{20}) was composed of the oven dried soil on the sieve, including the 2 g air dried soil for the ergosterol analysis in mycorrhized samples and the remaining surplus of the same air dried soil. The total amount of soil (m_d) consisted of m_{20} and the soil that passed the 20 mm sieve.

$$stab[gg^{-1}] = \frac{m_{20}}{m_d} \tag{2.12}$$

2.7 Degree of Mycorrhization

In order to quantify the degree of mycorrhization, the roots of *Alnus incana* and *Poa pratensis* were stained and subsequently analysed using a binocular microscope (WILD M3Z; Art. No: 473849; Heerbrugg, Switzerland) applying a modified gridline intersect method of Brundrett (2008). Prior to the clearing and staining of the roots, they were washed from soil particles and rinsed three times in deionised water. The root systems were then outspread all over a petri dish and a randomized sample of 10 mm x 10 mm was cut out with a scalpel.
In respect of arbuscular mycorrhiza first staining tests of roots of *Poa pratensis* were conducted with an ink and vinegar method according to Vierheilig et al. (1998). The roots were washed, then cleared by boiling them in 10 % Potassium hydroxide (KOH). Following this, the roots were rinsed three times with tap water and stained three minutes in a boiling 5 % ink-vinegar solution (Pelikan 4001; Brillant black). To destain the roots, they were rinsed in tap water and afterwards stored in a vinegar-filled falcon tube. After the staining procedure, all roots of the treatments with *Poa pratensis* were analyzed with a binocular microscope. However, as the quality of the staining was unsatisfactory, an additional staining method using Chlorazol Black E (CBE) was applied in compliance with Brundrett (2008).
To clear the roots and to make them transparent, each root sample was put in an autoclave-resistant glass tube containing a 10 % KOH solution and then autoclaved for 20 minutes at 121 ° C. One autoclave clearing cycle was sufficient to destain the roots of *Poa pratensis*. After destaining, the roots were washed three times in deionised water. To stain the endomycorrhiza, the roots were put in a glass tube containing a CBE dye, which binds to fungal hyphae. The CBE dye compounds lactic acid, glycerol and water were mixed in a ratio of 1:1:1 and then 0.03 % CBE was added according to Brundrett (2008). The staining was carried out for 20 minutes at 121 ° C in the autoclave. The roots were then washed three times in deionised water and stored in a falcon tube with vinegar (acidic acid 45 g l^{-1}). With regard to ectomycorrhiza, roots of *Alnus incana* were stained with CBE, as described in the previous paragraph for *Poa pratensis*.
To determine the degree of mycorrhization, a square of 25 mm x 25 mm on a scale paper with a 1 mm grid was fixed under a petri dish. The stained roots were distributed on this square and analysed with a binocular microscope. The degree of mycorrhization (Myc) was calculated by the fraction of mycorrhized root tips ($Tips_{Myc}$) and the total number of root tips ($Tips_{Total}$) according to the slightly modified formula 2.13 of Frei (2009). In contrast to this method, each root tip was counted in each grid cell and was defined as mycorrhized or not.

$$Myc[\%] = \frac{Tips_{Myc}}{Tips_{Total}} \cdot 100 \tag{2.13}$$

2.8 Ergosterol

2.8.1 Ergosterol Liquid Phase Extraction

After the wet-sieving procedure, a small amount of the soil remaining on the sieve was collected from each mycorrhized sample of the *Alnus incana* (AM), *Poa pratensis* (PM), *Alnus incana* and *Poa pratensis* (APM) and the control treatment (KM) to quantify the fungal biomass by applying an Ergosterol liquid phase extraction. The soil was taken close to the roots, immediately shielded from UV radiation in a carton box, to prevent a reduction of the Ergosterol amount (De Vries, 2000) and air dried during four and a half weeks. Air drying was conducted due to statements of several studies which observed a reduction of Ergosterol after oven drying. A study of Newell et al. (1988) revealed that oven drying of an ergosterol sample led to reduced recoveries between 20 to 80 %. Findings of Beglinger (2011) showed an Ergosterol decrease after oven drying by a factor of five. First ergosterol extraction methods were presented by Salmanowicz and Nylund (1988). The following Ergosterol extraction procedure was based on Daood et al. (2008) and conducted with slight modifications of Dubach (2013). The selection of the sample was conducted considering the sample number beginning at number one. Exact experimental steps are described in the Appendix V.I.

In a first step of the Ergosterol liquid phase extraction, 2 g of the air dried soil was weighed into a plastic dish and 0.2 g of ascorbic acid (L(+)-Ascorbic acid; $C_6H_8O_6$ - M 176.12 g mol^{-1} - density 1.65; Art.-Nr. 3525.2; Carl Roth GmbH + Co. KG, Schoemperlenstr. 3-5, D-76185 Karlsruhe) in a separate dish. After weighing, a mixture consisting of 10 ml methanol (CH_4O), 5 ml ethanol (C_2H_6O) and 2 g of potassium hydroxide (KOH) pellets was filled into a round bottom flask. The oil bath temperature and speed was adjusted to 110 ° C and 1460 rpm, respectively. The soil and ascorbic acid were then added to the methanol-ethanol-KOH mixture. To saponify the sample, the whole bottom flask was heated for 35 minutes in the oil bath (Figure 2.4). Following this, the sample was cooled in ice for 2 minutes, then filtered into an Erlenmeyer flask and poured into a separating funnel. A volume of 20 ml of n-hexane was added. The separation funnel was then gently shaken. After some minutes, two layers had formed. Both layers, n-hexane and the methanol-ethanol-KOH mixture, were collected in a separate glass flask. To bind the water, a small amount of sodium sulfat (Na_2SO_4 - M = 142.04 g mol^{-1}; Cas-No: 7757-82-6; Merck KGaA, 64271 Darmstadt, Germany; EMD Millipore Corporation, 290 Concord Road, Billerica MA 01821, USA.) was added to the n-hexane and stirred until the sodium sulfat was fluffy. The n-hexane including the sodium sulfat flakes was then filtered into a round bottom flask. The mixture was poured back in the separating funnel, 20 ml of n-hexane were added and the same separating procedure was conducted. Finally, the filtered n-hexane liquid was vacuum-dryed at 40 ° C (Buchi Rotavapor; BUCHI Labortechnik; Flawil, Switzerland. Vacuubrand; Wetheim, Germany) until all the n-hexane was evaporated (Figure 2.5).

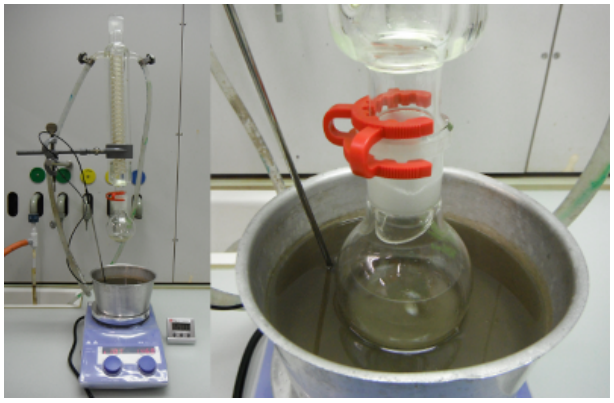


Figure 2.4: Saponification process of the Ergosterol liquid phase extraction. The round bottom flask was heated for 35 minutes in the oil bath adjusted to 110 ° C and a speed of 1460 rpm. Photo Bader A. 2013.

Figure 2.5: Left: Separation funnel with the transparent n-hexane layer on top and the yellow methanol-ethanol-KOH mixture at the bottom. Right: Vacuum dryer. Photo Bader A. 2013.

2.8.2 HPLC Analysis

The high-performance liquid chromatography (HPLC) was conducted according to Dubach (2013). First, a standard curve with an Ergosterol stock solution was produced, to confirm the Ergosterol content and determine the accuracy of the HPLC analysis. Therefore, 100.33 mg of commercial Ergosterol (SIGMA®; Life science; Prod. No.: 45480-10G-F; $\geq$ 95.0 %; SIGMA-ALDRICH Co., 3050 Spruce Street, St. Louis, United States) were mixed with 200 ml of methanol (2 g 100 g^{-1}; 10.04 μg ml^{-1}). A calibration line was designed using Ergosterol stock solution injections with a range from 100.4 to 5020 ng Ergosterol per ml solution, yielding the amount of 67 to 4997 ng Ergosterol per g sample (Appendix V.II).
To redissolve the vacuum dried sample, a 2 ml volume of HPLC grade-methanol was added.

The redissolution process was supported by ultrasonic sound. Following this, 10 μg of the dissolved sample were injected into the HPLC^{TM} UltiMate 3000 apparatus. Hibar® (HR 150-2.1, Purospher Star, RP-18 encapped 3 μm) HPLC columns thermostated to 40 ° C were used to carry out the analyses. The solvent was composed of 97 % methanol and 3 % water. With a flow rate of 0.15 ml min^{-1}, the analysis took 25 minutes for each sample (21.6 h for 52 samples). After analysis, chromatograms were illustrated using the software Chromaeleon 7.0. In the chromatograms the four ergosterol-specific wavelengths (262 nm, 271 nm, 282 nm and 294 nm) could be observed. Due to a high amount of noise, the wavelength at 294 nm was showing the best results. Additionally, Ergosterol peaks were flatter compared to chromatograms with less noise. Therefore, all chromatograms were integrated over the peak area revieling higher retrieval rates. During HPLC analysis, the ergosterol stock solution was injected regularly to record divergences. Ergosterol identification was conducted by overlaying a chromatogram of an Ergoserol stock solution (No. nearby sample No.) and a chromatogram of a soil sample and comparing the peaks (Daood et al., 2008; Dubach, 2013). The detection limit was determined by adding a standard Ergosterol stock solution to a soil sample resulting in 220 ng Ergosterol per g sample.

2.8.3 Determination of Hexane Extraction Losses

To determine the extraction losses during hexane extraction, the mixture consisting of 10 ml methanol (CH_4O), 5 ml ethanol (C_2H_6O), 2 g of potassium hydroxide (KOH) pellets and 0.2 g of ascorbic acid was filled directly into the separation funnel. After this, 1 ml of the Ergosterol stock solution (2 g 100 g^{-1}; 10.04 μg ml^{-1}) was added to the mixture, the separation funnel was gently shaken until the two layers had formed. Further extraction procedure equaled the described steps in the paragraph of the ergosterol liquid phase extraction. It is notable, that the mixture was not heated in the oil bath. Therefore, the Ergosterol losses due to hexane extraction could be determined after HPLC analysis (Table 3.6).

2.8.4 Determination of Liquid Phase Extraction Losses

The quantification of Ergosterol losses during liquid phase extraction was determined based on Dubach (2013). Therefore, 2 g of a non-mycorrhized sample which contained no ergosterol was added to the mixture consisting of 10 ml methanol (CH_4O), 5 ml ethanol (C_2H_6O), 2 g of potassium hydroxide (KOH) pellets and 0.2 g of ascorbic acid with additional 1 ml of the Ergosterol stock solution (2 g 100 g^{-1}; 10.04 μg ml^{-1}). Liquid phase extraction procedure was then conducted and HPLC analysis with an injection volume of 10 μm of the sample. After the HPLC analysis, the recovery values and extraction losses were calculated using the resulted yield of Ergosterol (Table 3.7).

2.9 Root Length Scanning

2.9.1 Sample Preparation

The root length was determined by a root scanning procedure using the image analysis system WinRhizo© (WinRhizo, 2000) and a flatbed EPSON scanner (EPSON PERFECTION V700 PHOTO). Prior to the scanning, the roots of *Alnus incana* and *Poa pratensis* were cleaned from debris and soil particles. After the washing process, the root systems of each sample were separated. The separation of the three *Alnus incana* root systems could be carried out without any problems. In contrary, the separation of the five root systems of *Poa pratensis* was far too complicated. Therefore, the grass roots were subdivided into six parts of the same size (Figure 2.6). To scan the roots, they were placed in a plastic dish filled with outgased water. Following this, the roots were evenly distributed on the plastic dish using a pair of tweezers and a brush.

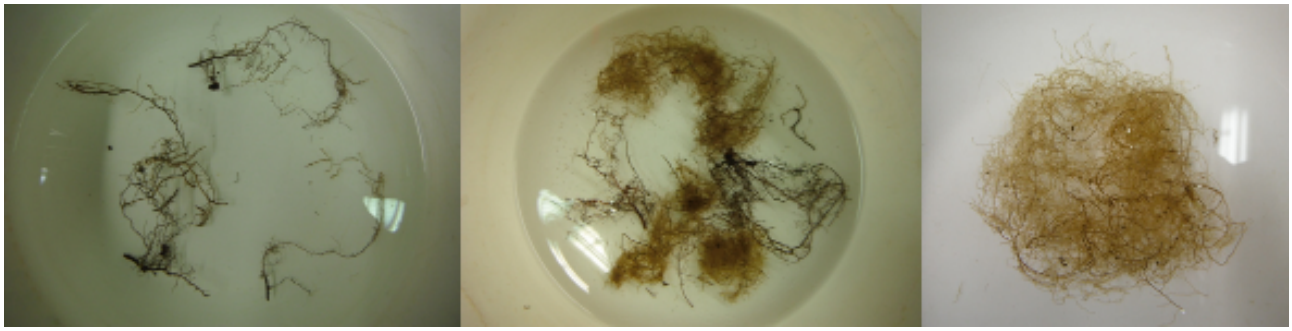


Figure 2.6: Left: Three root systems of *Alnus incana*. Center: Root systems of *Alnus incana* and *Poa pratensis*. Right: Root systems of *Poa pratensis*. Photos Bader A. 2013.

2.9.2 Image Acquisition

After the positioning, the roots were scanned using a filter of 0.04 cm^2 resulting in a digital image. The roots were then identified by colored lines, whereas each color was representing a range of the root diameter as illustrated in figure 2.7. Root length and diameter were determined with Regent's unique method and with Tennant's statistical method (WinRhizo, 2000). Root length density was calculated by the fraction of root length (L_{root}) divided by the soil volume (V_0) according to the formula 2.14 of Frei (2009).

$$Rootdens[cmcm^{-3}] = \frac{L_{root}}{V_0} \tag{2.14}$$

To test the accuracy of the root lenght determination, the roots from one selected sample of each mycorrhized treatment of *Alnus incana* (AM), *Poa pratensis* (PM) and *Alnus incana* and *Poa pratensis* (APM) were measured ten times and distributed slightly different after each measurement.

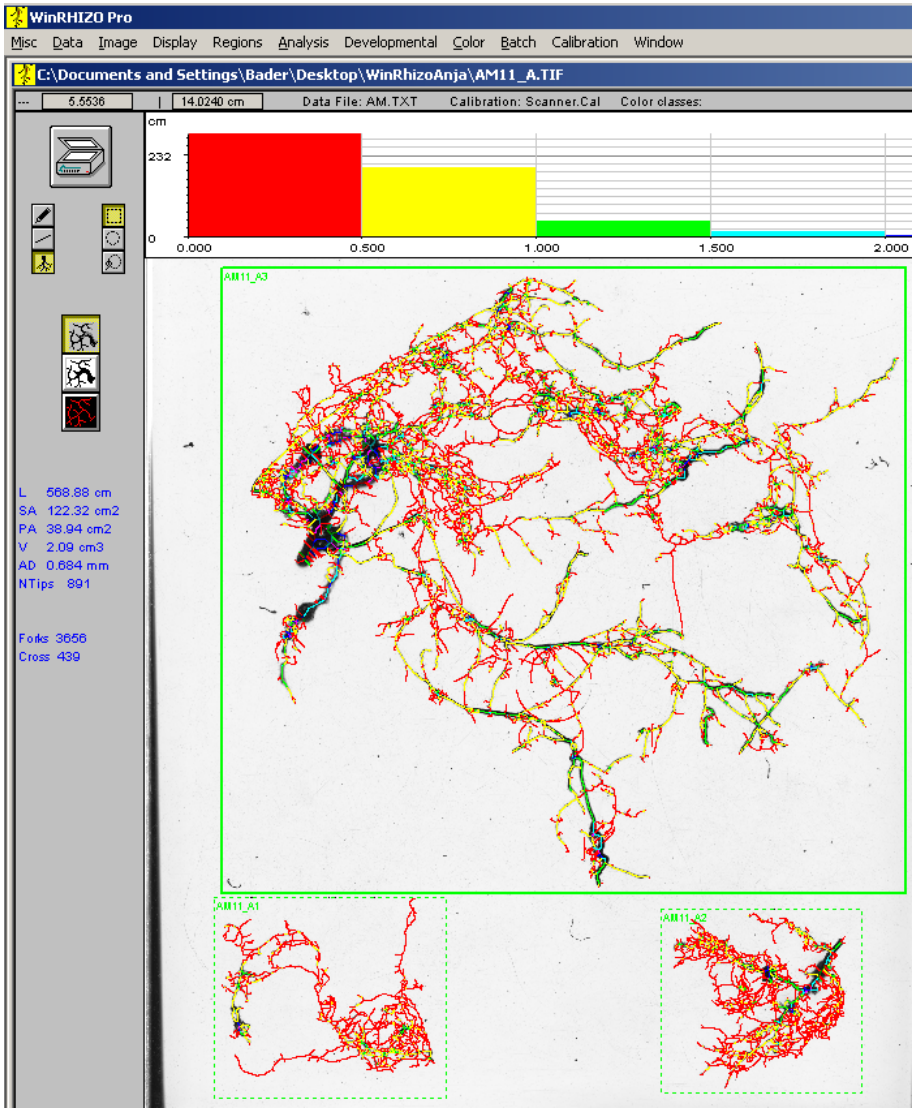


Figure 2.7: Screenshot of the root diameter identification of three *Alnus incana* root systems by WinRhizo. Red: diameter 0-0.5 mm; yellow: diameter 0.5-1 mm; and so on.

2.10 Plant Dry Weight

Prior to the wet-sieving procedure, the aboveground biomass was removed, oven dried (24 h at 105 ° C) and weighed. After root length scanning, the same drying and weighing procedure was conducted with the roots. To weigh the dryed roots a KERN PB balance (Gottl. KERN & Sohn GmbH, D-72458 Albstadt, Germany. KERN: PB400-3. Ser. No.: WP03061) was used. Stained roots used for determining the mycorrhization degree were oven dried likewise and added to the root dry weight. The addition of the aboveground plant dry weight and the root dry weight resulted in the dry weight of the total plant biomass.

2.11 Statistical Analysis

Statistical Analysis and visualizations were conducted using the software R (Version 2.15.2) and the user interface RStudio (RStudio, 2014). All values were rounded to three decimals. To determine if one treatment is significantly different compared to the other treatments, the Kruskal-Wallis rank sum test was used (Hollander and Wolfe, 1973). The pairwise Wilcoxon rank sum test with the p-value adjustment method of Holm (1979) was applied to distinguish the difference among each treatment, where a p-value lower than 0.05 % showed a significant difference. Significant differences were underlined in the tables of the p-values. The corresponding significant classes are illustrated in table 2.3.

Table 2.3: Significance classes of the p-value

Case	**Notation**	**Interpretation**
$P > 0.05$	n.s.	not significant
$0.05 \geq P > 0.01$	*	weakly significant
$0.01 \geq P > 0.001$	**	strongly significant
$0.001 \geq P$	***	very strongly significant

Simple linear regression models were used to compare different parameters. Soil aggregate stability was transformed using the arcsine square-root transformation ($\arcsin(\sqrt{gg^{-1}})$) due to the value range between 0 and 1. Root length per soil volume and plant dry weight were log transformed ($\log_{10}(x)$). Each treatment was considered separately to ensure independency of the individual regressions. The analysis of the covariance (Ancova) was conducted with the lm operator in R. As an example the model of the regression between x and y depending on different groups (A - D) was designed as follows:

model ← lm(y ∼ Groups * x)
summary(model)

The output of the Ancova listed all extimated parameters as illustrated in table 2.4. The first row shows the intercept of the first group (A), the following rows display the differences of the intercept between this group and the first group (B, C, D). The slope is visible in row five (Labelled x). This is the slope of the graph of y against x for group A. Additional rows show the differences between this slope and the slope of the other groups. Parameter values as estimate, standard error, t value and the significantly different parameters are listed in the corresponding columns. Additional, residual standard errors, multiple R-squared, F statistics and p-values are illustrated in the lower part of the table.

Table 2.4: Example of the summary of the linear regression model lm(y ∼ Groups * x). The groups are abbreviated by A, B, C and D.

Coefficients	**Estimate**	**Std. Error**	**t value**	**Pr(>|t|)**
Intercept	-	-	-	-
B	-	-	-	-
C	-	-	-	-
D	-	-	-	-
x	-	-	-	-
A:(x)	-	-	-	-
B:(x)	-	-	-	-
C:(x)	-	-	-	-
D:(x)	-	-	-	-
Residual standard error	-			
Multiple R-squared	-	Adjusted R-squared		-
F-statistic	-		P-value	-

Residual analysis was performed by the Tukey-Anscombe plot, normal QQ-plot, leverages, histograms and box plots. Each treatment was considered singly to neglect a linear dependency between the treatments. If the model revealed a significant Pearson correlation value (p-value <0.05) the linear regression line was plotted (Spearman, 1904). Tensiometer pore water pressure measurements were evaluated using the local polynomial regression function (R library(lpridge)) (Seifert and Gasser, 2000).

3 Results

3.1 Soil Analysis

The grain size distribution analysis by Frei (2009) and Graf and Frei (2013) of the soil material which originated from the landslide area Schwandruebi in the larger catchment of the Flueligraben is illustrated in figure 3.1. The moraine soil included grains up to 63 mm and was classified as clayey gravel with sand (GC-CL). Further, a liquid limit of 21.5 % and a plasticity index of 8.6 % were determined (Graf and Frei, 2013).

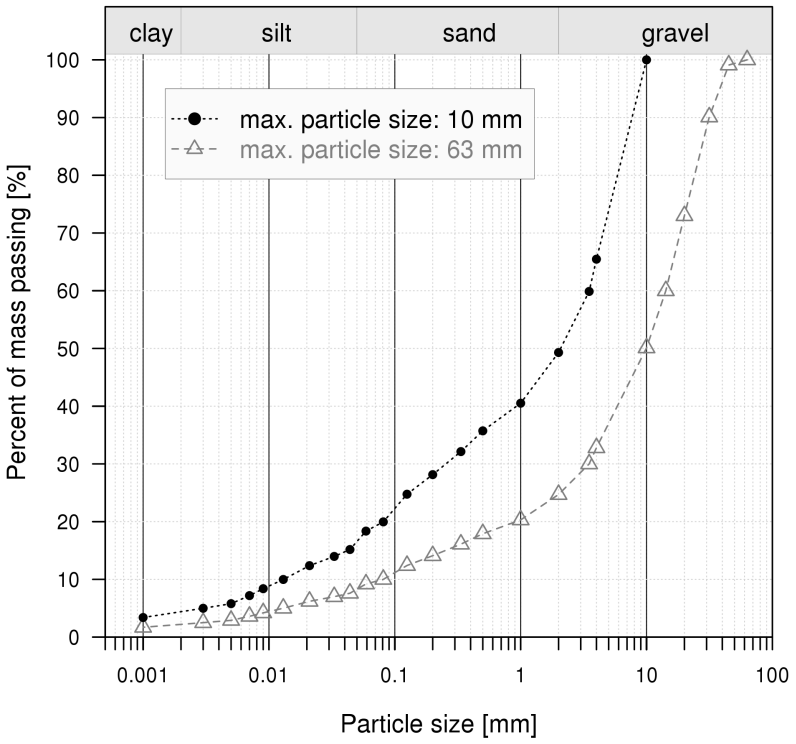


Figure 3.1: Grain size distribution curves of the soil material from the upper part of the Schwandruebi landslide area in the larger catchment of the Flueligraben. The gray curve represents the distribution including the coarse grains with a fraction up to 63 mm, the black curve includes grain fractions smaller than 10 mm which were used for the soil aggregate stability tests.

Further grain size distribution analysis of the moraine soil material with a fraction smaller than 10 mm resulted in two different soil sample curves (Soil A and B) which are illustrated in figure 3.2. The fraction of the fine grains <0.075 mm amounted to 11 % of the total mass and the coarse fraction to 89 %. Therefore the soil was classified as a coarse-grained.

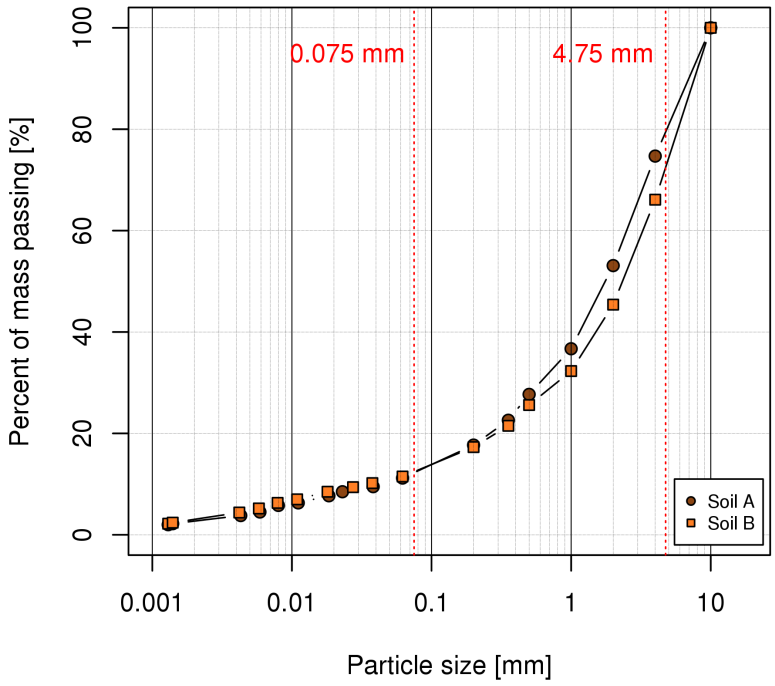


Figure 3.2: Grain size distribution of two different soil samples A and B from the moraine soil material with a fraction smaller than 10 mm.

The soil was then identified as sand because more than 50 % of the coarse fraction passed through a 4.75 mm sieve. Due to a smaller amount of 12 % passing through the 0.075 mm sieve, the coefficient of curvature (C_c) and the coefficient of uniformity (C_u) were calculated as illustrated in table 3.1. The values of the particle size diameters D_{10}, D_{30} and D_{60} of soil A were identified as 0.05 mm, 0.67 mm and 2.7 mm and of soil B as 0.04 mm, 0.9 mm and 3.7 mm, respectively (Figure 3.2, Table 3.1).

Table 3.1: Soil parameters of the two Hexenruebi soil samples A and B. Particle size diameter units are in [mm], coefficient of curvature and uniformity units in [g g^{-1}].

Parameters	**Soil A**	**Soil B**
D_{10}	0.05	0.04
D_{30}	0.67	0.9
D_{60}	2.7	3.7
C_u	54	92.5
C_c	3.325	5.473

Table 3.2: Different soil parameter values (mass units in [g], water content in [g g^{-1}], number of blows in [-]) of eight different soil samples (S1-S8), which were used to determine the liquid limit.

Sample No.	**S1**	**S2**	**S3**	**S4**	**S5**	**S6**	**S7**	**S8**
Mass of can	9.46	9.47	9.47	5.83	9.45	9.48	5.87	5.88
Mass of moist sample & can	12.72	12.71	13.63	9.26	14.3	14.12	12.58	12.46
Mass of dry sample & can	12.29	12.27	13.08	8.82	13.65	13.49	11.71	11.59
Mass of water	0.43	0.44	0.55	0.44	0.65	0.63	0.87	0.87
Mass of dry soil	2.83	2.80	3.61	2.99	4.20	4.01	5.84	5.71
Water content	0.152	0.157	0.152	0.147	0.155	0.157	0.149	0.152
Average water content	0.155		0.150		0.156		0.151	
Number of blows	20		30		13		26	

A dual classification with two group symbols was given, due to a fine fraction between 5-12 %. Based on the fact that the C_c and C_u values did not fulfill the requirements of well graded sand, the first group symbol was identified as poorly graded sands, gravelly sands with little or no fines (SP). The second group symbol was determined as silty sands (SM) due to a plasticity index smaller than 4. Therefore, the fraction of the Hexenruebi soil, which was smaller than 10 mm, was classified as poorly graded sand and silty sand (SP-SM) (ASTM D2487-06, 2006; Das, 2007). The resulting soil parameters of the liquid and plastic limit tests of the soil are displayed in table 3.2 and 3.3, respectively.

Table 3.3: Different soil parameter values (mass units in [g], water content in [g g^{-1}], number of blows in [-]) of four different soil samples (S9-S12), which were used to determine the plastic limit.

Sample No.	**S9**	**S10**	**S11**	**S12**
Mass of Can	5.87	5.84	5.88	5.90
Mass of Moist Sample and Can	6.73	6.67	8.43	8.39
Mass of Dry Sample + Can	6.6	6.55	8.09	8.03
Mass of Water	0.13	0.12	0.34	0.36
Mass of Dry Soil	0.73	0.71	2.21	2.13
Water Content	0.178	0.169	0.154	0.169
Average Water Content	0.174		0.161	
Number of Blows	13		13	

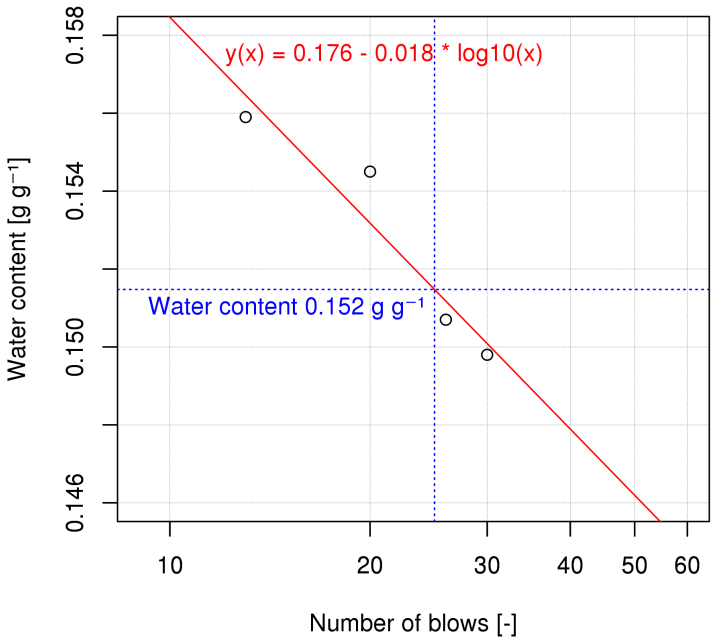


Figure 3.3: Semilogarithmic plot of the blow count [-] versus the water content [g g^{-1}]. At the crossing point of the abscissa at N = 25 blows and the fitting curve, the water content of the corresponding ordinate axis was 0.152 g g^{-1} which - multiplied by 100 % - equaled the liquid limit (15.15 %).

After plotting the blow count x versus the water content y(x) on a semilogarithmic plot, the fitted curve y(x) = 0.176 - 0.018 $*$ $\log_{10}$(x) resulted (Figure 3.3). At the crossing point of the abscissa at N = 25 blows and the fitted curve, the water content of the corresponding ordinate axis was 0.152 g g^{-1} which - multiplied by 100 % - equaled the liquid limit (15.15 %). The plastic limit and plasticity index were determined as 16.75 % and -1.60 %, respectively. Consequently, the soil was classified as non plastic (Bowles, 1992).

3.1.1 Water Content

Treatments of *Alnus incana* (A, AM) and the control treatments (K, KM) achieved higher water content values compared to the treatments of *Poa pratensis* (P, PM) and the combined treatments of *Alnus incana* and *Poa pratensis* (AP, APM) (Figure 3.4).

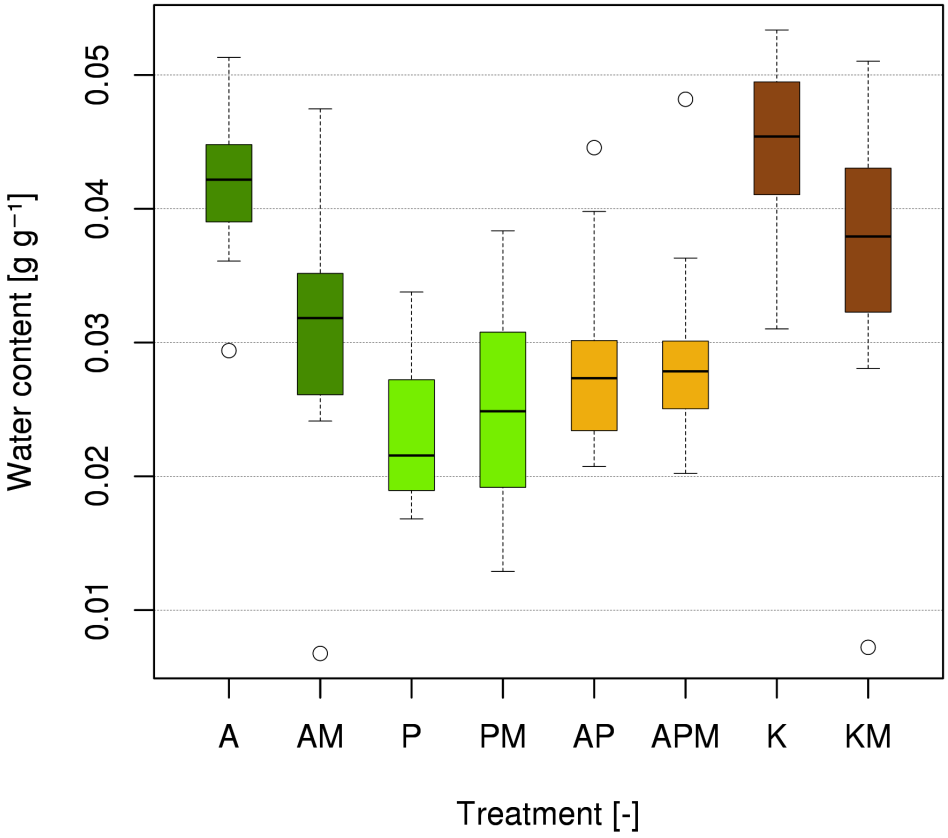


Figure 3.4: Water content [g g^{-1}] of the non-mycorrhized treatments A (*Alnus incana*), P (*Poa pratensis*), AP (*Alnus incana* and *Poa pratensis*), K (control) and the corresponding mycorrhized (M) treatments AM, PM, APM and KM.

Generally, mean and median values were similar within each treatment (Appendix I.III; Table 6.11). Treatment K revealed the highest median water content value of 0.045 g g^{-1}, closely followed by A (median = 0.042 [g g^{-1}]), AM (median = 0.032 [g g^{-1}]) and KM (median = 0.038 [g g^{-1}]). The median water content values of APM, AP and PM were calculated as 0.028, 0.027, 0.025 g g^{-1}, respectively, followed by the lowest median value of 0.022 g g^{-1} of the P treatment.
The treatment significantly affected the water content (Kruskal-Wallis rank sum test: chi-squared = 49.4316, df = 7, p-value = 1.867e-08) (Hollander and Wolfe, 1973). Pairwise Wilcoxon rank sum test with the p-value adjustment method of Holm (1979) revealed differences between the treatments A and AM (p-value = 0.030) as well as comparing A to P (p-value = 4.3e-05) and A to PM (p-value = 1.6e-04). Further differences were found between the treatment AM to K (p-value = 0.029), P to K (p-value = 2.3e-04), PM to K (p-value = 2.9e-04), AP to K (p-value = 0.001) and APM to K (p-value = 0.002) as displayed in table 3.4.

Table 3.4: P-values of the pairwise Wilcoxon rank sum test of the water content using the adjustment method of Holm (1979). Non-mycorrhized treatments are abbreviated by A (*Alnus incana*), P (*Poa pratensis*), AP (*Alnus incana* and *Poa pratensis*), K (control) and the corresponding mycorrhized (M) treatments by AM, PM, APM and KM.

Treatment	**A**	**AM**	**P**	**PM**	**AP**	**APM**	**K**
AM	0.030						
P	4.3e-05	0.128					
PM	1.6e-04	0.913	1.000				
AP	0.005	1.000	0.602	1.000			
APM	0.005	1.000	0.388	1.000	1.000		
K	1.000	0.029	2.3e-04	2.9e-04	0.001	0.002	
KM	1.000	0.602	0.012	0.059	0.267	0.129	0.602

Generally, the water content of the soil samples which were not watered from five to seven days decreased (Figures 3.5 and 3.6). Mean and median values off all treatments are listed in table 6.3 (Appendix I.IV; Table 6.3. Median values of the control treatments (K, KM) were slightly higher compared to the corresponding mean values. Similar results were observed within the AM treatment. All other treatments revealed lower median or equal median values compared to the mean.

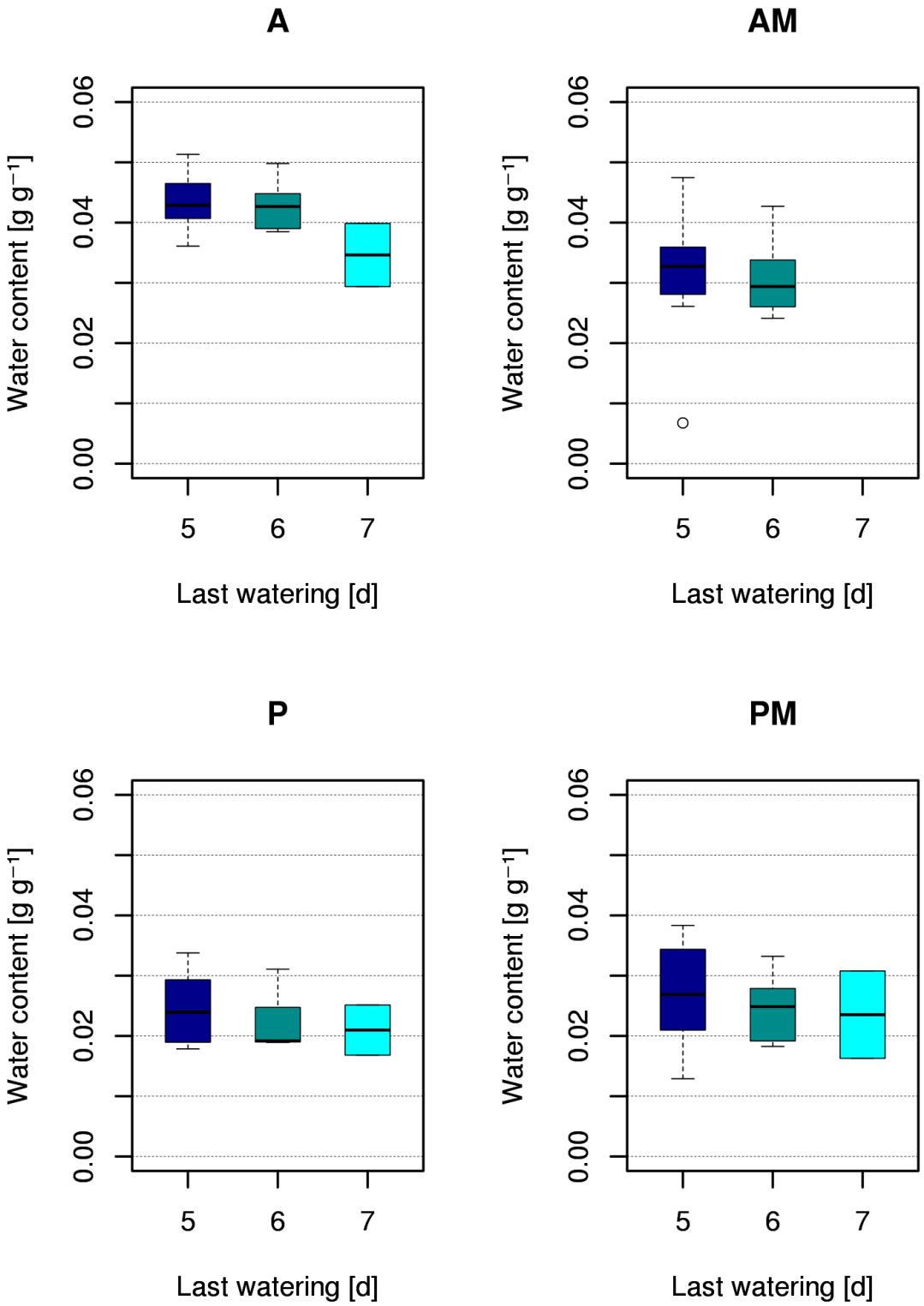


Figure 3.5: Water content [g g^{-1}] of the soil samples depending on the days since the last watering. Non-mycorrhized treatments are abbreviated by A (*Alnus incana*), P (*Poa pratensis*) and the corresponding mycorrhized (M) treatments by AM, PM.

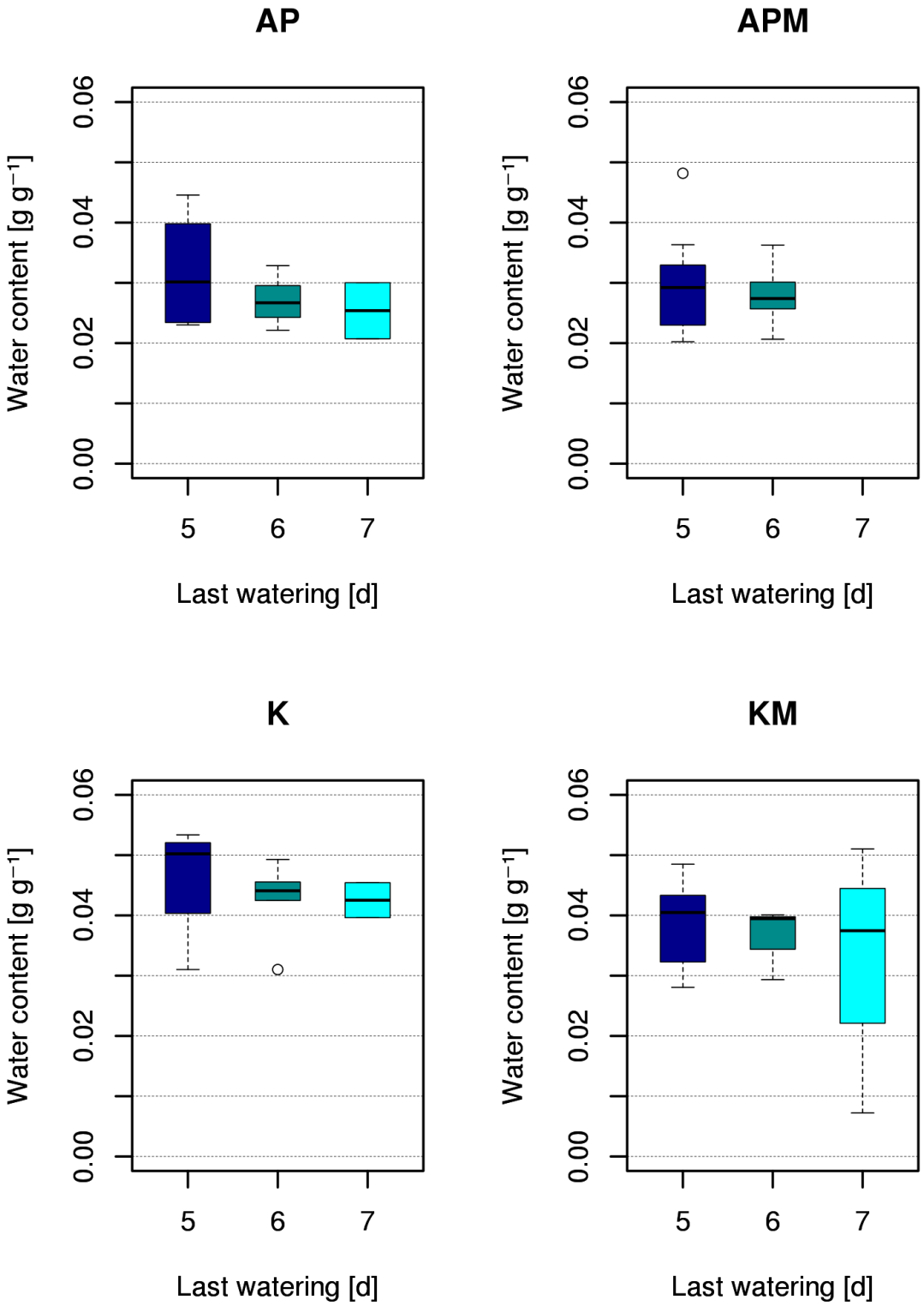


Figure 3.6: Water content [g g^{-1}] of the soil samples depending on the days since the last watering. Non-mycorrhized treatments are abbreviated by AP (*Alnus incana* and *Poa pratensis*), K (control) and the corresponding mycorrhized (M) treatments by APM and KM.

3.1.2 Soil Dry Unit Weight

The dry unit weight of the differently treated soil samples revealed no significant differences (Kruskal-Wallis rank sum test: chi-squared = 6.731, df = 7, p-value = 0.457) (Hollander and Wolfe, 1973). Mycorrhized treatments yielded slightly lower dry unit weight values compared to the corresponding non-mycorrhized treatments (Figure 3.7).

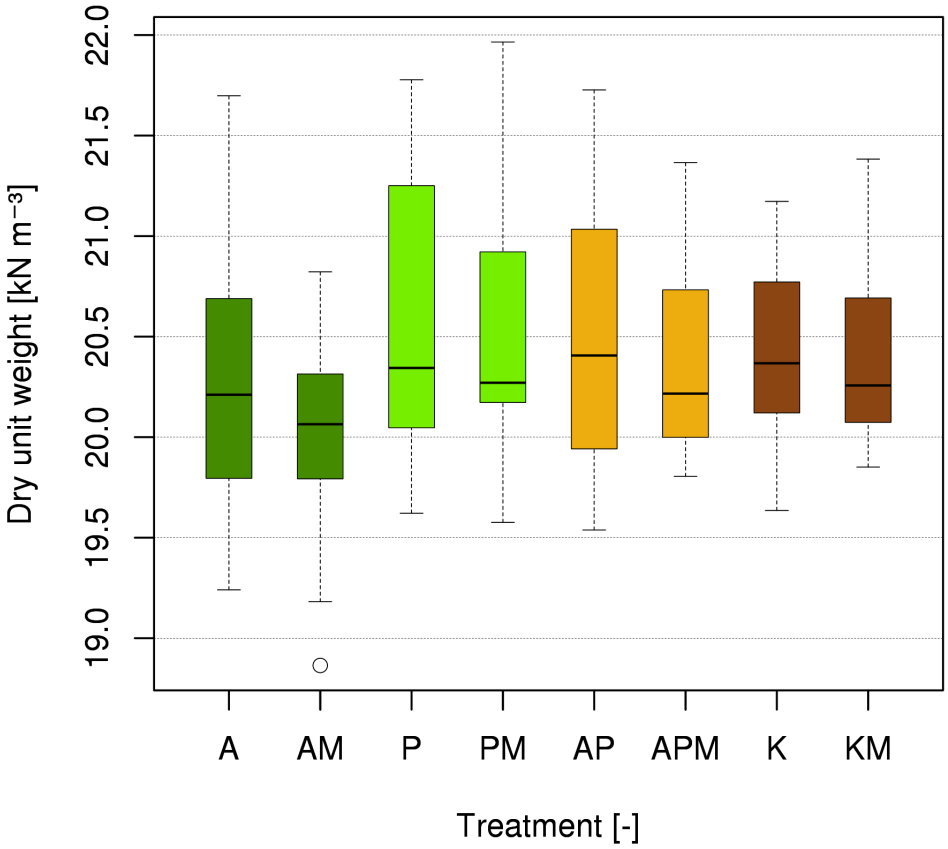


Figure 3.7: Soil dry unit weight [kN m^{-3}] of the non-mycorrhized treatments A (*Alnus incana*), P (*Poa pratensis*), AP (*Alnus incana* and *Poa pratensis*), K (control) and the corresponding mycorrhized (M) treatments AM, PM, APM and KM.

The lowest dry unit weight was observed within the AM treatment (median = 20.064 [kN m^{-3}]) and the highest dry unit weight in treatment AP (median = 20.406 [kN m^{-3}]) (Appendix I.V; Table 6.4). Generally, mean values were higher compared to the corresponding median values. Treatment P and PM yielded considerably higher mean values than median values. A notable exception was found within treatment AM which revealed a higher median compared to the mean.

3.2 Wet-Sieving

Many soil samples collapsed during the flooding of the wet-sieving procedure, after which the tensiometer was not in contact with the soil sample anymore. Nine to twelve samples of the non-mycorrhized and mycorrhized *Alnus incana* (A, AM) treatments and the control treatments (K, KM) collapsed while flooding. Concerning other treatments, mycorrhized samples of *Poa pratensis* (PM) were stable in eight measurements, the mycorrhized combined treatments of *Alnus incana* and *Poa pratensis* (APM) in eleven. The treatments of non-mycorrhized *Poa pratensis* (P) and the combination of *Alnus incana* and *Poa pratensis* (AP) did either not collaps at all during the wet-sieving procedure or the ceramic cup was at least in contact with the soil after water drainage such as illustrated in figure 3.8 which shows the sample AP10 during the flooding and afterwards. The pore water pressure measurements varied on a wide range concerning the initial pore water pressure. However, the general shape of the curves over the entire wet-sieving procedure did not obviously differ (Figure 3.9).

Figure 3.8: Wet-sieving procedure of the combined non-mycorrhized sample of *Alnus incana* and *Poa pratensis* (AP10). Left: During flooding. Right: After the wet-sieving the high grade porous ceramic cup of the tensiometer still remained in contact with the soil.

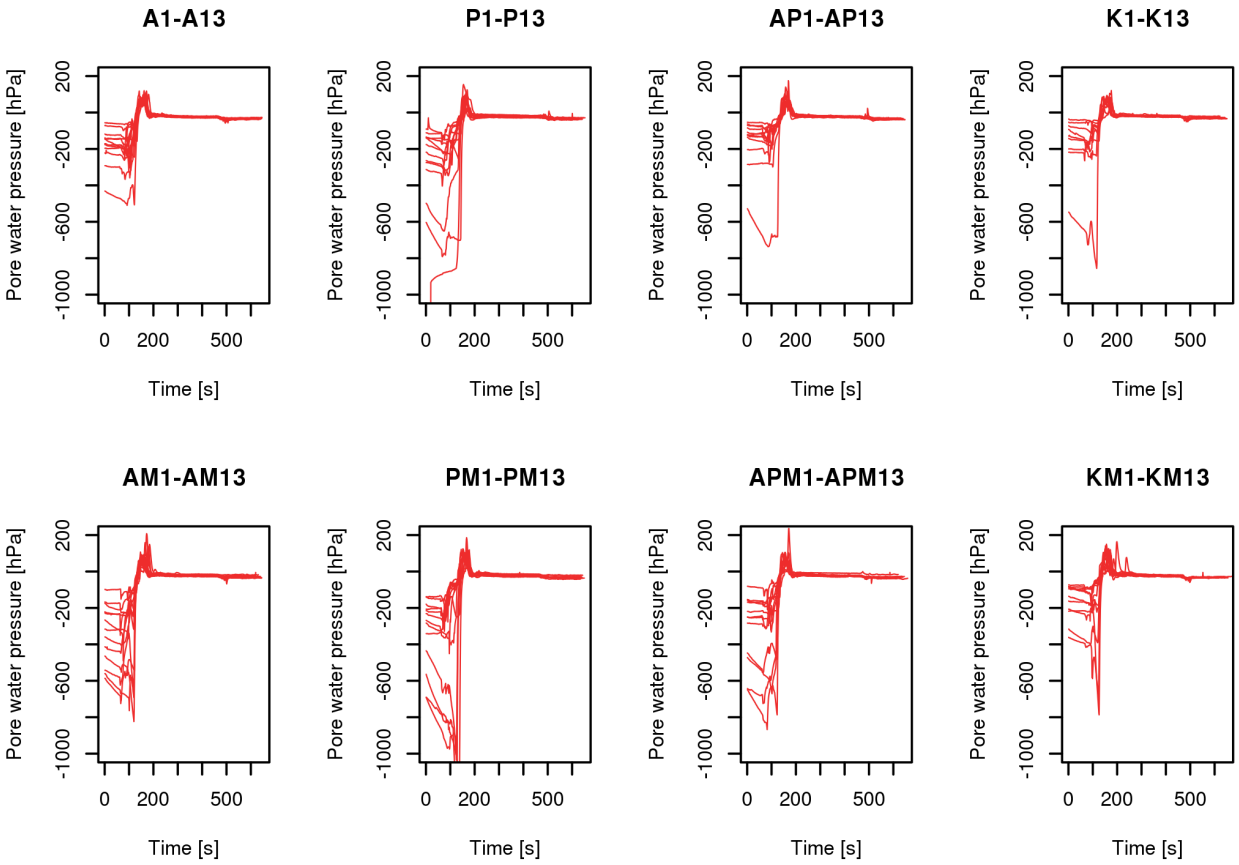


Figure 3.9: This figure illustrates the pore water pressure [hPa] measurements of all treatments during the wet-sieving procedure. The non-mycorrhized treatments are abbreviated by A (*Alnus incana*), P (*Poa pratensis*), AP (*Alnus incana* and *Poa pratensis*), K (control) and the corresponding mycorrhized (M) treatments by AM, PM, APM and KM.

Depending on the initial water content of the individual soil sample, the pore water pressure varied within a wide range (0-58 s). Figure 3.10 exemplarly illustrates the pore water pressure measurements of the *Alnus incana* treatments during the wet-sieving procedure. At the beginning, a slight decrease of the pore water pressure was observed. Within an average time span of 58 seconds the water level was at the second sieve which is illustrated by the first vertical line in figure 3.10 (No. 1). The water level at the specified numbers is displayed in figure 3.12. Abrupt changes in the pore water pressure measurements were a consequence of disaggregation processes and movements due to the flooding of the soil. After an average time span of 124 seconds the highly porous ceramic cup of the tensiometer was in contact with the water and consequently, the pore water pressure increases (No. 2). After 171 seconds the sample was completely flooded, resulting in the highest peaks (No. 3). During the flooding period of five minutes, pore water pressure levelled off within seconds between -30 and 0 hPa and decreased slightly when draining the water at 471 seconds (No. 4). The water level reached the 2nd sieve

within around 516 seconds (No. 5) and the wet-sieving procedure ended after a mean value of 587 seconds (No. 6).

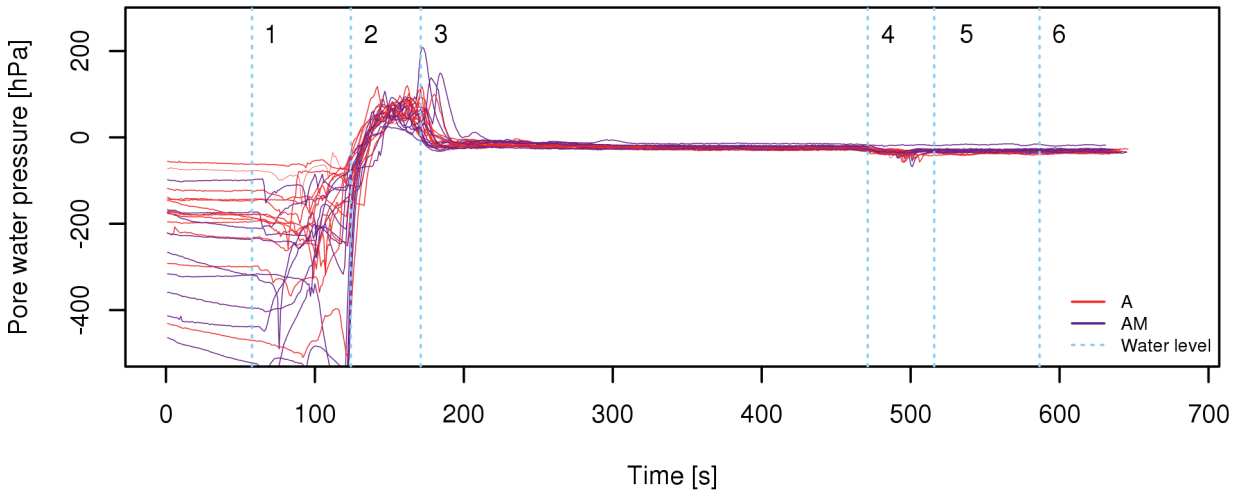


Figure 3.10: Pore water pressure [hPa] measurement of the non-mycorrhized and mycorrhized samples of *Alnus incana* (A,AM) during the wet-sieving procedure. Blue dotted vertical lines designate specific moments concerning the water level, the movement of the soil sample or the ceramic cup of the tensiometer. No. 1: Water at 2nd sieve (58 s); No. 2: Ceramic cup in water (124 s); No. 3, 4: Flooded soil sample (171 - 471 s); No. 5: Water at 2nd sieve (516 s); No. 6: End of wet-sieving procedure (587 s) (Figure 3.12).

The measurements of the pore water pressure were further analysed in more detail. An example of one individual non-mycorrhized *Alnus incana* soil sample (A1) is displayed in the figure 3.11. Vertical lines illustrate specific moments concerning the water level, the movement of the soil sample or the ceramic cup of the tensiometer (Figure 3.12). In general, the pore water pressure decreased until the water reached the second sieve (No. 1). After this, the water started to wet the sample. When a movement occured (Orange line in figure 3.11), the pore water pressure first immediately decreased and then increased again. The same was observed when the highly porous ceramic cup of the tensiometer was in the air, due to a second collaps of the soil sample which is referred to the violet line in figure 3.11. From this time on the tensiometer cup was either in the water or in the air but had no contact to the soil sample anymore until the end of the measurement. At the time when water reached the ceramic cup (No. 2), the pore water pressure increased immediately. In this sample the peak of the pore water pressure matches the end of the flooding (No. 3).

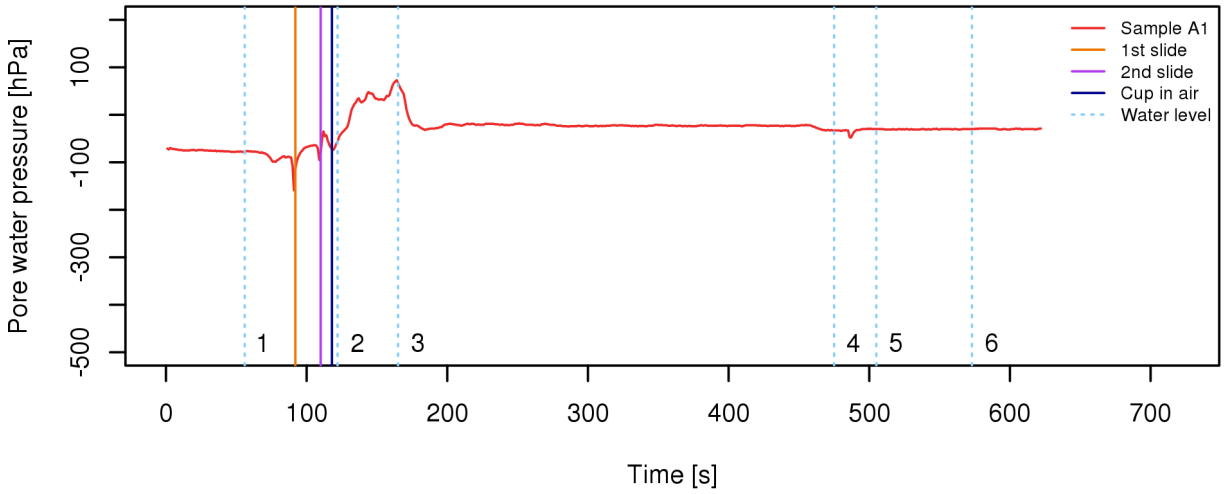


Figure 3.11: Pore water pressure [hPa] measurement of the non-mycorrhized sample A1 with *Alnus incana* during the wet-sieving procedure. Additional vertical lines show specific moments concerning the water level, the movement of the soil sample or the ceramic cup of the tensiometer. No. 1: Water at 2nd sieve (58 s); No. 2: Ceramic cup in water (124 s); No. 3, 4: Flooded soil sample (171 - 471 s); No. 5: Water at 2nd sieve (516 s); No. 6: End of wet-sieving procedure (587 s) (Figure 3.12).

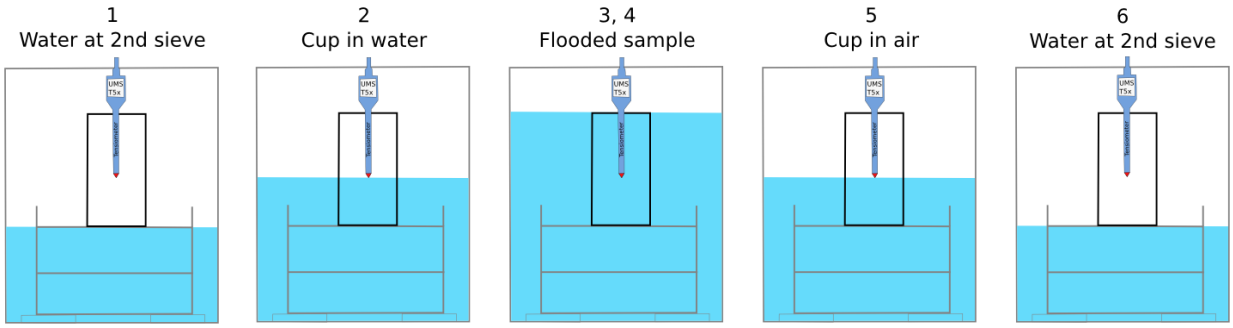


Figure 3.12: Water level during the wet-sieving procedure. No. 1: Water at 2nd sieve (58 s); No. 2: Ceramic cup in water (124 s); No. 3, 4: Flooded soil sample (171 - 471 s); No. 5: Water at 2nd sieve (516 s); No. 6: End of wet-sieving procedure (587 s) (Figures 3.10 and 3.11).

During the flooding period of five minutes, the pore water pressure levelled around - 30 hPa. After these five minutes (No. 4), water drainage often resulted in a collapse of the samples which could be identified in a decrease followed by an immediate increase of the pore water pressure. At the time when the ceramic cup was again in contact with the air (No. 5), no

further changes were observed anymore (Figure 3.11). Measurements of the same treatment but different samples resulted in similar behavior as described for the sample A1.
To compare the behavior of all different treatments during the wet-sieving procedure, the pore water pressure was plotted against the time using a local polynomial regression function (Figures 3.13 and 3.14). Mycorrhized treatments (dotted lines) showed a steeper curve and their initial pore water pressure was lower compared to the non-mycorrhized treatments which is visible in figure 3.14.

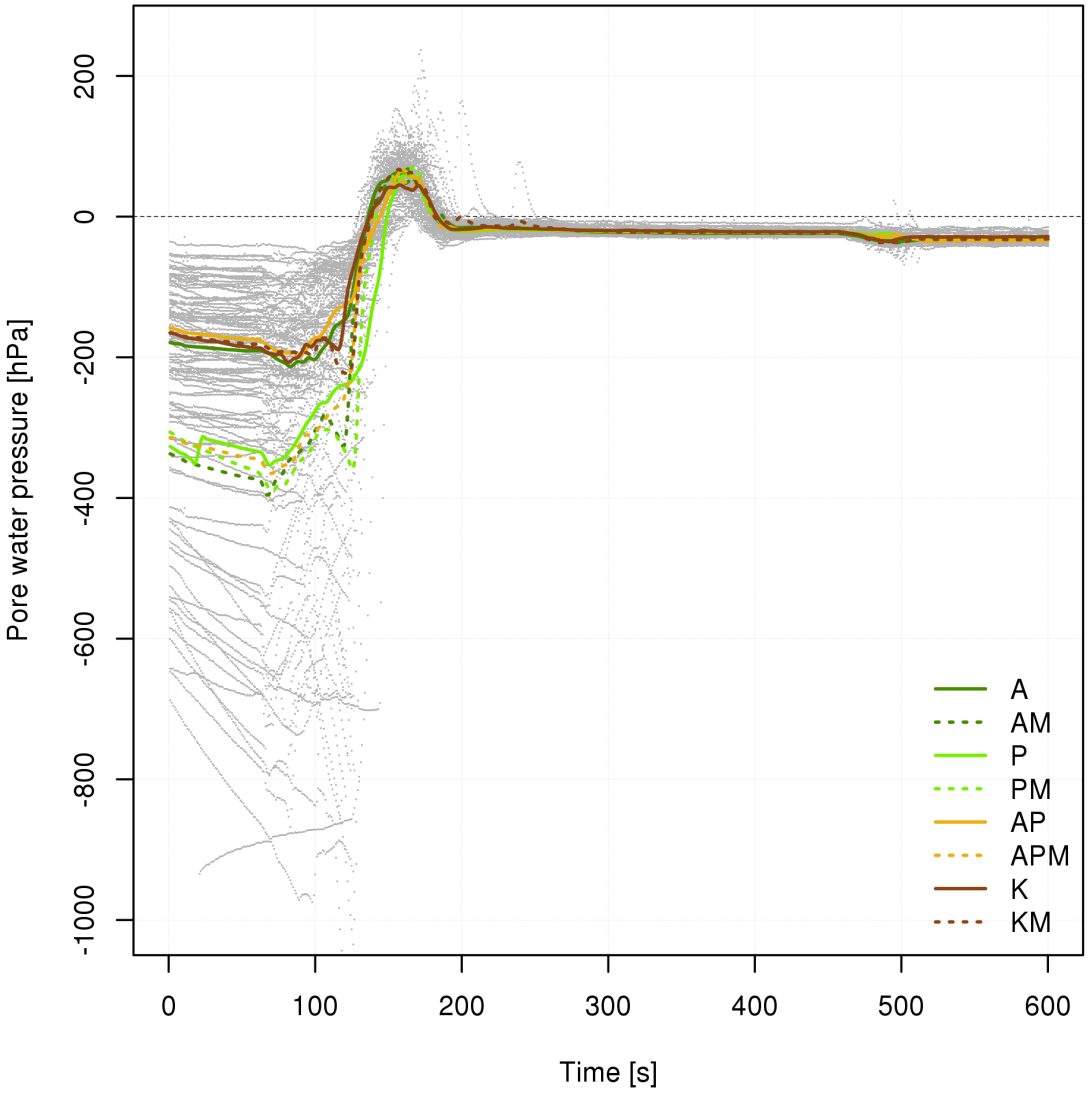


Figure 3.13: Pore water pressure [hPa] measurement of all treatments during the wet-sieving procedure [s] using a local polynomial regression function to smooth the curves. All curves belonging to one and the same treatment. Non-mycorrhized treatments are abbreviated by A (*Alnus incana*), P (*Poa pratensis*), AP (*Alnus incana* and *Poa pratensis*), K (control) and the corresponding mycorrhized (M) treatments by AM, PM, APM and KM.

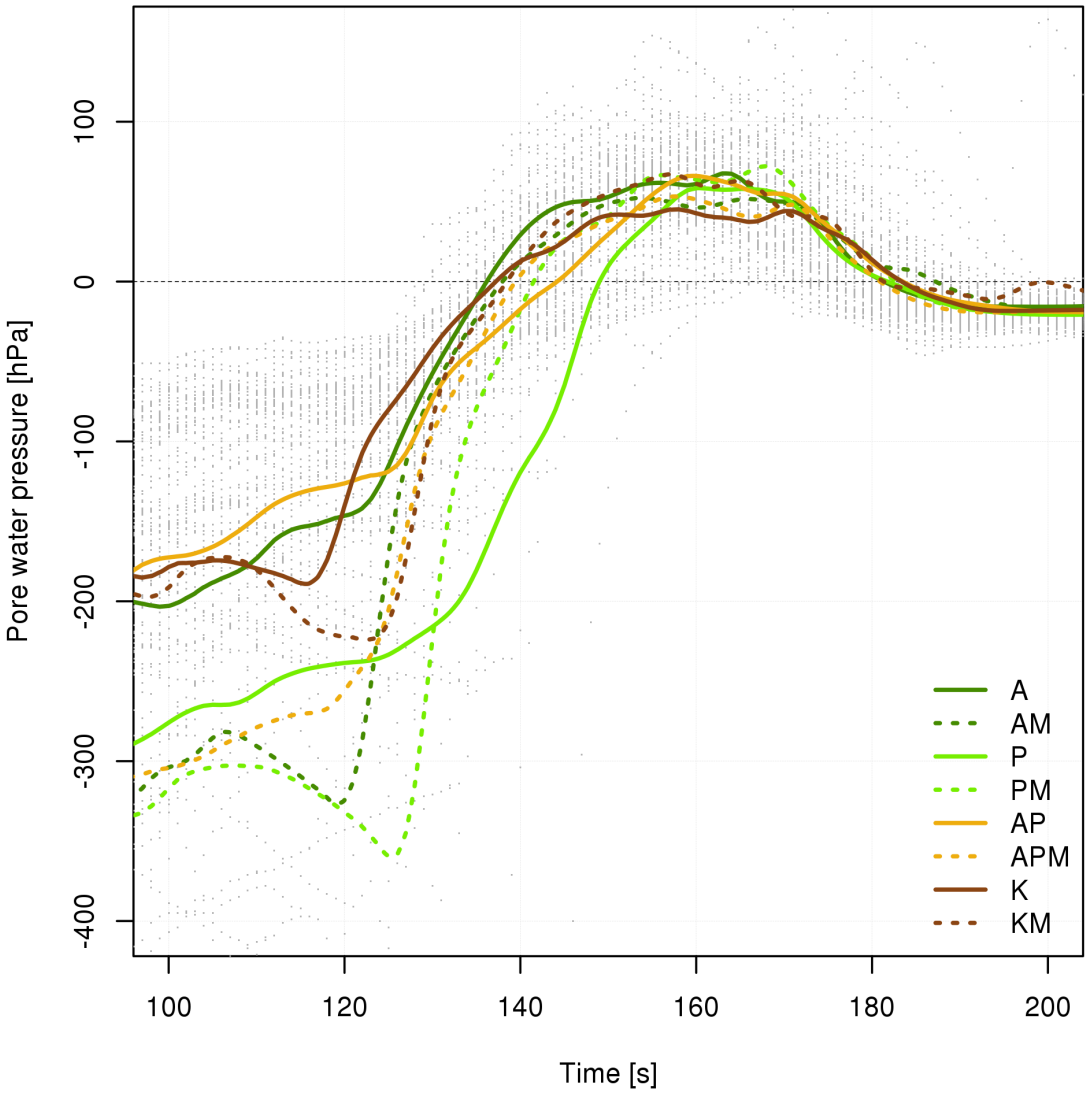


Figure 3.14: Detail of figure 3.13 of the pore water pressure [hPa] measurements of all treatments during the wet-sieving procedure [s] using a local polynomial regression function. All curves belonging to one and the same treatment. Non-mycorrhized treatments are abbreviated by A (*Alnus incana*), P (*Poa pratensis*), AP (*Alnus incana* and *Poa pratensis*), K (control) and the corresponding mycorrhized (M) treatments by AM, PM, APM and KM.

3.2.1 Soil Aggregate Stability

A comparison of the mean mass values of the remaining soil on the sieve to the soil which passed through the sieve after the wet-sieving procedure is illustrated in figure 3.15. Mean and standard deviation values are listed in table 6.5 (Appendix III.IV). The highest amount of soil which passed through the sieve was observed within the control treatments K (mean = 639.615 [g]) and KM (mean = 611.831 [g]) followed by AM and A. Treatments with *Alnus*

incana (A, AM) often collapsed during the wet-sieving procedure and consequently, more soil passed through the sieve than remained on the sieve.

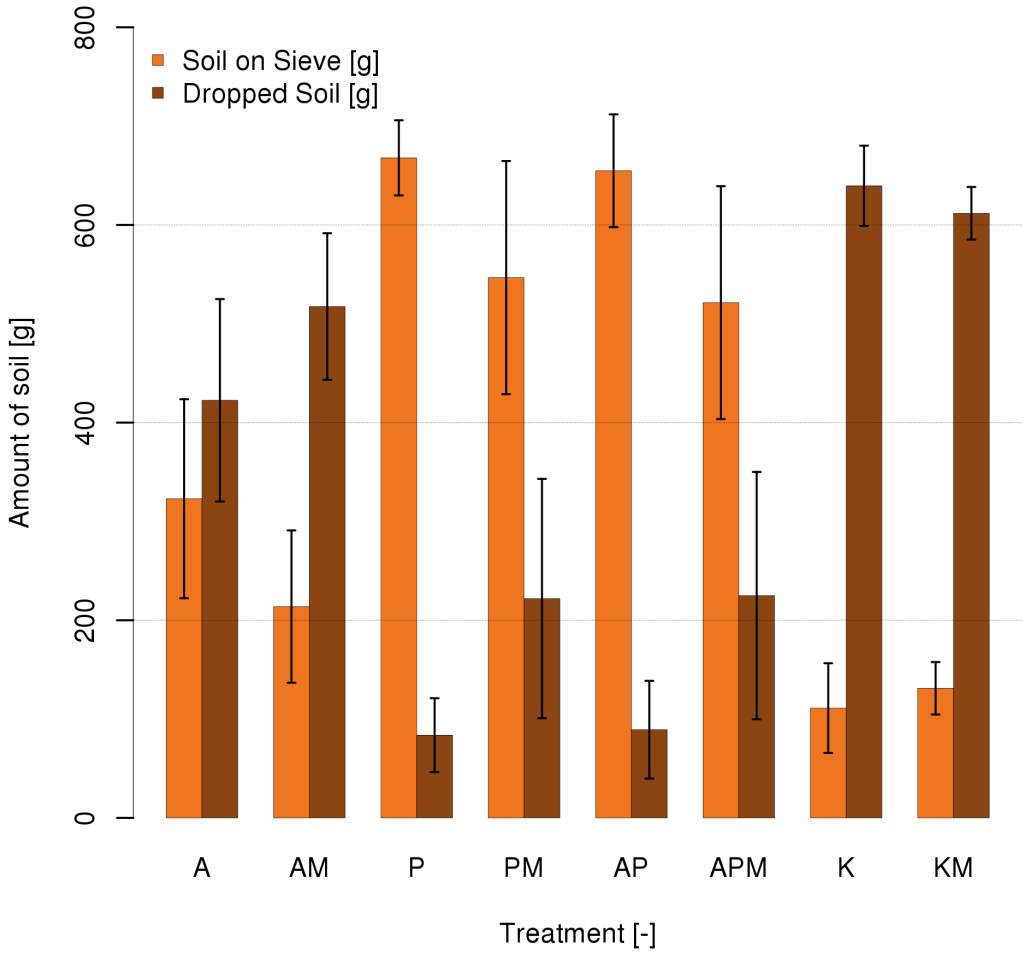


Figure 3.15: Mean mass of the amount of soil remaining on the sieve [g] compared to the dropped soil [g] after the wet-sieving procedure with an additional +/- 1 standard deviation interval at each mean. The non-mycorrhized treatments are abbreviated by A (*Alnus incana*), P (*Poa pratensis*), AP (*Alnus incana* and *Poa pratensis*), K (control) and the corresponding mycorrhized (M) treatments by AM, PM, APM and KM.

Poa pratensis (P, PM) and the combined treatments of *Alnus incana* and *Poa pratensis* (AP, APM) were more stable. The highest amount of soil remaining on the sieve after the wet-sieving procedure was yielded by the non-mycorrhized treatment P (mean = 667.866 [g]), closely followed by AP (mean = 654.823 [g]). The highest standard deviation values were found within the treatments PM and APM and the lowest within the KM treatment. It is notable that the amount of soil passing through the sieve was higher in all planted mycorrhized treatments

compared to the corresponding non-mycorrhized treatments.

Further soil analysis dealt with the calculated soil aggregate stability (Formula 2.12). The control treatments K and KM revealed the lowest soil aggregate stability with a median value of 0.134 g g^{-1} and 0.171 g g^{-1}, respectively (Figure 3.16). Treatments A and AM were more stable compared to the control treatments but the least stable of the planted treatments.

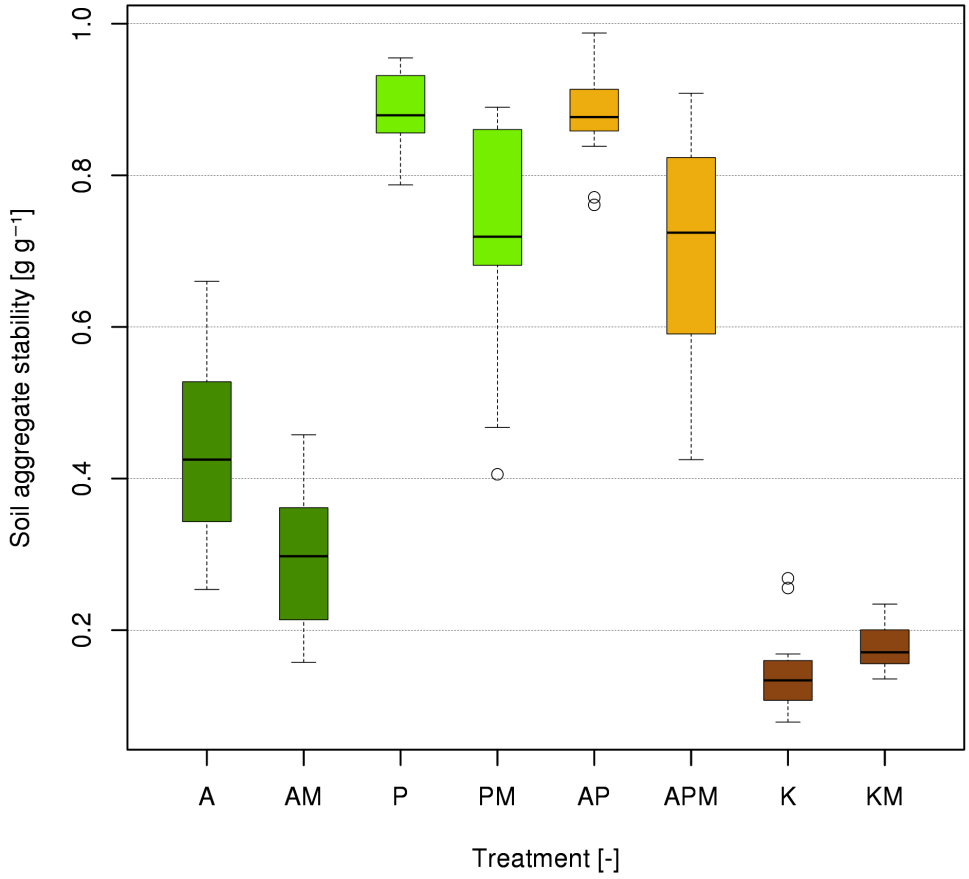


Figure 3.16: Soil aggregate stability [g g^{-1}] of all treatments. Non-mycorrhized treatments are abbreviated by A (*Alnus incana*), P (*Poa pratensis*), AP (*Alnus incana* and *Poa pratensis*), K (control) and the corresponding mycorrhized (M) treatments by AM, PM, APM and KM.

A high soil aggregate stability was found in the treatments P, PM, AP and APM, whereby the highest soil aggregate stability was observed within the non-mycorrhized treatments P (median = 0.879 [g g^{-1}]) and AP (median = 0.877 [g g^{-1}]). It is notable, that soil aggregate stability was lower in all mycorrhized planted treatments compared to the corresponding non-mycorrhized

treatments. Further, all mycorrhized planted treatments yielded a higher median than mean and vice versa considering non-mycorrhized planted treatments. Measurements of the K and KM treatment as well as the P and AP treatment showed a narrow distribution. In contrary, the treatments A, AM, PM and APM were more widely scattered. Additional mean, standard deviation (sd), median and median absolute deviation (mad) values of the soil aggregate stability are listed in table 6.6 (Appendix III.V).
The soil aggregate stability was significantly affected by the different treatments (Kruskal-Wallis rank sum test: chi-squared = 89.250, df = 7, p-value <2.2e-16) (Hollander and Wolfe, 1973). All pairwise comparisons were significantly different except the ones of treatment P to AP, PM to APM and K to KM (Table 3.5) (Holm, 1979).

Table 3.5: P-values of the pairwise Wilcoxon rank sum test of the soil aggregate stability [g g^{-1}] using the adjustment method of Holm (1979). Non-mycorrhized treatments are abbreviated by A (*Alnus incana*), P (*Poa pratensis*), AP (*Alnus incana* and *Poa pratensis*), K (control) and the corresponding mycorrhized (M) treatments by AM, PM, APM and KM.

Treatment	**A**	**AM**	**P**	**PM**	**AP**	**APM**	**K**
AM	0.041						
P	5.4e-06	5.4e-06					
PM	0.001	1.9e-05	0.004				
AP	5.4e-06	5.4e-06	1.000	0.010			
APM	0.002	1.2e-05	0.002	1.000	0.009		
K	1.9e-05	0.001	5.4e-06	5.4e-06	5.4e-06	5.4e-06	
KM	5.4e-06	0.009	5.4e-06	5.4e-06	5.4e-06	5.4e-06	0.132

3.3 Degree of Mycorrhization

After the staining procedure of *Poa pratensis* roots using Chlorazol Black E (CBE) no mycorrhized structures could be identified through the binocular microscope (Figures 3.17 and 3.18). With regard to ectomycorrhiza, root tips of *Alnus incana* with an ectomycorrhizal mantle of fungal tissue were clearly visible under the binocular microscope and could be distinguished from non-mycorrhized root tips as illustrated in figure 3.18. Consequently, the degree of mycorrhization was only calculated for treatments with *Alnus incana*.

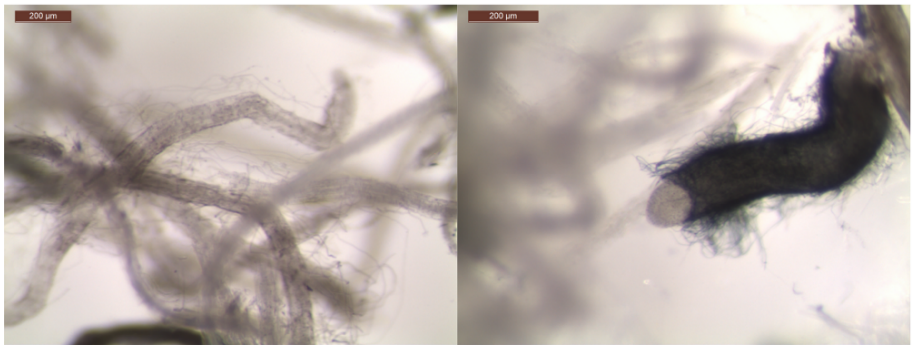


Figure 3.17: Left: Non-mycorrhized roots of *Poa pratensis* in the combined sample of *Alnus incana* and *Poa pratensis* (APM12). Right: Mycorrhized root tip of *Alnus incana* of the sample APM13. Both samples were stained in Chlorazol Black E. Photos through the binocular microscope (Leica $Mz9_5$) by the software Leica Application Suite©; (Version 4.0.0)

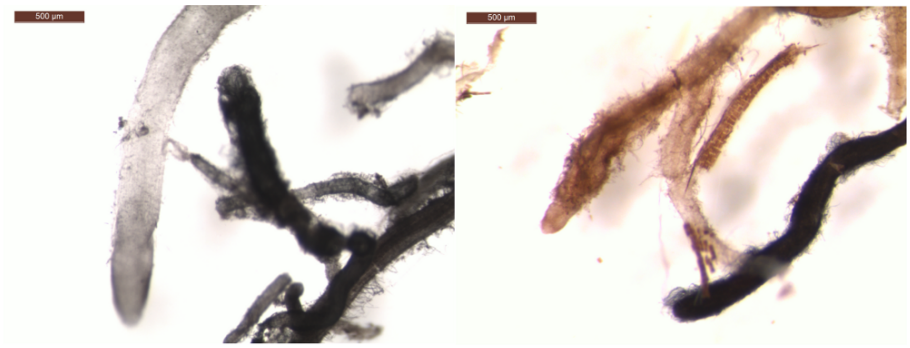


Figure 3.18: Comparison of non-mycorrhized and mycorrhized root tips of *Alnus incana* (AM). Left: Sample AM13. Right: Sample AM12. Photos through the binocular microscope (Leica $Mz9_5$) by the software Leica Application Suite©; (Version 4.0.0)

The degree of mycorrhization (Formula 2.13) of the mycorrhized *Alnus incana* treatment (AM) and the combined treatment of *Alnus incana* and *Poa pratensis* (APM) is illustrated in figure

3.19. Within both treatments the median values were higher than the mean values. Treatment AM yielded a higher degree of mycorrhization (median = 83.784 [%]) compared to the APM treatment (median = 48.649 [%]) (Appendix IV.I; Table 6.7). Comparing AM and APM, a significant difference between the two treatments was revealed (Chi-squared = 9.471, df = 1, p-value = 0.002) using the Kruskal-Wallis rank sum test (Hollander and Wolfe, 1973).

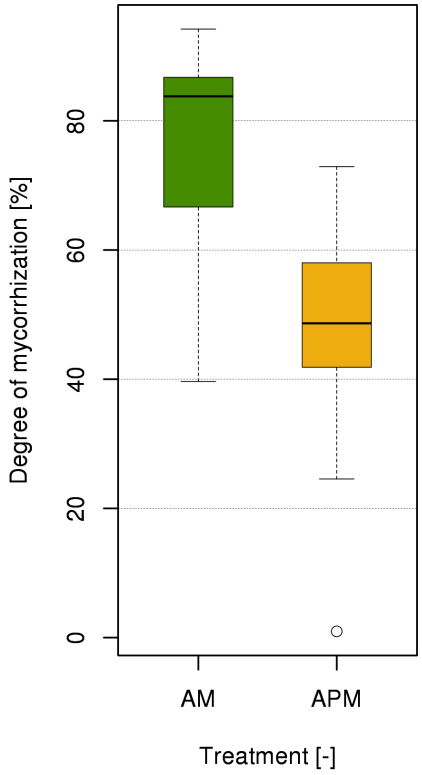


Figure 3.19: Degree of mycorrhization [%] of *Alnus incana* from the mycorrhized *Alnus incana* treatment (AM) and the combined treatment of *Alnus incana* and *Poa pratensis* (APM).

3.4 Ergosterol Extraction

Ergosterol liquid phase extraction and the following HPLC analysis revealed no significant amount of Ergosterol. All values of the measured Ergosterol amount were lower than the detection limit of 220 ng per g sample. Additional information regarding the calibration and Ergosterol standard and the Ergosterol stock solution can be seen in the Appendix V.II. To verify the exact position of the Ergosterol peak in the chromatogram a stock solution was added to a specific sample. Following this, the chromatograms of the soil sample, of the soil sample with the added stock solution and of an Ergosterol stock solution were displayed in one window. An example of the sample AM13 is illustrated in figure 3.20. The black curve of the soil sample shows one peak around 15.25 min and a smaller peak at 14.79 min on the left side (Vertical gray line). By comparing this black curve to the blue curve with an additional stock solution and the pink curve of the Ergosterol stock solution it is evident, that the higher peak of the black curve does not match with the peak of the added stock solution which is indicated by the vertical gray line. Due to the fact that the smaller peak disappears in the noise Ergosterol cannot be clearly differentiated from the noise. Hence, the quantification of its concentration is not possible in this case.

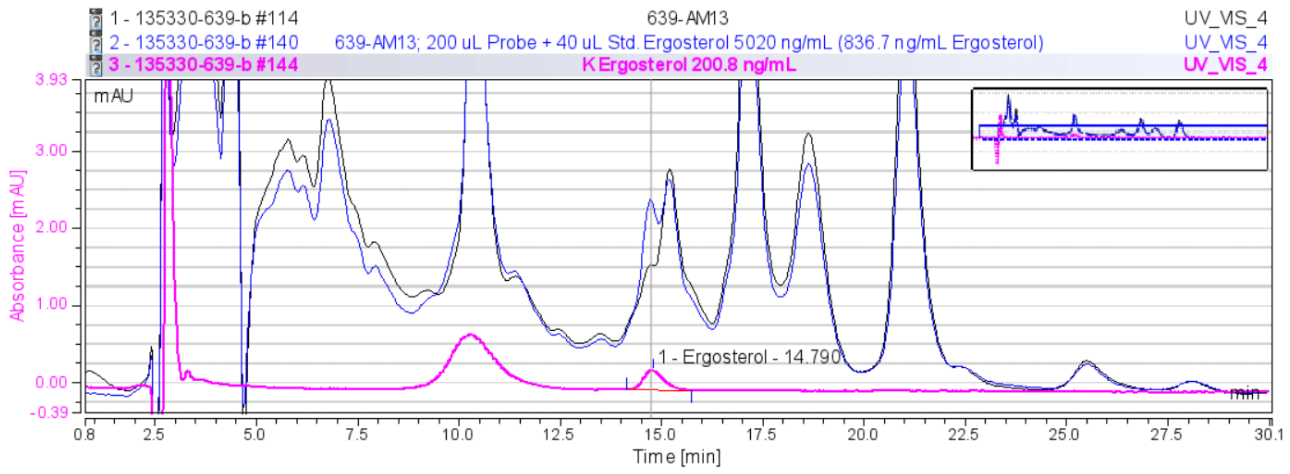


Figure 3.20: Overlap of the three chromatograms of the mycorrhized *Alnus incana* sample AM13 (black), the AM13 sample with an additional Ergosterol stock solution injection (blue) and the Ergosterol stock solution (pink). The vertical gray line indicates the location of the detected Ergosterol stock solution.

To address the real case of this designated pollution, chromatograms of the Ergosterol stock solution were consulted. This chromatogram in figure 3.21 shows no high peaks around the Ergosterol peak. Therefore, the noise resulted from the Ergosterol liquid phase extraction and not from the HPLC analysis. To address the real case of this designated pollution, chromatograms

of the Ergosterol stock solution were consulted. This chromatogram in figure 3.21 shows no high peaks around the Ergosterol peak. Therefore, the noise resulted from the Ergosterol liquid phase extraction and not from the HPLC analysis.

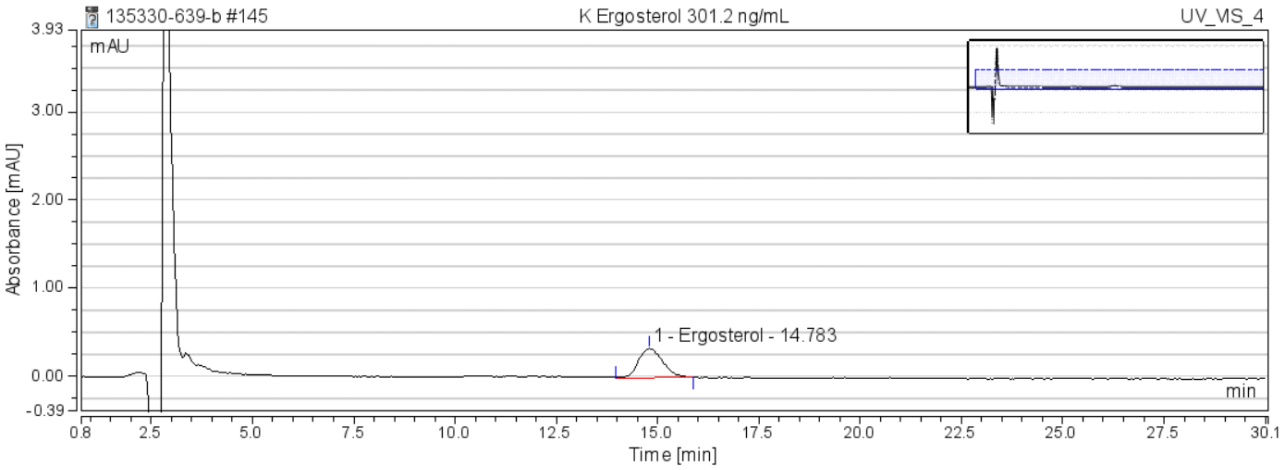


Figure 3.21: This figure displays the chromatogram of an Ergosterol stock solution.

3.4.1 Ergosterol Recovery After Hexane Extraction

The results of the hexane extraction, which was conducted with four different samples, are illustrated in table 3.6. Ergosterol recovery after hexane extraction revealed values between 10'012.2 and 11'228.6 ng Ergosterol per g sample. Deviation values ranged between 0.1 and 10.8 %. In the chromatogram of the figure 3.22 the blue curve represents the hexane sample number 21 (Appendix V.III.1).

Table 3.6: Recovery in [ng g^{-1}], recovery in [%] and deviation values [%] of four samples after hexane extraction and HPLC analysis. In each sample 1 ml Ergosterol stock solution (10.04 μg ml^{-1}) was added to the ethanol-methanol-KOH mixture.

Sample No.	**Recovery [ng g^{-1}]**	**Recovery [%]**	**Deviation [%]**
20	11080.6	110.8	10.8
21	10012.2	100.1	0.1
22	10722.5	107.2	7.2
23	11228.6	112.3	12.3

3.4.2 Ergosterol Recovery After Liquid Phase Extraction

After the HPLC analysis of the non-mycorrhized soil samples with the additional volume of 1 ml Ergosterol stock solution, the recovery values ranged between 7'405.7 and 8'673.9 ng Ergosterol per g sample. Therefore, the retrieval rates were between 74.1 and 86.7 %, and the corresponding total extraction losses between 25.9 % and 13.3 %, respectively (Table 3.7). In the chromatogram of the figure 3.22 the black curve represents the liquid phase extraction using a non-mycorrhized soil sample (Appendix V.III.2).

Table 3.7: Recovery in [ng g^{-1}], recovery in [%] and deviation values [%] of six soil samples after Ergosterol liquid phase extraction and HPLC analysis. Each sample consisted of the ethanol-methanol-KOH mixture, 2 g of non-mycorrhized soil and 1 ml Ergosterol stock solution (10.04 μg ml^{-1}).

Sample No.	**Recovery [ng g^{-1}]**	**Recovery [%]**	**Deviation [%]**
119	8663.2	86.6	13.4
121	7405.7	74.1	25.9
134	8648.4	86.5	13.5
135	7491.4	74.9	25.1
138	8673.9	86.7	13.3
139	7500.8	75.0	25.0

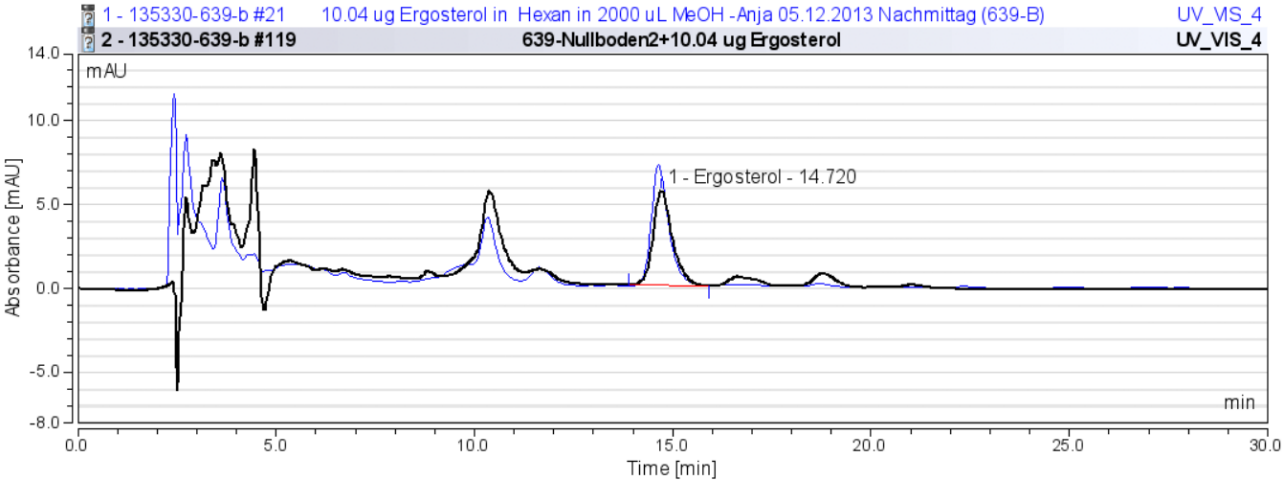


Figure 3.22: This figure shows two chromatograms. The blue curve displays the hexane extraction (Sample No. 21) and the black one the liquid phase extraction using non-mycorrhized soil (Sample No. 119).

Recovery values, standard deviation (sd) and coefficient of variance (CV) of the hexane extractions and the extractions of the non-mycorrhized soil samples are summarized in the table 3.8. The coefficient of variance was calculated by the fraction of the standard deviation and the mean value. Hexane extraction revealed a mean recovery value of 10'761.0 ng Ergosterol per g sample, which is much higher compared to the recovery values of the soil extraction (mean = 8'063.9 [ng g^{-1}]). It can therefore be concluded, that the conducted Ergosterol liquid phase extraction resulted in an averaged extraction loss of 19.4 %.

Table 3.8: Summarized mean recovery in [ng g^{-1}], recovery in [%], standard deviation (sd) [ng g^{-1}] and coefficient of variance (CV) [%] of the mean values of the hexane extraction and the extraction with non-mycorrhized soil

Extraction	**Recovery Mean [ng g^{-1}]**	**Recovery [%]**	**Sd [ng g^{-1}]**	**CV [%]**
Hexane	10761.0	107.6	542.5	5.0
Soil	8063.9	80.6	655.9	8.1

3.5 Plant Biomass

3.5.1 Germination and Growth of Plants

The transplanting of some surplus *Alnus incana* seedlings from different samples but the same treatment was successful, resulting in three *Alnus incana* individuals per sample. Less successful was the transfer of all samples into the climate chamber which resulted in the death of some *Alnus incana* seedlings of the combined treatment of *Alnus incana* and *Poa pratensis* (AP) (Figure 3.23). After the growing period, growth of *Alnus incana* in the AM treatment was markedly higher compared to the A treatment (Figure 3.24). The germination success of *Poa pratensis* was high and satisfactory. Further, growth of *Poa pratensis* was similar between the non-mycorrhized and the corresponding mycorrhized treatment.

Figure 3.23: Left: Sample AP3 of the combined non-mycorrhized treatment of *Alnus incana* and *Poa pratensis* showing dead *Alnus incana* saplings. Right: Corresponding mycorrhized sample APM3 with healthy *Alnus incana* saplings.

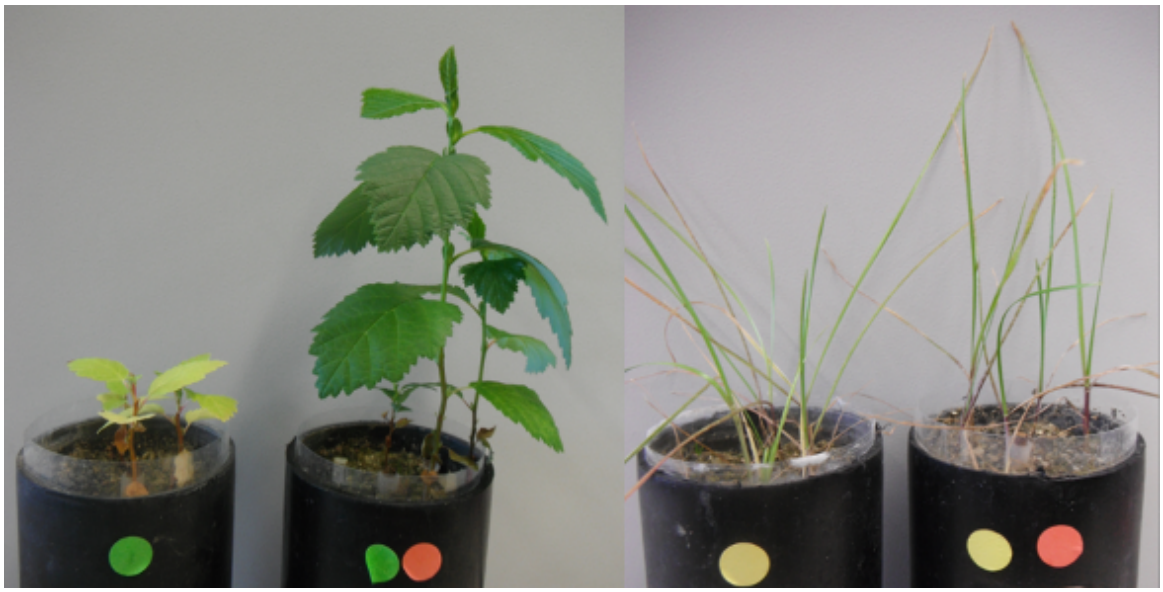


Figure 3.24: From left to right: Non-mycorrhized sample of the *Alnus incana* treatment (A4), compared to the mycorrhized sample of the *Alnus incana* treatment (AM5), followed by the non-mycorrhized sample of the *Poa pratensis* treatment (P3), compared to the corresponding mycorrhized treatment (PM3). The sticker colours refer to the specific treatments (*Alnus incana* - green; *Poa pratensis* - yellow; Mycorrhized samples - orange).

3.5.2 Root Length

Root length per soil volume varies considerably between the treatments of *Alnus incana* (A, AM) and the other treatments (Figure 3.25). Mean and median values of the root length per soil volume were considerably higher in the non-mycorrhized treatments (A, P, AP) compared to the corresponding mycorrhized teratments (AM, PM, APM).

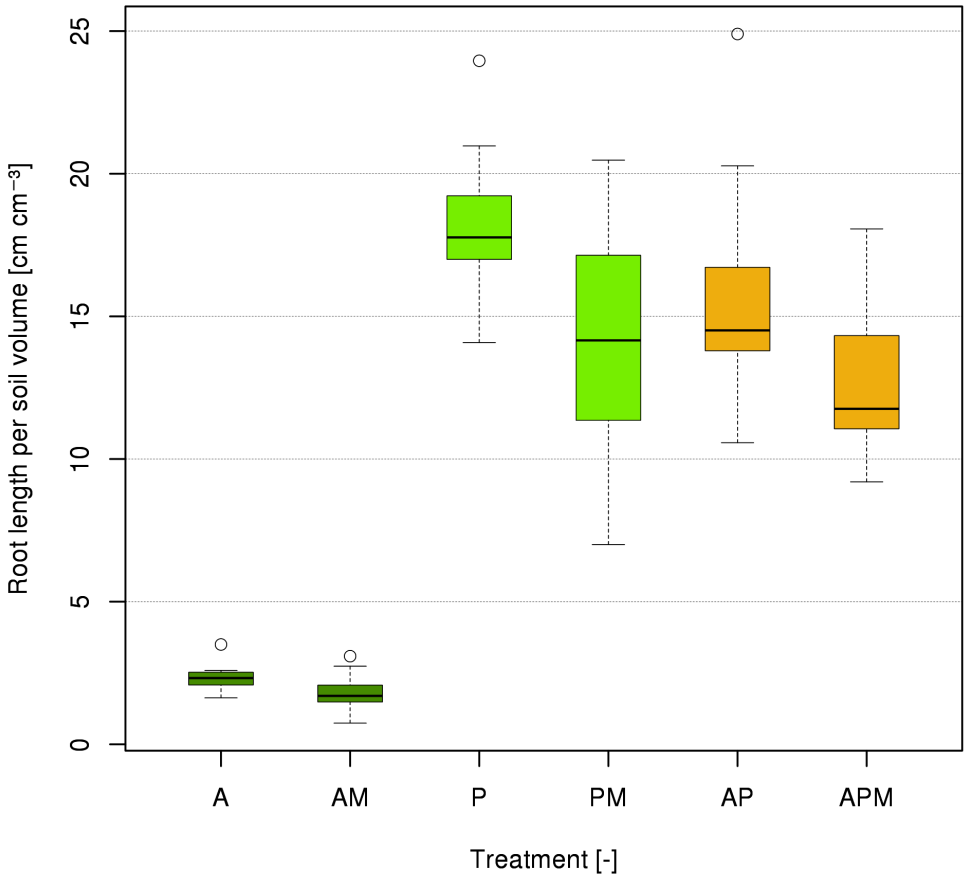


Figure 3.25: Root length per soil volume [cm cm^{-3}] of all non-mycorrhized treatments abbreviated by A (*Alnus incana*), P (*Poa pratensis*), AP (*Alnus incana* and *Poa pratensis*) and the corresponding mycorrhized (M) treatments by AM, PM and APM.

Considering each treatment separately, mean values were higher than the median values except within the PM treatment. The root length per soil volume of A (median = 2.321 [cm cm^{-3}]) and AM (median = 1.697 [cm cm^{-3}]) showed low values which were narrowly distributed. In contrary, the scattering of the treatments P, PM, AP and APM was much broader. The highest median value of the root length per soil volume could be observed in the non-mycorrhized treatment of P (median = 17.768 [cm cm^{-3}]). The treatment of non-mycorrhized AP reached slightly lower

values (median = 14.509 [cm cm^{-3}]) (Appendix VI.I; Table 6.9).
A significant difference among the treatments was observed with respect to the root length per soil volume (Kruskal-Wallis rank sum test: chi-squared = 58.219, df = 5, p-value = 2.834e-11) (Hollander and Wolfe, 1973). According to the pairwise Wilcoxon rank sum test, using the p-value adjustment method of Holm (1979), a significant difference was revealed between the treatment A compared to P, PM, AP and APM (p-value = 2.9e-06). Similar results were observed for the AM treatment and P, PM, AP and APM. The comparison of the treatment P to PM and APM revealed a p-value of 0.04 and 0.004, respectively (Table 3.9).

Table 3.9: P-values of the pairwise Wilcoxon rank sum test of the root length per soil volume using the adjustment method of Holm (1979). Non-mycorrhized treatments are abbreviated by A (*Alnus incana*), P (*Poa pratensis*), AP (*Alnus incana* and *Poa pratensis*) and the corresponding mycorrhized (M) treatments by AM, PM and APM.

Treatment	**A**	**AM**	**P**	**PM**	**AP**
AM	0.169				
P	2.9e-06	2.9e-06			
PM	2.9e-06	2.9e-06	0.044		
AP	2.9e-06	2.9e-06	0.169	0.724	
APM	2.9e-06	2.9e-06	0.004	0.724	0.169

To evaluate the accuracy of the root length scanning with the software WinRhizo, mean, median and standard deviation values of the ten times repeated root length measurements are displayed in table 3.10. The standard deviation of the root length of the sample AM13 varied from 0.8 to 12.2 cm (1.6-5.9 % relative to the mean). Similar results were observed in the sample PM13, in which the root lenght standard deviation differed between 27.6 to 68.3 cm (4-9.6 %). The root length of *Alnus incana* in the combined sample APM13 (*Alnus incana* and *Poa pratensis*) varied between 3.1 to 4.5 cm (2-8.2 %), the root length of *Poa pratensis* between 40.1 to 70.1 cm (3.3-6.8 %). High coefficients of variance were found in PM13 and APM13 samples with *Poa pratensis*, low coefficients in AM13 or APM13 samples with *Alnus incana*. Within all measurements, the standard deviation values of the corresponding mean values were smaller than 10 %, indicating a high accuracy.

Table 3.10: Mean, median and standard deviation (sd) of the total root length in [cm] and coefficient of variance (CV) in [%]. Values are from the ten times measured mycorrhized samples AM13 (*Alnus incana*), PM13 (*Poa pratensis*) and the sample APM13 (*Alnus incana* and *Poa pratensis*). The APM13 sample is further divided into APM13A which refers to the roots of *Alnus incana* and APM13P for the roots of *Poa pratensis*.

Treatment	**Mean**	**Median**	**sd**	**CV**
AM13A1	206.556	207.654	12.2	5.9
AM13A2	34.121	34.165	0.8	2.4
AM13A3	66.749	66.826	1.1	1.6
PM13P1	671.71	677.336	27.6	4.1
PM13P2	816.036	823.624	32.4	4.0
PM13P3	779.703	802.841	68.3	8.8
PM13P4	690.090	707.144	66.1	9.6
APM13A1	90.590	91.003	3.8	4.2
APM13A2	37.749	38.081	3.1	8.2
APM13A3	223.384	224.063	4.5	2.0
APM13P1	1027.415	1040.025	57.3	5.6
APM13P2	1214.834	1209.025	40.1	3.3
APM13P3	1005.763	1032.376	68.8	6.8
APM13P4	1122.776	1152.926	70.1	6.2

3.5.3 Dry Weight

3.5.3.1 Aboveground Plant Dry Weight

The dry weight of the aboveground plant biomass varied considerably among the treatments and, particularly, between non-mycorrhized and the corresponding mycorrhized treatments (Figure 3.26). The mycorrhized treatment of *Alnus incana* (AM) as well as the combined treatment of *Alnus incana* and *Poa pratensis* (APM) showed a higher plant dry weight compared to the corresponding non-mycorrhized treatments.

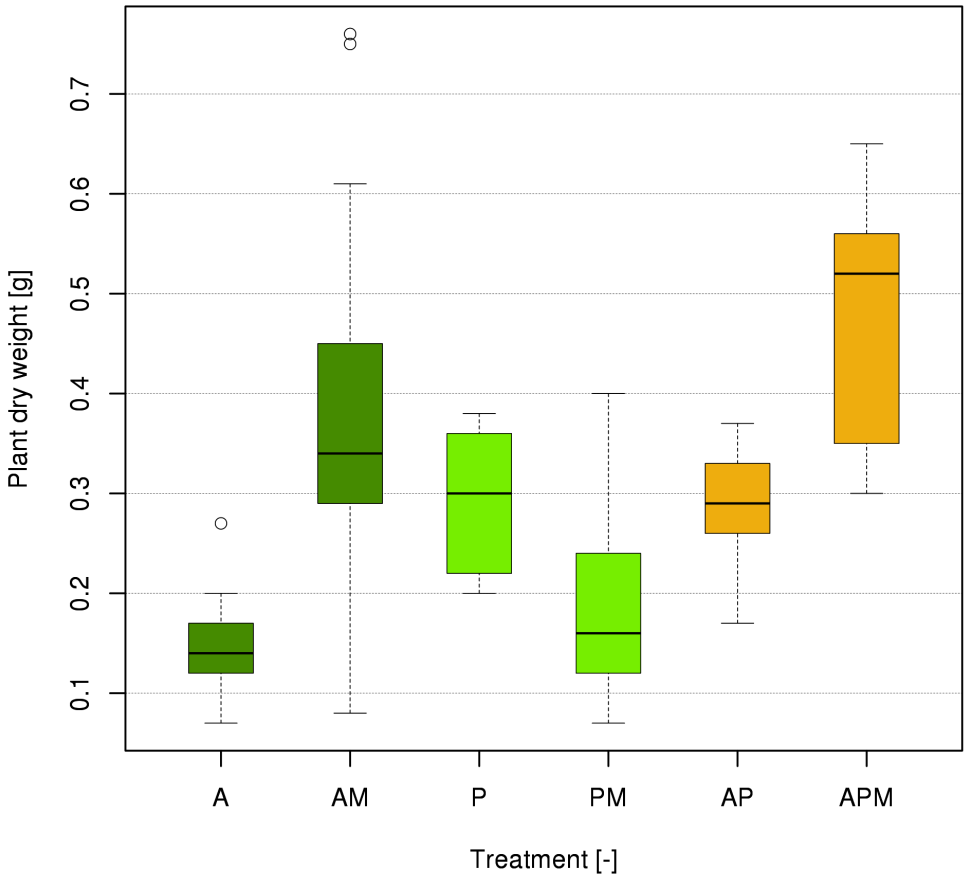


Figure 3.26: Aboveground plant dry weight [g] of the non-mycorrhized treatments A (*Alnus incana*), P (*Poa pratensis*), AP (*Alnus incana* and *Poa pratensis*) and the corresponding mycorrhized (M) treatments AM, PM and APM.

In contrary, the mycorrhized treatment of *Poa pratensis* (PM) showed a lower plant dry weight than the non-mycorrhized treatment P. The highest amount of aboveground plant dry mass was observed in the APM treatment resulting in a median value of 0.52 g. Treatment A revealed the lowest aboveground plant dry mass with a median value of 0.14 g. Mean values of P, AP and APM yielded a lower aboveground plant dry weight compared to the corresponding median values. With respect to the A, AM and PM treatment mean values were higher than median values (Appendix VI.II; Table 6.11).

Table 3.11: P-values of the pairwise Wilcoxon rank sum test of the aboveground plant dry weight using the adjustment method of Holm (1979). Non-mycorrhized treatments abbreviated by A (*Alnus incana*), P (*Poa pratensis*), AP (*Alnus incana* and *Poa pratensis*) and the corresponding mycorrhized (M) treatments by AM, PM and APM.

Treatment	**A**	**AM**	**P**	**PM**	**AP**
AM	0.008				
P	7.2e-04	0.683			
PM	0.910	0.035	0.036		
AP	1.24e-03	0.618	0.910	0.036	
APM	2.4e-04	0.618	0.008	0.001	0.002

According to the Kruskal-Wallis rank sum test, aboveground plant dry weight significantly differed within the treatments (Chi-squared = 43.566, df = 5, p-value = 2.838e-08) (Hollander and Wolfe, 1973). The pairwise Wilcoxon test with the p-value adjustment method of Holm (1979) showed a significant difference between the treatment A and AM (p-value = 0.008), P (p-value = 7.2e-04), AP (p-value = 1.24e-03), APM (p-value = 2.4e-04). Similar results were observed between the treatment AM and PM (p-value = 0.035), P and PM (p-value = 0.036), the treatment AP and PM (p-value = 0.036), APM (p-value = 0.002) and between the treatment APM and P (p-value = 0.008), PM (p-value = 0.001) (Table 3.11).

3.5.3.2 Root Dry Weight

The results of the root dry weight were rather contradictory to those of the aboveground plant dry weight. Treatments of *Alnus incana* (A, AM) revealed the lowest root dry weight (Figure 3.27). Mycorrhized treatments of the *Poa pratensis* (PM) and the combined treatment of *Alnus incana* and *Poa pratensis* (APM) showed smaller root dry weight values compared to the corresponding non-mycorrhized treatments. The highest amount of root dry weight was achieved by the treatment of P with a median value of 0.343 g, followed by the AP treatment with a median value of 0.313 g. Kruskal-Wallis and pairwise Wilcoxon rank sum tests were applied (Hollander and Wolfe, 1973). The treatment significantly affects the root dry weight (Chi-squared = 57.033, df = 5, p-value = 4.979e-11). The pairwise Wilcoxon rank sum test with the p-value adjustment method of Holm (1979) neither revealed a significant difference between the treatment A and AM (p-value = 0.817) nor between PM and APM (p-value= 0.766). All other treatment combinations were significant (Table 3.12).

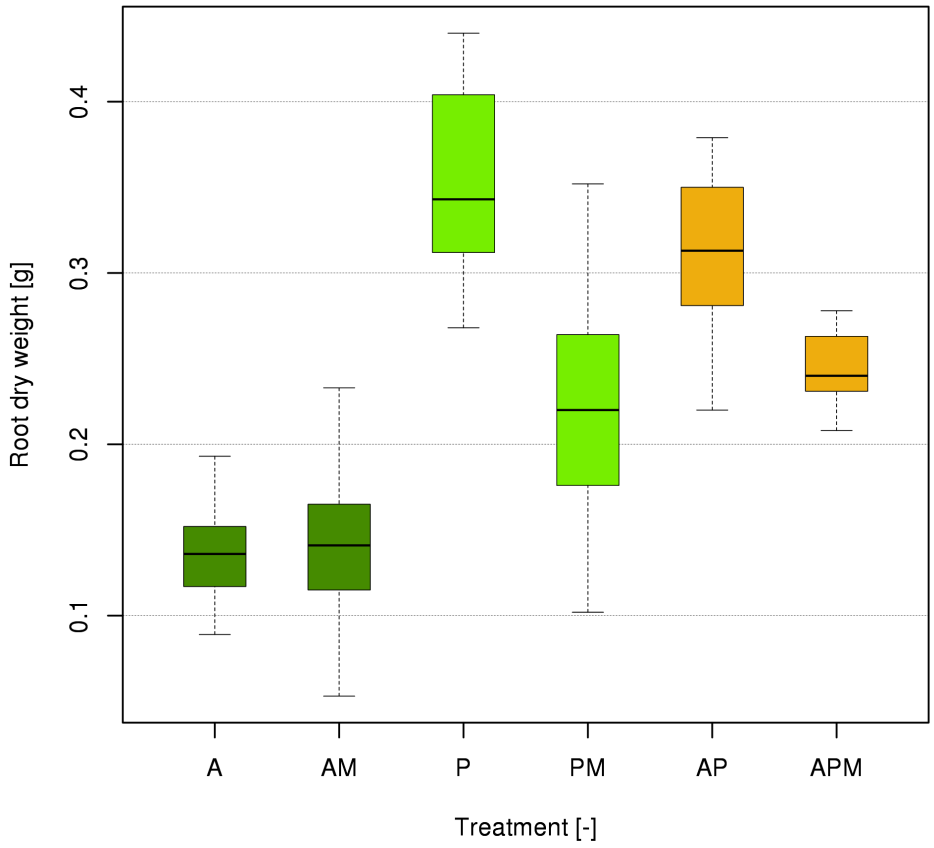


Figure 3.27: Root dry weight [g] of the non-mycorrhized treatments A (*Alnus incana*), P (*Poa pratensis*), AP (*Alnus incana* and *Poa pratensis*) and the corresponding mycorrhized (M) treatments AM, PM and APM.

Table 3.12: P-values of the pairwise Wilcoxon rank sum test of the root dry weight using the adjustment method of Holm (1979). Non-mycorrhized treatments abbreviated by A (*Alnus incana*), P (*Poa pratensis*), AP (*Alnus incana* and *Poa pratensis*) and the corresponding mycorrhized (M) treatments by AM, PM and APM.

Treatment	**A**	**AM**	**P**	**PM**	**AP**
AM	0.817				
P	2.9e-06	2.9e-06			
PM	0.011	0.038	0.001		
AP	2.9e-06	4.2e-06	0.339	0.019	
APM	2.9e-06	0.001	7.7e-06	0.766	0.001

3.5.3.3 Total Plant Biomass

The mycorrhized *Alnus incana* treatment (AM) revealed a higher plant biomass compared to the corresponding non-mycorrhized treatment A. Similar results were observed concerning the mycorrhized combined treatment APM compared to the non-mycorrhized AP treatment. The treatments of *Poa pratensis* (P, PM) showed contradictory results. A higher plant biomass was observed within the non-mycorrhized treatment P, compared to the mycorrhized treatment PM (Figure 3.28).

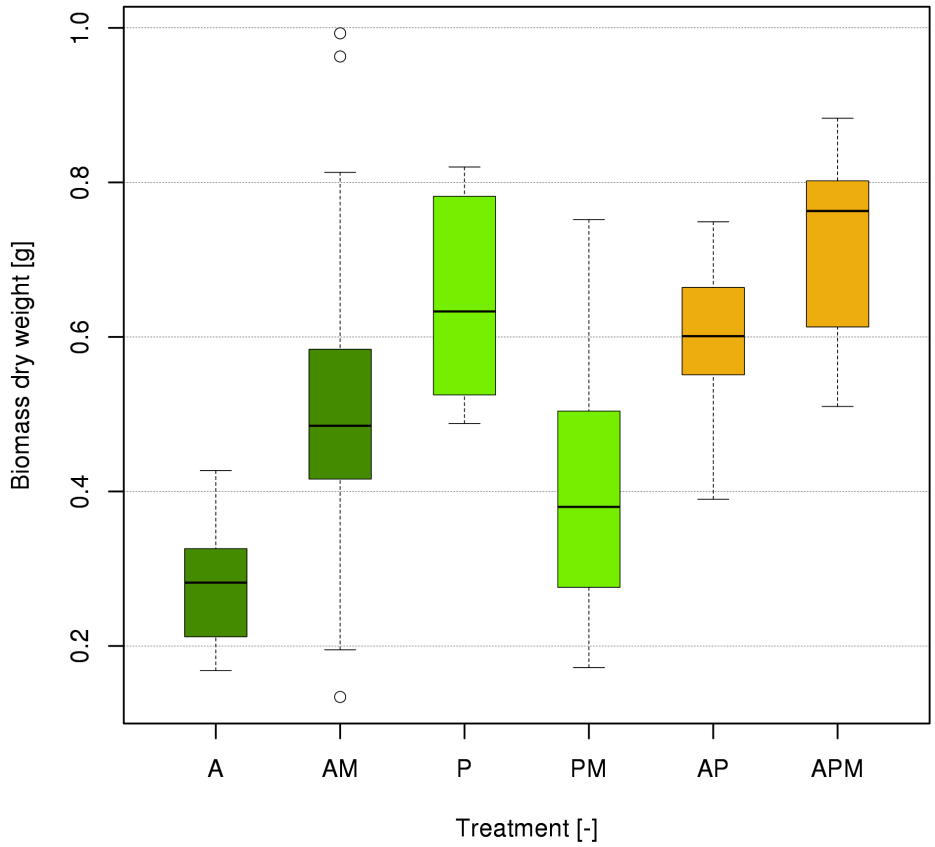


Figure 3.28: Dry weight of the total plant biomass [g]. Non-mycorrhized treatments are abbreviated by A (*Alnus incana*), P (*Poa pratensis*), AP (*Alnus incana* and *Poa pratensis*) and the corresponding mycorrhized (M) treatments by AM, PM and APM.

The highest total plant biomass was achieved by the combined treatment APM with a median value of 0.763 g, followed by the treatments P and AP with median values of 0.633 g and 0.601 g, respectively. Treatment A revealed the lowest total plant biomass (median = 0.282 [g]). Mean values of the treatments AM, P and PM were higher compared to the median values and vice versa considering the treatments A, AP and APM. All mean and median values concerning the

dry weight of the total plant biomass are displayed in table 6.12 (Appendix VI.IV).
The Kruskal-Wallis rank sum test showed a significant difference at least between two different treatments (Chi-squared = 39.587, df = 5, p-value = 1.809e-07) (Hollander and Wolfe, 1973). Treatments were further analyzed by applying the pairwise Wilcoxon rank sum test with the adjustment method of Holm (1979) (Table 3.13). A significant difference was revealed between treatment A and P (p-value = 2.9e-06), AP (p-value = 1.0e-05) and APM (p-value = 2.9e-06). Similar significance was observed by comparing the treatment PM to P (p-value = 0.005), AP (p-value = 0.035) and APM (p-value = 0.002).

Table 3.13: P-values of the pairwise Wilcoxon rank sum test of the total plant biomass dry weight using the adjustment method of Holm (1979). Non-mycorrhized treatments abbreviated by A (*Alnus incana*), P (*Poa pratensis*), AP (*Alnus incana* and *Poa pratensis*) and the corresponding mycorrhized (M) treatments by AM, PM and APM.

Treatment	**A**	**AM**	**P**	**PM**	**AP**
AM	0.065				
P	2.9e-06	0.565			
PM	0.385	0.565	0.005		
AP	1.0e-05	0.565	0.565	0.035	
APM	2.9e-06	0.206	0.565	0.002	0.131

3.6 Linear Regression Models

3.6.1 Soil Aggregate Stability Versus Root Length

The global linear regression model showed a significant effect of the root length per soil volume on soil aggregate stability with a p-value of <2.2e-16 and a significant intercept (Table 3.14). Table 3.14 gives an overview of the corresponding linear regression model parameters. In contrast, regarding the linear regression for the root length per soil volume and soil aggregate stability within each treatment, only the models of the AP and PM treatment were significant, showing a significant slope but a not significant intercept (Figure 3.29). The squared Pearson correlation (R^2) values of the AP and PM treatment were 0.525 and 0.461, and the corresponding Spearman rho rank correlation coefficients were 0.74 and 0.63, respectively (Spearman, 1904) (Appendix VII).

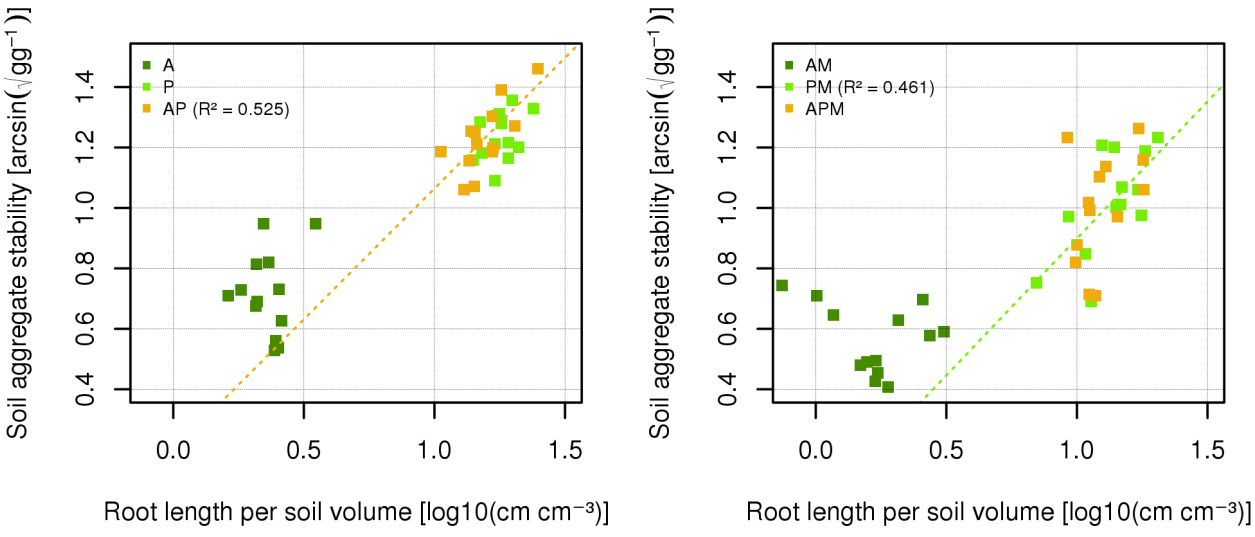


Figure 3.29: The linear regression models showed significant effects of the root length per soil volume on soil aggregate stability for two models: The models of the AP and PM treatment were significant with a significant slope and a squared Pearson correlation (R^2) of 0.525 and 0.461. Left: Non-mycorrhized treatments abbreviated by A (*Alnus incana*), P (*Poa pratensis*), AP (*Alnus incana* and *Poa pratensis*). Right: Corresponding mycorrhized (M) treatments AM, PM, APM.

Table 3.14: Summary of the linear regression model lm(asin(sqrt(Soil aggregate stability)) ∼ Groups * $\log_{10}$(Root length)). Non-mycorrhized treatments are abbreviated by A (*Alnus incana*), P (*Poa pratensis*), AP (*Alnus incana* and *Poa pratensis*) and the corresponding mycorrhized (M) treatments by AM, PM, APM.

Coefficients	**Estimate**	**Std. Error**	**t value**	**Pr(> |t|)**
Intercept	0.642	0.163	3.939	2.0e-4 ***
AM	-0.032	0.173	-0.185	0.854
AP	-0.44	0.486	-0.911	0.366
APM	-0.477	0.430	-1.109	0.271
P	0.002	0.733	0.003	0.997
PM	-0.649	0.355	-1.828	0.072
$\log_{10}$(Root length)	0.208	0.442	0.471	0.639
AM: $\log_{10}$(Root length)	-0.405	0.488	-0.830	0.409
AP: $\log_{10}$(Root length)	0.657	0.585	1.123	0.265
APM: $\log_{10}$(Root length)	0.556	0.571	0.975	0.333
P: $\log_{10}$(Root length)	0.266	0.721	0.369	0.714
PM: $\log_{10}$(Root length)	0.698	0.522	1.337	0.186
Residual standard error	0.1246 on 66 degrees of freedom			
Multiple R-squared	0.834	Adjusted R-squared		0.807
F-statistic	30.22 on 11 and 66 DF		P-value	< 2.2e-16

3.6.2 Soil Aggregate Stability Versus Water Content

Soil aggregate stability was significantly affected by the water content showing some significant slopes and intercepts, revealing a p-value of <2.2e-16 (Table 3.15). An overview of the linear regression model parameters is illustrated in table 3.15. Considering each treatment separately, models for A, AP and PM were significant (Figure 3.30). All these models revealed a significant slope and intercept. The corresponding squared Pearson correlation (R^2) of A, AP and PM was 0.559, 0.393 and 0.515, respectively. Applying the Spearman correlation, the rho rank coefficient values of A, AP and PM were -0.75, -0.74 and -0.81, respectively (Spearman, 1904) (Appendix VIII).

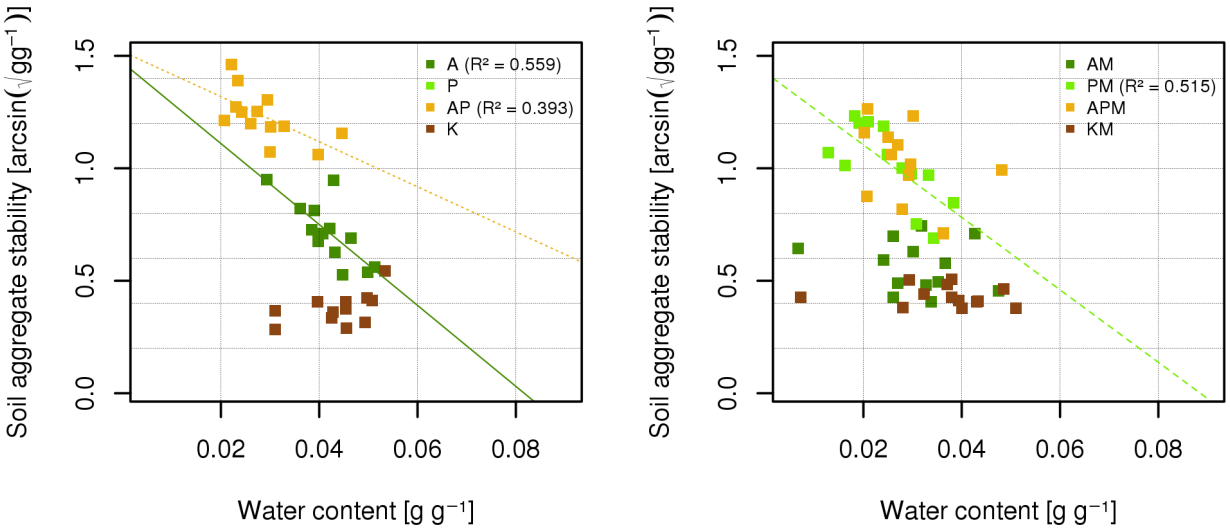


Figure 3.30: The linear regression models showed significant effects of the water content on soil aggregate stability. Considering each treatment separately, the models of the treatments A, AP and PM revealed significant slopes and a significant intercept. The corresponding squared Pearson correlation (R^2) of A, AP and PM was 0.559, 0.393 and 0.515, respectively. Left: Non-mycorrhized treatments are abbreviated by A (*Alnus incana*), P (*Poa pratensis*), AP (*Alnus incana* and *Poa pratensis*), K (control). Right: Corresponding mycorrhized (M) treatments AM, PM, APM, KM.

Table 3.15: Summary of the linear regression model lm(asin(sqrt(Soil aggregate stability)) ∼ Groups * (Water content)). Non-mycorrhized treatments are abbreviated by A (*Alnus incana*), P (*Poa pratensis*), AP (*Alnus incana* and *Poa pratensis*), K (control) and the corresponding mycorrhized (M) treatments by AM, PM, APM, KM.

Coefficients	**Estimate**	**Std. Error**	**t value**	**Pr(> |t|)**
Intercept	1.470	0.225	6.540	4.2e-09 ***
AM	-0.835	0.246	-3.393	0.001 **
AP	0.049	0.259	0.190	0.850
APM	-0.171	0.254	-0.671	0.504
K	-1.323	0.300	-4.402	3.1e-05 ***
KM	-1.018	0.248	-4.100	9.4e-05 ***
P	-0.161	0.262	-0.613	0.541
PM	-0.044	0.248	-0.176	0.861
Water content	-17.992	5.323	-3.380	0.001 **
AM: Water content	15.722	6.164	2.551	0.013 *
AP: Water content	7.974	6.886	1.158	0.250
APM: Water content	7.818	6.633	1.179	0.242
K: Water content	23.226	6.966	3.334	0.001 **
KM: Water content	17.445	6.002	2.906	0.005 **
P: Water content	15.119	7.744	1.952	0.054
PM: Water content	1.882	6.644	0.283	0.778
Residual standard error	0.1062 on 86 degrees of freedom			
Multiple R-squared	0.919	Adjusted R-squared		0.905
F-statistic	65.24 on 15 and 86 DF		P-value	< 2.2e-16

3.6.3 Soil Aggregate Stability Versus Dry Unit Weight

The global linear regression model showed a significant effect of the dry unit weight on soil aggregate stability with a p-value of <2.2e-16 and some significance in slope and intercept (Table 3.16). In contrast, regarding the linear regression for each treatment separately, no significant model was observed (Figure 3.31).

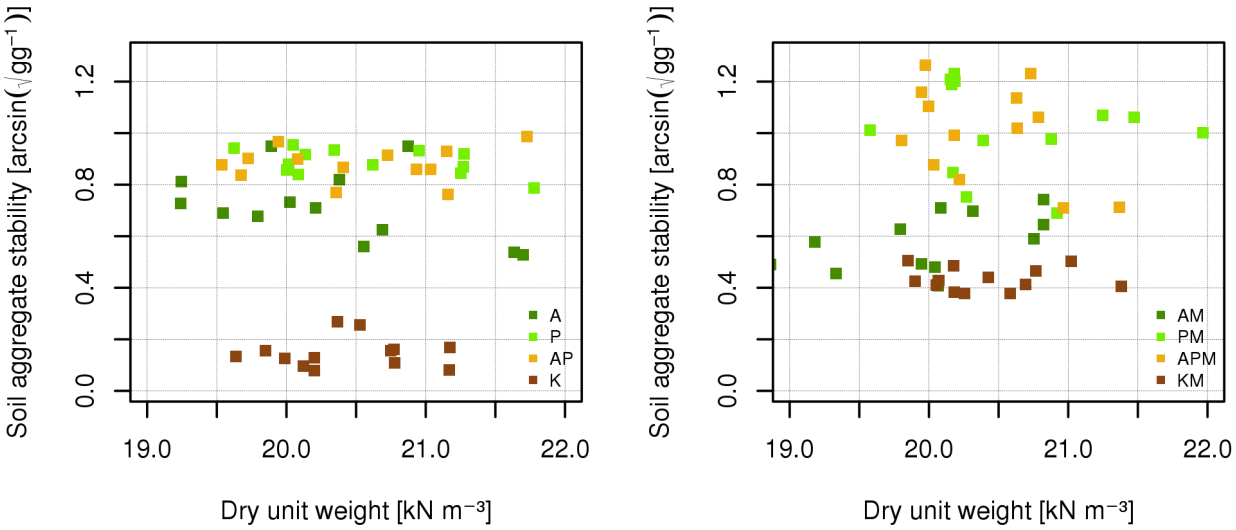


Figure 3.31: The linear regression models revealed no significant effects of the dry unit weight on soil aggregate stability. Left: Non-mycorrhized treatments are abbreviated by A (*Alnus incana*), P (*Poa pratensis*), AP (*Alnus incana* and *Poa pratensis*). Right: Corresponding mycorrhized (M) treatments AM, PM, APM, KM.

Table 3.16: Summary of the linear regression model lm(asin(sqrt(Soil aggregate stability)) ∼ Groups * (Dry unit weight)). Non-mycorrhized treatments are abbreviated by A (*Alnus incana*), P (*Poa pratensis*), AP (*Alnus incana* and *Poa pratensis*), K (control) and the corresponding mycorrhized (M) treatments by AM, PM, APM, KM.

Coefficients	**Estimate**	**Std. Error**	**t value**	**Pr(> |t|)**
Intercept	2.338	0.890	2.626	0.010 *
AM	-3.567	1.448	-2.463	0.016 *
AP	-1.737	1.374	-1.264	0.210
APM	1.896	1.737	1.091	0.278
K	-2.061	1.722	-1.197	0.235
KM	-1.852	1.797	-1.031	0.306
P	0.034	1.397	0.024	0.981
PM	-0.489	1.398	-0.350	0.728
Dry unit weight	-0.080	0.044	-1.822	0.072
AM: Dry unit weight	0.170	0.072	2.356	0.021 *
AP: Dry unit weight	0.111	0.067	1.644	0.104
APM: Dry unit weight	-0.078	0.085	-0.919	0.360
K: Dry unit weight	0.085	0.084	1.011	0.315
KM: Dry unit weight	0.077	0.088	0.877	0.383
P: Dry unit weight	0.025	0.068	0.363	0.718
PM: Dry unit weight	0.039	0.068	0.578	0.565
Residual standard error	0.121 on 88 degrees of freedom			
Multiple R-squared	0.895	Adjusted R-squared		0.877
F-statistic	49.96 on 15 and 88 DF		P-value	< 2.2e-16

3.6.4 Plant Dry Weight Versus Root Length

The simple linear regression model showed a significant effect of the root length per soil volume on the aboveground plant dry weight with a p-value <2.2e-16 and a significance in slope and intercept (Figure 3.32). An overview of the corresponding model parameters is illustrated in table 6.21. Considering each treatment separately, the model of AP, AM and PM were significant yielding a significant difference in slope and intercept. The corresponding squared Pearson correlation (R^2) of AP, AM and PM was 0.570, 0.708 and 0.534, respectively. Applying Spearman correlation the treatments A, AP, AM and PM revealed a rho rank coefficient value of 0.55, 0.76, 0.87 and 0.72, respectively (Spearman, 1904) (Appendix IX).

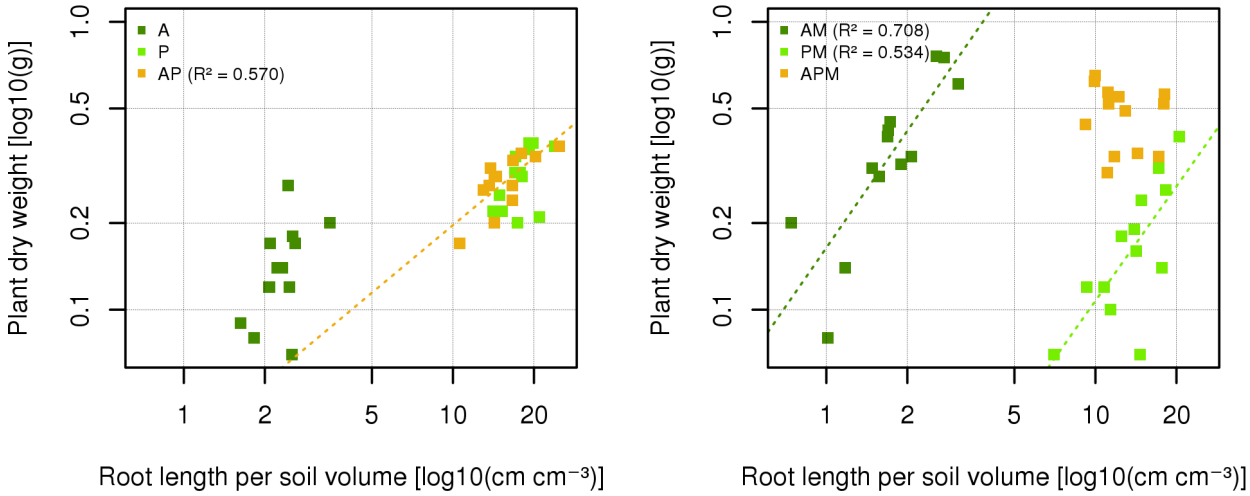


Figure 3.32: The linear regression model lm($\log_{10}$(Plant dry weight) $\sim$ Groups * $\log_{10}$(Root length)) showed a significant effect of root length per soil volume on aboveground plant dry weight. Considering each treatment separately, the models of the AP, AM and PM treatment were significant in slope and intercept. The corresponding squared Pearson correlation (R^2) was 0.570, 0.708 and 0.534, respectively. Left: Non-mycorrhized treatment are abbreviated by A (*Alnus incana*), P (*Poa pratensis*), AP (*Alnus incana* and *Poa pratensis*). Right: Corresponding mycorrhized (M) treatments AM, PM, APM. Significant positive effects were found in the treatments of A, AP and AM.

Table 3.17: Summary of the linear regression model lm($\log_{10}$(Plant dry weight) $\sim$ Groups * $\log_{10}$(Root length)). Non-mycorrhized treatments are abbreviated by A (*Alnus incana*), P (*Poa pratensis*), AP (*Alnus incana* and *Poa pratensis*) and the corresponding mycorrhized (M) treatments by AM, PM, APM.

Coefficients	**Estimate**	**Std. Error**	**t value**	**Pr(> |t|)**
Intercept	-1.260	0.169	-7.454	2.51e-10 ***
AM	0.475	0.180	2.648	0.010 *
AP	-0.225	0.504	-0.447	0.656
APM	1.138	0.446	2.553	0.013 *
P	-0.378	0.760	-0.497	0.621
PM	-1.030	0.368	-2.796	0.007 **
$\log_{10}$(Root length)	1.082	0.459	2.358	0.021 *
AM: $\log_{10}$(Root length)	0.268	0.506	0.529	0.599
AP: $\log_{10}$(Root length)	-0.302	0.607	-0.498	0.620
APM: $\log_{10}$(Root length)	-1.272	0.592	-2.148	0.035 *
P: $\log_{10}$(Root length)	-0.207	0.748	-0.277	0.782
PM: $\log_{10}$(Root length)	0.239	0.542	0.442	0.660
Residual standard error	0.1293 on 66 degrees of freedom			
Multiple R-squared	0.7782	Adjusted R-squared		0.7412
F-statistic	21.05 on 11 and 66 DF		P-value	< 2.2e-16

4 Discussion

4.1 Plant and Fungus Association

This section focuses on the germination, growth and mycorrhizal fungi of the two plant species *Alnus incana* and *Poa pratensis*. The germination success was high and satisfactory considering plants of *Poa pratensis* but not satisfactory with respect to *Alnus incana*. This problem was solved by transplanting surplus *Alnus incana* seedlings from different samples but the same treatment. After transferring the samples to the climate chamber the mortality of *Alnus incana* increased especially in the combined non-mycorrhized treatment (AP). The mortality may be attributed to a high heat radiation due to the spotlights. The soil surface dryed out rapidly which caused the death of some *Alnus incana* saplings. Similar problems were described by Frei (2009) who also observed high mortality in the climate chamber. Another possible reason of the higher mortality might be explained by the mycorrhization. Besides an improved nutrient availability for the plant, mycorrhizas also enhance water uptake (Lehto and Zwiazek, 2011). Consequently, the soil sample might have been too dry for the plants roots of the non-mycorrhized combined treatment of *Alnus incana* and *Poa pratensis* (AP) to enable water uptake but within the APM treatment ectomycorrhizal fungi could explore a greater soil volume and therefore provided enough water (Kipfer et al., 2012).
Regarding the total biomass, it is likely that the mycorrhized PM treatment revealed no increased biomass compared to the non-mycorrhized P treatment due to the fact that no arbuscular mycorrhizas could be identified in the root systems of *Poa pratensis*. Therefore, it can be assumed that the commercial fungal inoculum did not germinate or *Poa pratensis* did not match with the endomycorrhizal fungus species (*Glomus etunicatum*, *Glomus intraradices* and *Glomus claroideum*) in the commercial inoculum "Forst" (INOQ Forst, 2013) (Appendix II.I). Further influence can be attributed to the greenhouse and the small pot size of the samples (Baath and Hayman, 1984). These suggestions are contradictory to the statements in the study of Smith and Read (2008) where arbuscular mycorrhiza in general are extremely diverse and have a high range of host plants. These findings are supported by detailed distribution analyses of *Glomus claroideum* in Poland which were carried out by Blaszkowski et al. (2003). In this study, *Glomus claroideum* was identified as the third most frequent endomycorrhizal fungus species within 199 root-rhizosphere samples collected from plant species which were cultivated in 106 sites in the western Pomerania voivodeship. The collected soil represented cultivated

sites as well as maritime dunes of northern Poland. Further studies revealed a colonisation of *Glomus claroideum* on *Ammophila arenaria* in maritime dunes in Denmark (Blaszkowski and Czerniawska, 2011), in the Tuchola Forests in Poland (Tadych and Blaszkowski, 2000) where the plants from the families of the Cupressaceae (*Juniperus communis*) and Plantaginaceae (*Plantago lanceolata*) were investigated as well as in Northern Europe and the USA on arable land (Walker and Vestberg, 1998). Concludingly, *Glomus claroideum* has a wide range of host plants and it is not likely that *Poa pratensis* is an exception to that.
Several other studies investigated the symbiosis of *Glomus etunicatum*, *Glomus intraradices* and *Glomus claroideum* with different host plants (Schuessler, 2000; Shirmohammadi and Aliasgharzad, 2013). Antunes et al. (2011) even observed the growth of *Poa pratensis* which was colonised by several arbuscular mycorrhizal fungi including *Glomus claroideum*. Most of these studies show examples of well working mycorrhizations using one or several of the discussed fungal species. The only difference to the present study is that a cultivated inoculum was used instead of a commercial one. It might be possible that mycorrhization was not successful due to fungal spores inside the inoculum which were not capable of germination. Unfortunately, no further research concerning the germination capability of the spores could be conducted due to the dense time management of the thesis. Additional discussion considering the possible reasons why no arbuscular mycorrhizal structures could be identified in the root systems of *Poa pratensis* can be seen in the section 4.3 Degree of Mycorrhization.
Within the treatments of *Alnus incana*, root mycorrhization was identified. *Alnus incana* formed at least one of several possible symbioses with the ectomycorrhizal fungus species *Amanita muscaria*, *Boletus edulis*, *Hebeloma crustuliniforme*, *Laccaria laccata*, *Paxillus involutus*, *Pisolithus tinctorius*, *Thelephora terrestris* and *Xerocomus badius* of the commercial inoculum "Forst" (INOQ Forst, 2013) and two additional cultivated fungi *Melanogaster variegatus* and *Paxillus involutus*. In the study of Graf and Frei (2013) *Alnus incana* species were inoculated with cultivated *Melanogaster variegatus*. The results showed a significantly higher root length and a higher aggregate stability compared to non inoculated samples and mycorrhization of *Melanogaster variegatus* was identified. Unfortunately, mycorrhized roots were not further investigated in the present thesis and no fungus species identification was conducted whereby only the following assumptions can be made. With respect to the study of Graf and Frei (2013) it is likely that some symbiosis was formed between *Alnus incana* and *Melanogaster variegatus*. Roots of *Alnus* are further infected by the N_2-fixing actinomycete *Frankia*, which could also be observed in the present study.
As investigated in many studies *Alnus* species not only form a symbiosis with *Frankia* and ectomycorrhiza but also with arbuscular mycorrhiza (Isopi et al., 1994; Monzón and Azcón, 2001; Orfanoudakis et al., 2010). Analyses from Orfanoudakis et al. (2010) revealed a growth increase in *Alnus glutinosa* plants inoculated with *Frankia* and arbuscular mycorrhiza. In a study of Monzón and Azcón (2001) shoot and root dry weight strongly increased due to an

inoculation with *Glomus mosseae* and *Glomus intraradices*. Also nutrient uptake efficiency of nitrogen and phosphor was observed to be much higher in inoculated plants compared to control samples. Similar results could be observed in a study of Isopi et al. (1994) where *Alnus cordata* was inoculated with *Frankia* and two different arbuscular mycorrhiza strains. Root length was increased and root architecture as well as root branching strongly influenced. It is therefore likely, that *Alnus incana* used endo- and ectomycorrhizal fungi as symbionts.

4.2 Wet-Sieving

In this section, pore water pressure measurements during the wet-sieving procedure are discussed in more detail. Classical soils in nature are often unsaturated which results in a negative pore water pressure (Rahardjo et al., 2013). This negative pore water pressure is highly affected by infiltration, evaporation or transpiration. Pore water pressure in a slope for example increases downhill due to an increased infiltration of rainwater downhill (Rahardjo et al., 2013). Similar findings were observed within the pore water pressure measurements during the wet-sieving procedure of the present thesis. Initial pore water pressure was negative in dry samples and increased continuously during the flooding of the sample. Measurements were slightly different depending on different factors such as the initial water content, the amount of macro- and micropores in the soil, the plant root system and the hyphal network. As illustrated in the results of the wet-sieving procedure the measurement of the non-mycorrhized *Alnus incana* treatment A1 shows a typical curve of the pore water pressure (Figure 3.11). In this example the whole soil sample collapsed before the water reached the ceramic cup of the tensiometer. One reason for a fast slacking is a dry initial soil sample and the resultant pressure of the entrapped air (Tisdall and Oades, 1982). It is further probable, that the root system of *Alnus incana* was not dense enough to hold the soil aggregates together.
Additional pore water pressure analyses were conducted by visualising all treatments in one plot (Figures 3.13, 3.14). After the highly porous ceramic cup of the tensiometer came in contact with the water the pore water pressure strongly increased resulting in high peaks. These peaks revealed pore water pressure values in the positive range which flattened after some seconds into the slightly negative range. It is likely that the overshoot of the high peaks resulted due to the fast wetting and some time was required until the reading of the pore water pressure stabilised. It may also be possible that some soil samples collapsed right after the soil was completely flooded which then resulted in a positive peak. The plot of the smoothing function of all pore water pressure measurements of the planted mycorrhized treatments shows a steeper curve than the non-mycorrhized treatments. This effect may be explained due to a higher aboveground biomass in the treatments with *Alnus incana*, therefore a higher transpiration and consequently

a dryer initial soil sample compared to the non-mycorrhized treatments.
Considering the calculated water content, a significant difference between the mycorrhized planted treatments and the corresponding non-mycorrhized treatments could be observed only within the treatment of *Alnus incana*. AM revealed a significantly lower water content compared to A. Interestingly, the treatments P, PM, AP and APM showed the lowest water content values which indicates a low initial pore water pressure. These findings are in accordance to the smoothed pore water pressure curve. A notable exception is the AP treatment which slightly differed concerning the initial pore water pressure. The highest water content was observed within the control treatment K, followed by the treatments A and KM. These results match with the smoothed curve where the corresponding pore water pressure measurements were less negative. The steeper curve of the pore water pressure with respect to the planted mycorrhized treatments may be further attributed due to a higher amount of macro aggregates in mycorrhized treatments and therefore a faster water absorption. Small pores withhold water more strongly than large pores. After water drainage, the large pores are drained sooner compared to small pores (Ritz and Young, 2004). The higher amount of macro soil aggregates in mycorrhized treatments may explain why these treatments were much less stable compared to the corresponding non-mycorrhized treatments.
It is very difficult to compare the tensiometer results with previous studies because most tensiometer measurements were conducted in the field and not in a pot. The tensiometer measurements of the present thesis were an improvement of earlier volumetric water content measurements during the wet-sieving procedure which were conducted by Beglinger (2011) using a 5TM Decagon device. Before the wet-sieving, water content values averaged at 7.8 %. One minute after the complete flooding, values ranged between 44 and 94 % (average = 53 %) and did not differ significantly after three minutes when the average was 54 %. Beglinger (2011) mentioned that in general the curves of the measurements from the samples did not cross within the plot. Samples with a higher initial volumetric water content revealed a disproportionate increase of the water content during the flooding of the wet-sieving procedure (Beglinger, 2011). Several studies used tensiometers for measurements of the pore water pressure in a soil on the basis of different precipitations. In a study of Weller et al. (2013) tensiometers were buried in the ground of a levee beside a dike. Pore water pressure measurements showed a similar curve compared to the measured water content. An increased water flow through the dike resulted in an increasing pore water pressure (Weller et al., 2013). Pore water pressure in a slope for example increased due to an infiltration of rainwater into the slope. Such findings were also observed by Rahardjo et al. (2013) where several tensiometers were buried in a slope which consisted of different soil materials.

Figure 4.1: This figure illustrates the contact between the tensiometer cup and the surrounding soil after the wet-sieving procedure. Left: Sample AP2. Right: Sample AP4. Photo Bader A. 2013.

With respect to these two outdoor studies it must be pointed out, that the pore water pressure measurements of this thesis were conducted within an indoor experiment in which the volume of the sample was highly different. The soil samples had a diameter of around 70 mm and a height of 140 mm. Many soil samples collapsed before the water even reached the top of the tensiometer shaft. After the collapse, the tensiometer cup was either in contact with the air or with the water. Therefore, from this point on the pore water pressure measurements are not as meaningful as in the samples where the tensiometer was still in contact with the soil after the wet-sieving procedure. Most samples which did not collapse entirely but only slacked down some centimeters. Through this slacking and the wetting the hole in which the tensiometer was placed enlarged. As a result, the cup of the tensiometer was not entirely in contact with the soil anymore (Figure 4.1). It can thus be suggested that pore water pressure measurements were most valuable at the beginning of the wet-sieving procedure until the tensiometer cup lost contact with the soil. Concludingly, pore water pressure measurements are extremely dependent on the soil sample, the corresponding soil characteristics, the influence of water, the experimental settings and the accuracy of the measuring devices.

4.3 Degree of Mycorrhization

The mycorrhized treatment of *Alnus incana* (AM) revealed a higher mycorrhization degree (median = 83.784 %) compared to the mycorrhized combined treatment of *Alnus incana* and *Poa pratensis* (APM) (median = 48.649 %). This difference can be explained by the fact that a specific AM sample was comprised of *Alnus incana* roots, whereas an APM sample included root systems of *Alnus incana* and *Poa pratensis*. As mentioned before, *Poa pratensis* roots were not mycorrhized which resulted in a lower mycorrhization degree over the specific sample size. In a pot experiment of Kipfer et al. (2012) with *Pinus sylvestris*, high colonisation rate values between 80 and 100 % were revealed. However, these are very high colonisations compared to e.g. the study of Frei (2009) where a mycorrhization degree of 32 % was observed. As a possible explanation for the low colonisation, the adding of the fungal inoculum with the MMN agar (Marx and Bryan, 1975) was discussed. The MMN agar cubes may have nourished the fungus in the beginning which could have delayed the fungal-plant symbiosis (Frei, 2009). In contrary, the same addition of agar cubes with the fungal inoculum of *Melanogaster variegatus* was applied by Graf and Frei (2013) to their samples, resulting in mycorrhization.
Analyses from Orfanoudakis et al. (2010) revealed a substantial growth increase in *Alnus glutinosa* plants due to inoculation with *Frankia* and arbuscular mycorrhiza. Considering the low degree of mycorrhization (45 %) they concluded, that symbiosis must have been very effective. It was further demonstrated that due to an infection by *Frankia* (nodulation) root branching was much more frequent (Orfanoudakis et al., 2010). Therefore, nutrient and water uptake in the soil was facilitated and the soil aggregate stability was probably increased as well. These findings are very important in respect to revegetation sites or landslide areas.
It can only be assumed, why no arbuscular mycorrhizal structures could be observed in roots of *Poa pratensis*. One assumption would be that the staining procedure was not appropriate although two different staining procedures were applied and many studies could identify arbuscular structures using ink and vinegar or CBE (Vierheilig et al., 1998, 2005; Brundrett, 2008). Vierheilig et al. (2005) mention the possibility of an over-staining with CBE if roots are not cleared properly. But if the clearing is conducted properly the authors assume that the staining with CBE results in images with high contrast. In a study of Blaszkowski et al. (2003) the roots were cleared in 10 % KOH, acidified in 20 % HCl and stained in trypan blue. Vesicular and arbuscular structures could be clearly identified in roots which were colonised by *Glomus claroideum*.
More likely explanations are that the commercial inoculum which was partly discussed in the section 4.1 Plant and Fungus Association or the high soil dry unit weight might have affected the treatments of *Poa pratensis*. Due to the addition of a small amount of 40 ml of the commercial inoculum it might be possible, that there were not enough arbuscular mycorrhizal spores present per soil sample although the 40 ml were within the range of the recommended inoculum amount

for new plantings. Further, the applied inoculum "Forst" is intended for applications in forests whereas another commercial inoculum "Agri" only consists of arbuscular mycorrhizas and is more frequently used for agricultural purposes. In addition, the spore germination of the inoculum was not tested on agar plates. A low germination success would have negatively influenced the mycorrhization. A similar negative influence may be attributed to the high soil dry unit weight with an average value of 20.399 kN m^{-3}. According to Shierlaw and Alston (1984) root growth of *Zea mays* and *Lolium rigidum* completely failed due to a compacted soil with a bulk density $\geq$ 1550 kg m^{-3} which would be equal to 15.50 kN m^{-3} after conversion. Consequently, it is likely that root growth might have been hampered in the present thesis due to soil compaction.

4.4 Ergosterol Extraction

Surprisingly, no Ergosterol was found concerning the ectomycorrhized treatments revealing a higher amount than 220 ng per g sample. Ectomycorrhized root tips of *Alnus incana* were identified under the binocular microscope in the treatments AM and APM and therefore, at least a small amount of Ergosterol was expected as was found in the study of Beglinger (2011). However, there are several possible explanations for this result. A first explanation might be that the extracted soil sample remained too long in the carton box. The duration of four and a half weeks at room temperature of 20 °C may have caused a reduction of the Ergosterol concentration. The delay of the Ergosterol analyses was due to problems with the mycorrhization degree determination and problems concerning the procedure of the Ergosterol liquid phase extraction. However, according to Newell et al. (1988), an Ergosterol storage in darkness at 25 °C for 14 days resulted in no detectable loss of Ergosterol. It is therefore likely, that a storage of four and a half weeks would result only in a small amount of Ergosterol loss. Another possible explanation is a decrease of Ergosterol due to UV radiation, although the samples were shielded from sunlight in a carton box. A study of Mille-Lindblom et al. (2004) showed that the Ergosterol concentration of soil samples totally shielded from sunlight decreased 34 % in a time frame of two months, whereas concentration of samples exposed to the sunlight decreased by 43 % in 24 hours. It is also possible that the soil extraction near the root systems of the soil remaining on the sieve after the wet-sieving procedure was unsuitable. However, the soil extraction by Beglinger (2011) was conducted in a similar way and a small amount of Ergosterol between 0 and 135 ng g^{-1} was found. A loss of Ergosterol can be further explained by the saponification process. Comparing the extraction loss of a saponified non-mycorrhized soil sample to the extraction loss of a non-saponified sample, a huge difference was observed. According to De Vries (2000) a loss of Ergosterol might be possible due to the presence of KOH

and the saponification.
These findings support the statements made by Beglinger (2011). In conclusion, liquid phase extraction does not reveal satisfactory results concerning Ergosterol detection in coarse grained moraine soil material. For future studies it is therefore recommended to undergo an alternative Ergosterol extraction procedure when dealing with this type of soil. In a recent study Gong et al. (2001) developed a new method to extract Ergosterol from soil samples by physical disruption using glass beads. Further suggestions are solid phase extraction as mentioned in Beglinger (2011).

4.5 Hypothesis 1: Planted Soil Samples Mobilise Higher Soil Aggregate Stability Compared to Bare Soil Samples

Four different mechanisms causing aggregate breakdown by water were identified in the study of Le Bissonais (1996) as slacking, breakdown by differential swelling or due to raindrop impact and physio-chemical dispersion. In the wet-sieving procedure slacking occurs when a dry soil sample is rapidly wetted. Analyses of Kemper (1966) showed that soil aggregate stability is strongly influenced by the rate of wetting and decreases at higher rates (Matkin and Smart, 1987). Several studies found a decrease of slacking if the moisture content of a soil sample was increased (Panabokke and Quirk, 1957; Truman et al., 1990; Le Bissonais, 1996). Analyses of Truman et al. (1990) indicate that the soil aggregate stability with respect to flowing water will be higher with increasing initial water content (Air dried, -1 kPa and -0.5 kPa). This can be explained due to a decreasing volume of air inside the soil sample (Le Bissonais, 1996).
In the study of Kemper (1966) more detailed factors were identified which may have an effect on soil aggregate stability. These factors include the moisture content of a sample, the soil aggregate size, the sieve and the sample size itself, the methods of sample wetting, the water temperature and the duration of the wet-sieving procedure. According to Tisdall and Oades (1982) the relation between micro- and macroaggregates in a soil sample needs to be considered. Macroaggregates are defined having a diameter >250 μm, microaggregates a diameter between 20-250 μm. The water stability of macroaggregates is highly dependent on the root systems and hyphal network which was confirmed by Jastrow et al. (1998) and Rillig and Mummey (2006). Further parameters which affect soil aggregate stability are soil structure and constituents (Kemper, 1966).
Before starting the wet-sieving procedure, all samples were not watered for five to seven days and the water content of each sample was measured. Due to this absence of watering, the water content revealed low values between 0.7 and 9.6 % with a mean value of 3.2 %. These findings match those observed in the study of Frei (2009) and Beglinger (2011). Frei (2009) found a

water content between 0.7 and 7 %, although the watering was stopped only three days before. Beglinger (2011) did not water the plants five days before the analyses, which resulted in water content values between 0.1 and 13 % and a mean value of 7.8 %.
Comparing the soil aggregate stability of the control treatments K and KM with the planted treatments, a significant difference was revealed using the pairwise Wilcoxon rank sum test. These present findings are somewhat consistent with the study of Burri et al. (2009) which found a significantly higher soil aggregate stability on revegetated sites compared to control sites without vegetation. The dry unit weight was lower in the control site compared to the revegetated site and, conversely, the water content was higher in the control site (Burri et al., 2009). These results are in contrast to the findings in this thesis. Dry unit weight did not differ between control and planted treatments, whereas the water content revealed the highest mean value in the treatment of non-mycorrhized *Alnus incana* closely followed by the treatments K, KM and AM.
In conclusion, referring to the 1st hypothesis, soil aggregate stability of the planted treatments (A, AM, P, PM, AP, APM) was significantly higher compared to the bare soil samples of the control treatments (K, KM). Therefore, the vegetation cover has a strong effect on the soil aggregate stability. Consequently, the 1st hypothesis can be confirmed.

4.6 Hypothesis 2: Planted Soil Samples Inoculated with Mycorrhizal Fungi Mobilise Higher Soil Aggregate Stability than Non-Inoculated Samples

Mycorrhizal fungal mycelium may have a direct effect on the soil aggregate stability which can be categorised into biological, biochemical and biophysical processes according to Rillig and Mummey (2006). The biological effect is due to the symbiosis with the plant root and therefore a more complex root system of the fplant-fungus association. Possible other stabilising effects can be attributed to a symbiosis with bacteria and archaea. Furthermore, Glomalin, a fungal protein, may act as a “glue” to stick the soil aggregates together (Rillig and Mummey, 2006).
The results of the present thesis do not support the 2nd hypothesis. The inoculated treatments did not exhibit a significantly higher soil aggregate stability compared to the corresponding non-inoculated treatments. In contrast, with respect to all planted treatments the soil aggregate stability was lower in mycorrhized treatments compared to the corresponding non-mycorrhized treatments. Soil aggregate stability of the control treatments did not differ significantly. With regard to the results of the root length per soil volume, mycorrhized treatments revealed lower

values compared to the corresponding non-mycorrhized treatments. In conclusion, mycorrhizas did not have the expected effect of increased root growth and increased soil aggregate stability. Similar observations concerning the soil aggregate stability were described by Bast et al. (2014). Non-mycorrhized treatments had the highest positive effect on soil aggregate stability, followed by the mycorrhized and the control treatments. Concerning the decreased root length in mycorrhized treatments findings are consistent with those of Burri (2011). In wind tunnel experiments Burri (2011) observed significantly smaller root systems in mycorrhized plants compared to non-mycorrhized plants. However, aboveground biomass was lower in mycorrhized plants and showed the same significant differences which does not confirm the findings made in the present thesis. However, mycorrhized plants were able to reduce wind erodibility of soil, although mycorrhiza did not affect plant growth (Burri, 2011).

In contrast, different results were observed by Burri et al. (2009) in the outdoor experiments of three test sites with different vegetation covers. The root network positively correlated within the different test sites from the soil with sparse pioneer vegetation, to the revegetated site and the climax forest. Soil aggregate stability was higher in the climax forest compared to the site with sparse pioneer vegetation and the revegetated site and therefore had a lower susceptibilty to landslides. These results agree with the findings of Graf and Frei (2013). In a later study a significant increase of the soil aggregate stability was revealed comparing bare soil samples with planted and the mycorrhized samples by testing *Alnus incana* inoculated with *Melanogaster variegatus*. Furthermore, a high correlation between soil aggregate stability and root length per soil volume was found (Graf and Frei, 2013). In the present thesis the soil aggregate stability and the root length per soil volume did not reveal such results. Within the non-mycorrhized treatments only the regression model of the combined treatment AP was significant and within the mycorrhized treatments the one of PM.

With respect to the statement that the 2nd hypothesis is not supported it has to be pointed out that mycorrhization is not the only factor which influences the soil aggregate stability. The initial water content can highly influence the behaviour of the soil sample during the wet-sieving, as discussed before. According to Brunner (2001) mobilisation and absorbation of water is much more effective in mycorrhized plants. Hence, treatments with mycorrhizas may dry out sooner compared to non-mycorrhized treatments. Therefore, water content values may be lower and consequently, soil aggregate stability revealed lower values in mycorrhized treatments compared to the corresponding non-mycorrhized treatments. Due to this confounding effect it is not permissible to entirely reject the 2nd hypothesis. Further, it is possible that the difference between mycorrhized and non-mycorrhized treatments could be observed due to a longer period without watering before the wet-sieving procedure. It is therefore likely that differences would have been smaller if watering would have stopped only three days before or if the initial water content would have been equal within each sample.

4.7 Hypothesis 3: Planted Soil Samples Inoculated with Mycorrhizal Fungi Produce a Higher Amount of Biomass than Non-inoculated Samples

To discuss this hypothesis it has to be mentioned that the biomass referred to includes the aboveground plant dry weight and root dry weight. Findings of the aboveground plant dry weight do not match with the ones of the root dry weight. The main differences were observed within the treatments of *Alnus incana* (A, AM). Aboveground plant biomass was significantly higher within the mycorrhized treatment AM compared to the non-mycorrhized treatment A. In contrary, root dry weight did not differ significantly although values of the AM treatment varied more. Consequently, AM revealed a higher median value of the total amount of plant biomass compared to A, but the difference was not significant based on the pairwise Wilcoxon rank sum test. Similar findings were observed within the combined treatments of *Alnus incana* and *Poa pratensis* (AP, APM). The aboveground plant dry weight was significantly higher in the APM treatment compared to AP. In contrast to the comparison between A and AM, the root dry weight of APM showed significantly lower values than in AP. Therefore, the total amount of biomass did not differ significantly. Treatments of *Poa pratensis* (P, PM) behaved quite contradictory. Aboveground plant dry weight and the root dry weight were significantly lower in the mycorrhized treatment PM compared to the non-mycorrhized treatment P. Consequently, the total amount of biomass differed significantly, with P having higher values. Conclusively, these results falsify the third hypothesis because the differences within the treatments of *Alnus incana* and the combined treatments of *Alnus incana* and *Poa pratensis* were not significant and the results of *Poa pratensis* were contradictory.

Similar findings of an increased aboveground biomass due to mycorrhization were revealed in a pot experiment of Kipfer et al. (2012) using *Pinus sylvestris* seedlings and four ectomycorrhizal fungi. Aboveground plant biomass only increased in seedlings which were colonised by the ectomycorrhizal fungus *Suillus granulatus*. Therefore, the authors conclude that an increase of the aboveground biomass is highly dependent on the specific plant-fungus association. Further limiting factors which influence the ectomycorrhizal fungal species composition are described by Kipfer et al. (2012) as the ability of the fungus to make the limiting resources for the plant growth available and to transfer these nutrients to the host plant. If these factors are not granted, the plant-fungus symbiosis is not mutualistic. Comparing these findings with the present thesis, treatments including *Alnus incana* showed similar results concerning the aboveground biomass. Therefore, it can be assumed, that the plant-fungus symbiosis was mutualistic with one or several mycorrhizal fungi.

These findings of an increased aboveground biomass and a decreased root dry weight within mycorrhized treatments do not agree with many previous studies (Frei, 2009; Burri, 2011;

Graf and Frei, 2013). Graf and Frei (2013) report that the root length of mycorrhized *Alnus incana* was more than twice as long compared to the non-mycorrhized samples. Plants of the mycorrhized *Alnus incana* were also inoculated with cultivated *Melanogaster variegatus* on MMN agar plates (Marx and Bryan, 1975), but no commercial inoculum was added. This difference contradicts the assumption of Frei (2009) that MMN agar may nourish the fungus and therefore hamper the inoculation. A possible influence can only be predicted due to the combination of the cultivated *Melanogaster variegatus* inoculum and the commercial inoculum "Forst". In another study of Burri (2011) findings agree with respect to the lower root length in mycorrhized treatments but also revealed a lower aboveground biomass in mycorrhized treatments compared to non-mycorrhized treatments, which contradicts the results of the present study. However, mycorrhized plants had a positive effect on the erodibility of the soil, although mycorrhiza did not affect plant growth (Burri, 2011).

Only assumptions can be made on why mycorrhization had a negative effect on root growth and on soil aggregate stability but a positive effect on the aboveground biomass in treatments which included *Alnus incana*. One possible explanation might be the addition of the liquid fertilizer (Maag Wuxal, NPK 10107.5: 2 ml l^{-1}). According to Cernusca (1986) and Graf and Gerber (1997) fertilization increases the aboveground plant biomass but also decreases root growth which would negatively affect soil aggregate stability. Exactly these characteristics were observed within the treatment of *Alnus incana* of the present thesis. It is therefore possible that the application of 10 ml fertilizer after the sample preparation and further 5, 10 and 5 ml after two, six and nine weeks, respectively, was excessive.

Another explanation might be that the mycorrhization of *Alnus incana* might have been very effective. Hence, instead of investing energy into root growth, the plant primarily invested its energy into aboveground biomass production, because nutrient uptake was satisfactory using the symbiosis with the mycorrhizal fungi (Orfanoudakis et al., 2010).

4.8 Further Research

The scope of this thesis was limited. Hence, there is room for further research which is discussed in this chapter. Considering the pore water pressure measurements, the fixture of the tensiometer needs to be improved. The fixation using the two wires which were pulled through the small plastic tube and fixed above the wooden construction around the cable of the tensiometer was an improvised solution. Due to the fact that the first two soil samples, which were not fixed, collapsed completely during the wet-sieving procedure, this wire solution was conceived to provide stabilisation to the tensiometer, which would have otherwise fallen in sync with the soil sample. As a result, the flooding period was shortened to allow the water table to reach exactly

the upper end of the tensiometer shaft, but this change in flooding time was not acceptable for the tests. In addition, after some measurements it was noted that the tensiometer ceramic cap was slightly damaged by the wire and probably by soil particles as well. For further tensiometer analyses it is therefore recommended to fix the tensiometer with a thread or something similar. Due to the fact that pore water pressure measurements were most valuable as long as the cup of the tensiometer was still in contact with the soil, it would be advisable to prevent a collapse of the soil samples within the first few seconds of the flooding. Therefore, a successful root growth and mycorrhization is essential.

It is also recommended to test the tensiometer's accuracy in advance. According to National Instruments (2009) the offset check can be conducted by placing the tensiometer into a cup which is filled with deionized water up to the center of the sensor body (Appendix III.I; Figure 6.2). After the voltage record is stable the pore water pressure measurements should range between +3.0 and -3.0 kPa (National Instruments, 2009) which equals +30.0 and -30.0 hPa. Further possibilities are to fix the tensiometer in a horizontal or vertical position and pore water pressure measurements could be conducted for 5 to 10 minutes on each increasing water level (Appendix III.II).

Further, tensiometer pre-tests are recommended considering the contact point of the tensiometer ceramic cup and the soil material. In the present thesis an additional silt drop was added in each hole to increase the conductivity between the coarse-grained soil and the ceramic cup of the tensiometer but it was difficult to add the silt the same way across all samples. It also has to be considered, that a higher amount of silt could strongly bind the wooden stake to the surrounding soil particles. Therefore, during the growing period it would be advisable to periodically check if the wooden stake is still removable.

Another important aspect worth mentioning is the temperature and air circulation during the wet-sieving. In the present thesis it was observed that pore water pressure measurements were highly affected by small changes in temperature or even breezes of air in the room. Consequently, the door of the laboratory was closed during the wet-sieving procedure.

With respect to the soil aggregate stability after the wet-sieving procedure it can be assumed that the soil samples were too dry in the present thesis. Samples were not watered for five to seven days which could have affected the soil aggregate stability. In the studies of Frei (2009) and Beglinger (2011) samples were not watered for three to five days which resulted in higher soil aggregate stability compared to the present thesis. This longer dry period might have further increased the differences between non-mycorrhized and mycorrhized treatments concerning the water content. Mycorrhized soil samples were in general dryer compared to the non-mycorrhized samples which then resulted in a lower soil aggregate stability. Due to this confounding effect it is not possible to declare, that the mycorrhized treatments result in less stable samples compared to the non-mycorrhized treatments. Additionally, pore water pressure measurements were merely suitable prior to the collapse of the soil samples. For further research

a dry period between three to five days would be recommended. Therefore, a collapse of the soil samples may happen less frequently. Another solution to this problem would be a similar water content within all treatments at the beginning of the wet-sieving procedure. If all wet-sieving tests would have been conducted with the same initial water content improved conclusions could be made considering the mycorrhization effect.

With respect to the mycorrhization, the commercial inoculum could be tested with respect to the germination success of the fungal spores. In further pot experiments it would be recommended to add a mixture of the inoculum "Forst" and "Agri" to the soil samples, when dealing with plants which associate with arbuscular and ectomycorrhizal fungi (Appendix II.I and II.II).

Concerning the dry unit weight of the soil samples it would be advisable to determine the net weight and height of the soil sample after the sample preparation as well as before the wet-sieving procedure to estimate the increase of the dry unit weight during the growing period. The former was measured but the soil height was omitted. It can be speculated that the high dry unit weight of the soil samples resulted due to the watering during the growing period and a resultant compaction of the soil.

Regarding the germination success it would be useful to write down all individual transferred plants and the corresponding pot labels. Additionally, the mortality of seedlings should be observed and noted. Considering the determination of the mycorrhization degreee, root of *Alnus incana* and *Poa pratensis* of the combined treatment should have been analysed separately under the binocular microscope. Last but not least the conducted experiments were extremely time consuming which was underestimated in the present thesis. Therefore, a generously time table is recommended.

5 Conclusion

This Master's thesis investigated a new plant-fungus association of *Alnus incana* and *Poa pratensis* and their contribution to soil aggregate stability. The objective of the thesis was to either confirm or reject the following hypotheses:

1. Planted soil samples mobilise higher aggregate stability compared to bare soil samples.
2. Planted soil samples inoculated with mycorrhizal fungi mobilise higher aggregate stability than non-inoculated samples.
3. Planted soil samples inoculated with mycorrhizal fungi produce a higher amount of biomass than non-inoculated samples.

Multiple experiments revealed interesting and occasionally unexpected results. Tensiometer measurements showed in general a lower initial pore water pressure and a steeper curve during the wetting in mycorrhized treatments compared to non-mycorrhized treatments. In terms of soil aggregate stability, planted treatments revealed a significantly higher stability than the control treatments. Additionally, a significant negative stabilising effect was observed in all mycorrhized planted treatments compared to the corresponding non-mycorrhized treatments. Regarding the determination of the mycorrhization degree, ectomycorrhizas were present on a high percentage of root tips of *Alnus incana* but no arbuscular mycorrhizas could be identified in the root systems of *Poa pratensis*. Considering the Ergosterol liquid phase extraction, no Ergosterol could be detected. The highest root length values were measured in the treatments which included *Poa pratensis*. All mycorrhized treatments yielded lower root length values compared to the corresponding non-mycorrhized treatments. Similar findings were observed considering the root dry weight. In contrast, abovegroud plant dry weight was considerably increased within mycorrhized treatments which included *Alnus incana* (AM, APM) compared to the corresponding non-mycorrhized treatments (A, AP). The total amount of biomass was affected in the same way but differences were not significant in most cases. Regarding the linear regression models, significant positive effects of the root length and significant negative effects of the water content on soil aggregate stability were found. Further, root length significantly affected aboveground plant dry weight.

Concerning the hypotheses, soil aggregate stability was significantly higher in planted treatments compared to the control treatments. Therefore, the vegetation cover had a strong effect and the

first hypothesis can be confirmed. Further, mycorrhizas did not increase root growth and soil aggregate stability. Consequently, the second hypothesis can not be supported. In terms of the total amount of biomass, only mycorrhized treatments which included *Alnus incana* produced more biomass than the corresponding non-mycorrhized treatments. Due to the fact that these differences were not significant the third hypothesis can not be supported. The results of the first and second hypothesis may be explained by more factors than merely mycorrhizas which influence soil aggregate stability: For example root growth, soil water content and dry unit weight. The lower initial water content in the mycorrhized treatments may have negatively influenced soil aggregate stability as well as affected pore water pressure measurements. Additionally, the high soil dry unit weight might have hampered root growth and disabled a mycorrhization of *Poa pratensis*. Considering the third hypothesis, the increased aboveground biomass coupled with a decreased root growth of the mycorrhized treatments may be the result of an excessive fertilizer application.

In conclusion, the investigations were slightly limited by the lack of mycorrhization in the treatments of *Poa pratensis*. However, the present thesis enhances the knowledge in terms of a new plant-fungus combination. Additionally, the thesis makes noteworthy contributions to the understanding of pore water pressure measurements in small soil samples during wet-sieving using a high suction tensiometer. Nevertheless, further research regarding the measurements of the pore water pressure and the plant-fungus association with *Poa pratensis* is strongly recommended. In particular, pore water pressure measurements using soil samples with similar initial water content could result in meaningful observations.

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List of Figures

List of Tables

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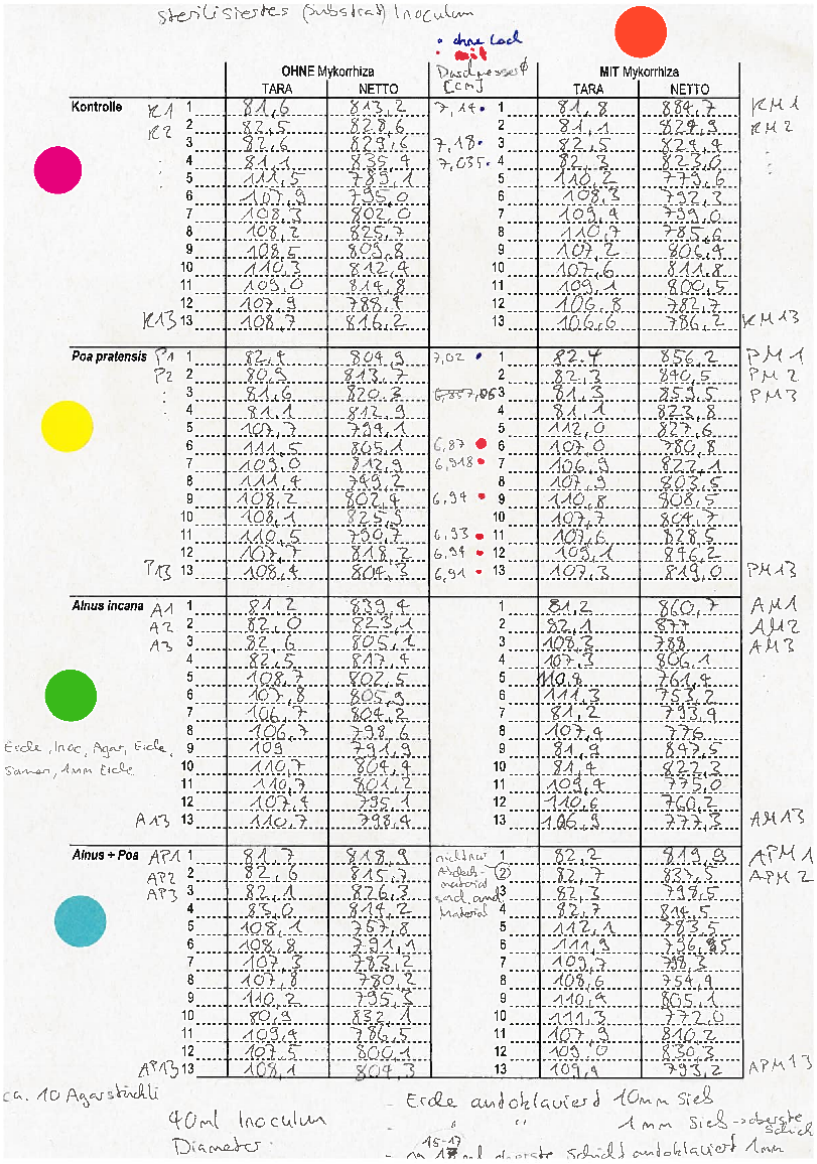
Appendix

I Soil Analysis

I.I Net and Tare Weight of Soil Samples after Preparation



sterilisiertes (substrat) Inoculum

		OHNE Mykorrhiza TARA	OHNE Mykorrhiza NETTO	Dosdurchmesser ø [cm]	MIT Mykorrhiza TARA	MIT Mykorrhiza NETTO	
Kontrolle	K1 1	81,6	843,2	7,14	81,8	884,7	KM1
	K2 2	82,5	828,6		81,1	829,3	KM2
	3	87,6	829,6	7,18	82,5	829,9	
	4	81,1	855,9	7,035	82,3	825,0	
	5	111,5	793,1		110,2	795,6	
	6	107,9	795,0		108,3	792,3	
	7	108,3	802,0		109,9	799,0	
	8	108,2	825,3		110,7	785,6	
	9	108,5	805,8		107,2	806,9	
	10	110,3	812,4		107,6	811,8	
	11	105,0	819,8		109,1	800,5	
	12	107,9	788,1		106,8	782,7	
	K13 13	108,7	816,2		106,6	786,2	KM13
Poa pratensis	P1 1	82,4	809,9	7,02	82,4	856,2	PM1
	P2 2	80,9	813,7		82,3	840,5	PM2
	3	81,6	820,2	6,857,105	81,3	854,5	PM3
	4	84,1	813,9		84,1	823,8	
	5	107,3	799,1		112,0	827,6	
	6	111,5	805,1	6,87	102,0	780,8	
	7	103,0	812,9	6,918	106,9	822,1	
	8	111,4	799,2		107,9	805,5	
	9	108,2	802,9	6,94	110,8	808,5	
	10	108,1	825,5		107,7	804,7	
	11	110,5	790,7	6,93	107,6	828,5	
	12	107,7	811,2	6,94	105,1	816,2	
	P13 13	108,4	804,5	6,91	107,3	819,0	PM13
Alnus incana	A1 1	81,2	839,4		81,2	860,7	AM1
	A2 2	82,0	825,1		82,1	877	AM2
	A3 3	82,6	805,1		108,2	788	AM3
	4	82,5	817,4		107,7	806,1	
	5	108,7	802,5		110,9	761,9	
	6	107,8	805,9		111,3	757,2	
	7	106,7	804,2		81,2	793,9	
	8	106,7	798,6		107,9	776	
Erde, Inoc, Agar, Erde, Samen, 1mm Erde	9	109	791,9		81,9	849,5	
	10	110,7	804,9		81,4	824,3	
	11	110,7	801,2		109,9	775,0	
	12	107,4	795,1		110,6	760,2	
	A13 13	110,7	798,9		106,9	777,2	AM13
Alnus + Poa	APM1 1	81,7	818,9	autoklav Erde/Substrat/Inoculum	82,2	819,8	APM1
	APM2 2	82,6	815,7		82,7	837,6	APM2
	APM3 3	82,1	826,3		82,3	799,5	
	4	85,0	814,2		82,7	814,5	
	5	108,1	797,8		112,1	793,5	
	6	108,8	791,1		111,9	786,85	
	7	107,3	793,2		109,7	800,5	
	8	107,8	780,2		108,6	754,9	
	9	110,2	795,5		110,9	805,1	
	10	80,9	832,1		111,3	772,0	
	11	109,9	786,5		107,3	810,2	
	12	107,5	800,1		109,0	830,3	
	APM13 13	108,1	804,3		109,9	795,2	APM13

ca. 10 Agarstückli

40ml Inoculum

Diameter

Erde autoklaviert 10mm Sieb

1mm Sieb -> oberste Schicht

15-17 oberste Schicht autoklaviert 1mm

I.II Grain Size Distribution Values

Table 6.1: Grain size distribution values of the Hexenruebi soil material with different grain sizes (labelled S63, S10) according to the analysis of Frei (2009) and Graf and Frei (2013). The soil material of S63 includes coarse grains with a maximum of 63 mm, the grain sizes of the soil material S10 are smaller than 10 mm.

Soil 63		**Soil 10**
Mesh opening [mm]	**Ratio S63**	**Ratio S10**
63	1.000	-
45	0.991	-
31.5	0.901	-
20	0.730	-
14.23	0.600	-
10	0.501	1
4	0.328	0.655
3.5	0.300	0.599
2	0.247	0.493
1	0.203	0.405
0.5	0.179	0.357
0.335	0.161	0.321
0.2	0.141	0.281
0.125	0.124	0.248
0.081	0.100	2.000
0.059	0.092	0.184
0.044	0.076	0.152
0.033	0.070	0.140
0.021	0.062	0.124
0.013	0.050	0.100
0.009	0.042	0.084
0.007	0.036	0.072
0.005	0.029	0.058
0.003	0.025	0.050
0.001	0.017	0.034

I.III Mean and Median Values of the Water Content

Table 6.2: Mean and median values of the water content [g g^{-1}]. Non-mycorrhized treatments are abbreviated by A (*Alnus incana*), P (*Poa pratensis*), AP (*Alnus incana* and *Poa pratensis*), K (control) and the corresponding mycorrhized (M) treatments by AM, PM, APM, KM.

Treatment	**Mean**	**Median**
A	0.042	0.042
AM	0.031	0.032
P	0.023	0.022
PM	0.025	0.025
AP	0.029	0.027
APM	0.029	0.028
K	0.044	0.045
KM	0.037	0.038

I.IV Mean and Median Values of the Water Content Depending on the Last Watering

Table 6.3: Mean and median values of the water content [g g^{-1}] of the soil samples which were not watered for five to seven days (D5, D6, D7). Non-mycorrhized treatments are abbreviated by A (*Alnus incana*), P (*Poa pratensis*), AP (*Alnus incana* and *Poa pratensis*), K (control) and the corresponding mycorrhized (M) treatments by AM, PM, APM and KM.

Treatment	**D5 $_{\text{Mean}}$**	**D6 $_{\text{Mean}}$**	**D7 $_{\text{Mean}}$**	**D5 $_{\text{Median}}$**	**D6 $_{\text{Median}}$**	**D7 $_{\text{Median}}$**
A	0.043	0.043	0.035	0.043	0.043	0.035
AM	0.031	0.031	-	0.033	0.029	-
P	0.025	0.023	0.021	0.024	0.019	0.021
PM	0.027	0.025	0.024	0.027	0.025	0.024
AP	0.032	0.027	0.025	0.030	0.027	0.025
APM	0.030	0.028	-	0.029	0.027	-
K	0.046	0.043	0.043	0.050	0.044	0.043
KM	0.039	0.036	0.033	0.040	0.039	0.037

I.V Mean and Median Values of the Soil Dry Unit Weight

Table 6.4: Mean and median values of the dry unit weight [kN m^{-3}]. Non-mycorrhized treatments are abbreviated by A (*Alnus incana*), P (*Poa pratensis*), AP (*Alnus incana* and *Poa pratensis*), K (control) and the corresponding mycorrhized (M) treatments by AM, PM, APM, KM.

Treatment	**Mean**	**Median**
A	20.291	20.211
AM	20.007	20.064
P	20.569	20.344
PM	20.583	20.271
AP	20.496	20.406
APM	20.406	20.217
K	20.425	20.368
KM	20.412	20.257

II Commercial Inoculum

II.I INOQ Forst

INOQ Forst Charge IFP E1/10

Baumanzucht, -pflanzung, und -sanierung

Verbesserung der Bodenstruktur durch verschiedene Symbiosebildner
Erhöhung des Humusgehaltes
Erfüllt die FLL-Richtlinien
Gemäß EG-Öko-Durchführungs-VO 889/2008 Art. 3 (4) im Ökolandbau zugelassen

Ektomykorrhizapilze (heimische Stämme, enthält keine Gentechnisch Veränderten Organismen (GVO))	*Amanita muscaria, Boletus edulis Hebeloma crustuliniforme, Laccaria laccata Paxillus involutus, Pisolithus tinctorius Thelephora terrestris, Xerocomus badius*
Endomykorrhizapilze (heimische Stämme, enthält keine Gentechnisch Veränderten Organismen (GVO))	*Glomus etunicatum* (Becker & Gerdemann) *Glomus intraradices* (Schenck & Smith) *Glomus claroideum* (Schenck & Smith)
Endomykorrhiza Einheiten (pro cm³ Substrat)	180
Ektomykorrhiza-Einheiten (pro cm³ Substrat)	95
Mykorrhizawirkung (Wuchsförderung [%])	
Endomykorrhiza	32 ± 8
Ektomykorrhiza	24 ± 5
Trägermaterial	Torfsubstrat
Schüttgewicht [g/l]	250-400
pH-Wert	6,7

Dosierung	**Mischen mit Substrat**	2 bis 5 % (Jungpflanzenproduktion) 5 bis 10 % (Pflanzenproduktion)
	Einbringen in Pflanzloch	Jungpflanzen 20 – 100 ml Ballenware ab 40 cm ø 100-300 ml
	Bestehende Pflanzungen	pro 10 cm Stamm-ø 3 x 100 ml
	Verschulbeet	bis 100 ml / m Furche
Verträglichkeit mit Fungiziden		Wurde geprüft
Sicherheitshinweise		Keine besonderen Vorkehrungen nötig Verschlucken und inhalieren vermeiden Freiheit von Phytopathogenen durch DNA multiscan® nachgewiesen Material Safety Data Sheet kann angefordert werden

Kennzeichnung gemäß Düngemittelverordnung

Art	Bodenhilfsstoff
Zusammensetzung	Hochmoortorf (H3-H6) Ektomykorrhizapilze Arbuskuläre Mykorrhizapilze
Wirkungsbereich	Zur flächigen Bodenverbesserung und Pflanzlochbeigabe
Anwendung	Jährliche Applikation im Wurzelbereich
Nährstoffgehalte	Gesamt-N 0,29 %,
Organische Substanz	17,4 %
Lagerung	2 Jahre, kühl und trocken
Packungsgrößen	1 l Eimer 5 l Eimer 10 l Eimer 25 l Sack 1000 l Big Bag

Inverkehrbringer:
INOQ GmbH, Geschäftsführung: Dr. Carolin Schneider, Solkau 2, 29465 Schnega
Tel. 0 58 42/98 16 72, Fax. 0 58 42/4 93, info@inoq.de · www.inoq.de

II.II INOQ Agri

INOQ Agri Charge IFP V1/10

Rekultivierung, Mischung mit Saatgut, Mischung mit Dünger

Gute Rieselfähigkeit
Gute Haftung an Hanglagen
In der Betriebsmittelliste des FiBL Deutschland e.V. gelistet
Gemäß EG-Öko-Durchführungs-VO 889/2008 Art. 3 (4) im Ökolandbau zugelassen

Endomykorrhizapilze (heimische Stämme, enthält keine Gentechnisch Veränderten Organismen (GVO)		*Glomus etunicatum* (Becker & Gerdemann) *Glomus intraradices* (Schenck & Smith) *Glomus claroideum* (Schenck & Smith)
Mykorrhiza Einheiten (pro cm³ Substrat)		210
Mykorrhizawirkung (Wuchsförderung [%] im Standardtest)		40 ± 5
Trägermaterial		Vermiculite 1-2 mm
Schüttgewicht [g/l]		390-450
pH-Wert		5,6
Dosierung	**Mischung mit Saatgut**	10 – 20 ml / 1000 Korn
	Mischung mit Dünger	12 – 15 l / t
	Einbringen in Pflanzloch	20 ml / Pflanze (bis 12er Topf) bis 100 ml / Pflanze (bis 10 l Kübel)
	Bestehende Pflanzungen	Je nach Größe bis zu 100 ml / Pflanze in Bohrlöcher einbringen
	Flächenanwendung	Bis 100 ml / m²
Verträglichkeit mit Fungiziden		Wurde geprüft
Sicherheitshinweise		Keine besonderen Vorkehrungen nötig Verschlucken und inhalieren vermeiden Freiheit von Phytopathogenen durch DNA multiscan® nachgewiesen Material Safety Data Sheet kann angefordert werden

Kennzeichnung gemäß Düngemittelverordnung

Art	Bodenhilfsstoff
Zusammensetzung	Vermiculite Arbuskuläre Mykorrhizapilze
Wirkungsbereich	Zur flächigen Bodenverbesserung und Pflanzlochbeigabe
Anwendung	Jährliche Applikation im Wurzelbereich
Nährstoffgehalte	Gesamt-N 0,14 % $P_2 0_5$ 0,94 %, $K_2 0$ 5,33 %
Lagerung	2 Jahre, kühl und trocken
Packungsgrößen	1 l Eimer 5 l Eimer 10 l Eimer 25 l Sack 1000 l Big Bag

Inverkehrbringer:
INOQ GmbH, Geschäftsführung: Dr. Carolin Schneider, Solkau 2, 29465 Schnega
Tel. 0 58 42/98 16 72, Fax. 0 58 42/4 93, info@inoq.de · www.inoq.de

III Wet-Sieving

III.I Tensiometer Calibration Values at 10.6 V

Vertical installation: 0 hPa = 0.50 mV
500 hPa = 50.50 mV
1000 hPa = 100.50 mV
Offset = -5.0 hPa

Horizontal installation: 0 hPa = 0.00 mV
500 hPa = 50.00 mV
1000 hPa = 100.00 mV
Offset = 0.0 hPa

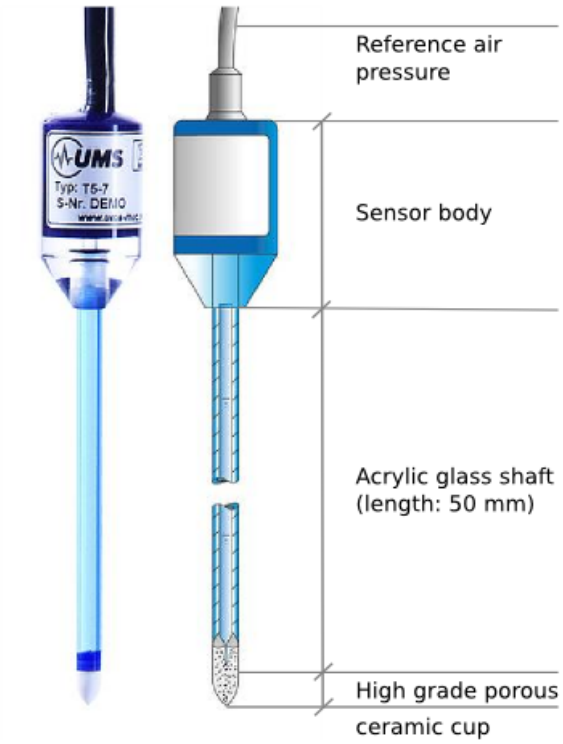


Figure 6.1: Schematic scetch of the tensiometer T5x (National Instruments, 2009).

III.II Tensiometer Pre-Tests

In this tensiometer pre-test the tensiometer was fixed horizontally in a plastic bottle. Therefore, a hole of the same size as the diameter of the tensiometer shaft was drilled into the plastic bottle. The tensiometer was placed into the bottle so that the ceramic cup was in the center of the bottle and the hole was sealed with silicon around the tensiometer. For each measurement, an additional volume of 40 ml water was added into the bottle. Pore water pressure measurements were conducted for 10 min on each increasing water level (Figure 6.2). Due to the horizontal installation the recorded voltage data of the tensiometer measurements was converted into hectopascals (hPa) according to the formula 6.1 (Appendix III.I).

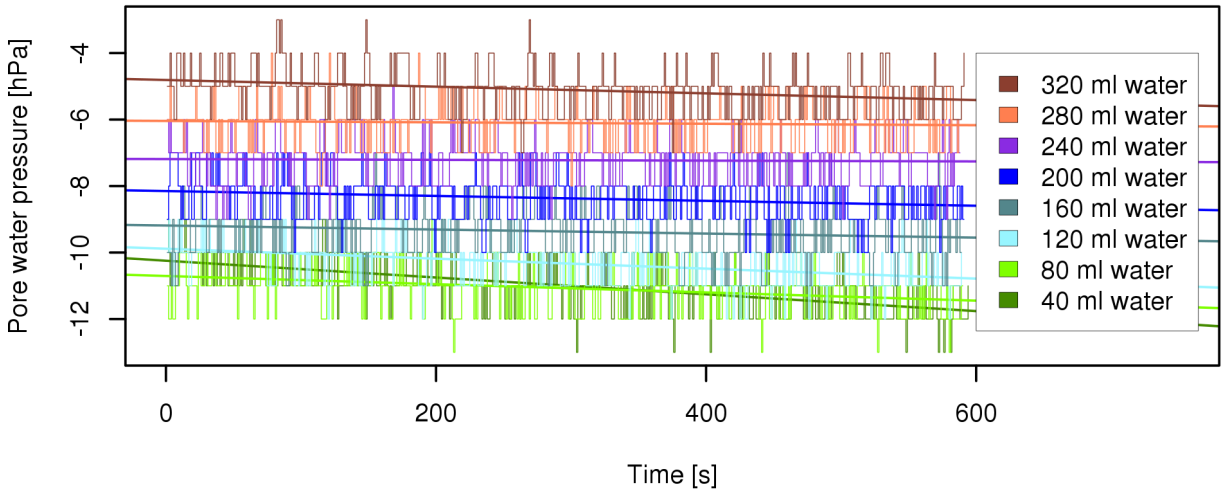


Figure 6.2: Results of the tensiometer pre-test. The tensiometer was fixed horizontally in a plastic bottle. Pore water pressure measurements were conducted for 10 min on each increasing water level.

$$y(x)[hPa] = \frac{10'000[hPa]}{[V]} \cdot x[V] \tag{6.1}$$

III.III Wet-Sieving Protocol Sheets

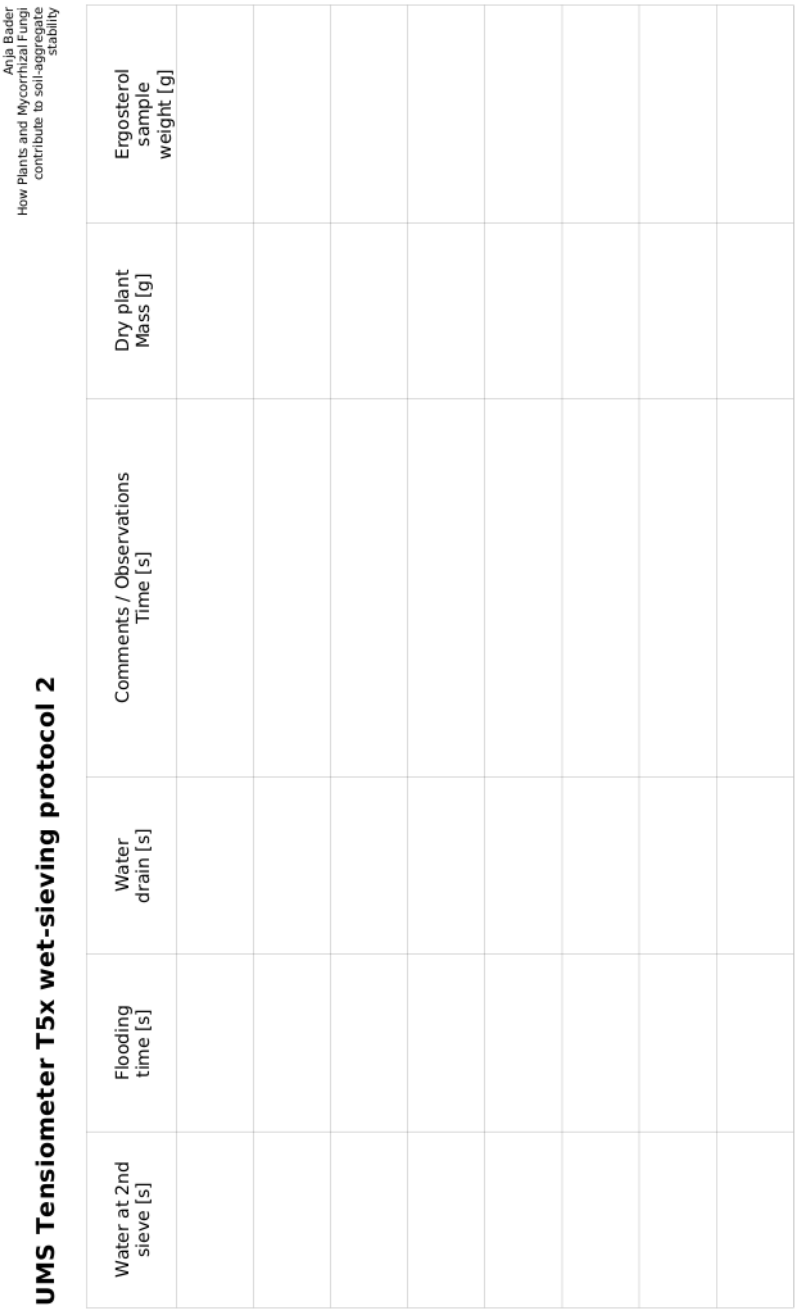
III.III.1 Protocol Sheet 1

Anja Bader
How Plants and Mycorrhizal Fungi contribute to soil-aggregate-stability

UMS Tensiometer T5x wet-sieving protocol 1

Sievecylinder mesh opening [mm]:		Sievecylinder weight [g]:		Barometer Pressure [hPa]:		Water / air temp [°C]:	
Date [dd.mm.yyyy]	Sample nr.	Days since last watering [d]	Gross weight (with plant) [g]	Gross weight (without plant) [g]	Distance Plastic to Soil [mm]	Net weight [g]	Soil height [mm]

III.III.2 Protocol Sheet 2



Anja Bader
How Plants and Mycorrhizal Fungi contribute to soil-aggregate stability

UMS Tensiometer T5x wet-sieving protocol 2

Water at 2nd sieve [s]	Flooding time [s]	Water drain [s]	Comments / Observations Time [s]	Dry plant Mass [g]	Ergosterol sample weight [g]

III.III.3 Protocol Sheet 3

Anja Bader
How Plants and Mycorrhizal Fungi contribute to soil-aggregate stability

UMS Tensiometer T5x wet-sieving protocol 3

Water content [%]	Dry weight Soil on sieve [g]	Dry weight Soil dropped [g]	Ergosterol sample dry weight [g]	Total soil dry weight [g]	Root dry weight [mg]	Volume [cm³]	Dry unit weight [kN/m³]

III.IV Mean and Standard Deviation Values of the Remaining and Dropped Soil after the Wet-sieving

Table 6.5: Mean and standard deviation (sd) values of the soil mass [g] remaining on the sieve (on) and the dropped soil (drop) after the wet-sieving procedure. Non-mycorrhized treatments are abbreviated by A (*Alnus incana*), P (*Poa pratensis*), AP (*Alnus incana* and *Poa pratensis*), K (control) and the corresponding mycorrhized (M) treatments by AM, PM, APM and KM.

Treatment	**Mean on**	**Mean drop**	**Sd on**	**Sd drop**
A	322.975	422.577	100.697	102.436
AM	213.844	517.462	77.067	74.141
P	667.866	83.738	38.026	37.393
PM	546.742	221.923	117.969	121.087
AP	654.823	89.285	57.096	49.446
APM	521.372	224.931	117.826	125.159
K	111.085	639.615	45.423	40.603
KM	131.180	611.831	26.539	26.603

III.V Mean, Standard Deviation, Median and Median Absolute Deviation Values of the Soil Aggregate Stability

Table 6.6: Mean, standard deviation (sd), median and median absolute deviation (mad) values of the soil aggregate stability in terms of all non-mycorrhized treatments A (*Alnus incana*), P (*Poa pratensis*), AP (*Alnus incana* and *Poa pratensis*), K (control) and the corresponding mycorrhized (M) treatments AM, PM, APM and KM.

Treatment	**Mean**	**sd**	**Median**	**mad**
A	0.433	0.135	0.425	0.152
AM	0.292	0.102	0.298	0.124
P	0.889	0.049	0.879	0.058
PM	0.712	0.155	0.719	0.209
AP	0.880	0.066	0.877	0.054
APM	0.700	0.163	0.724	0.170
K	0.147	0.059	0.134	0.039
KM	0.176	0.035	0.171	0.044

IV Degree of Mycorrhization

IV.I Mean and Median Values of the Mycorrhization Degree

Table 6.7: Mean and median values of the mycorrhization degree [%] of *Alnus incana* from the mycorrhized *Alnus incana* treatment (AM) and the combined treatment of *Alnus incana* and *Poa pratensis* (APM)

Treatment	**Mean**	**Median**
AM	75.576	83.784
APM	47.506	48.649

V Ergosterol

V.I Ergosterol Extraction Procedure

1. Weigh 2 g of the soil sample and 0.2 g of ascorbic acid (L(+)-Ascorbic acid ($C_6H_8O_6$ - M 176.12 g / mol - density 1.65; Pro.-No. 3525.2; Carl Roth GmbH + Co. KG, Schoemperlenstr. 3-5, D-76185 Karlsruhe)) in two separate dishes.

2. Prepare a mixture consisting of 10 ml methanol (CH_4O), 5 ml ethanol (C_2H_6O) and 2 g of potassium hydroxide (KOH) pellets in a graduated cylinder. If one has several samples, then a substantial amount of mixture can be prepared beforehand, i.e. 250 ml methanol, 125 ml ethanol, and 50 g of KOH pellets. It takes 15 to 30 min for the pellets to dissolved. The remaining mixture should be stored in the fridge if it is not being used immediately.

3. Adjust the temperature and the speed of the oil bath to 110 $^\circ$C and 1460 rpm, respectively, and open the cold water tap.

4. Prepare a mixture consisting of 2 g of the soil sample, 15 ml methanol-ethanol-KOH mixture, 0.2 g ascorbic acid in a round bottom flask.

5. Saponify the sample in the oil bath for 35 min. The surface of the oil bath should be at the same level or a little bit higher than the one of the mixture. Constant dripping should be observed during the whole saponification process (Figure 2.4).

6. Cool the sample mixture in ice for 2 min.

7. Filter the cooled sample mixture into an Erlenmeyer flask, add 1 ml of the mixture to the round bottom flask to rinse it.

8. Pour the filtered liquid into a separating funnel, rinse the Erlenmeyer with 4 ml deionized water and add it into the funnel.

9. Add 20 ml of n-hexane into the separating funnel.

10. Shake the separating funnel gently, open the valve three times to let air inside and place the separating funnel in a holder to keep it in a constant position. Wait until two different layers are formed (Figure 2.5).

11. Use a clean glass flask and collect the filtered liquid (methanol-ethanol-KOH).

12. Collect the n-hexane layer in another glass flask and add a small amount of anhydrous sodium sulphate (Na_2SO_4 - M = 142.04 g / mol; Cas-No: 7757-82-6; Merck KGaA, 64271 Darmstadt, Germany; EMD Millipore Corporation, 290 Concord Road, Billerica MA 01821, USA.) to bind the water. Stir it constantly until it becomes fluffy.

13. Filter the n-hexane liquid into a round bottom flask.

14. Pour the filtered liquid (methanol-ethanol-KOH) back into the separating funnel and add 20 ml of n-hexane. Repeat the same separation procedure as described above.

15. Vacuum-dry the liquid at 40 °C until all the n-hexane has evaporated (Figure 2.5). (First slowly decrease the pressure to 300 mbar for 10 min, then drain the collected n-hexane into the corresponding canister and decrease the pressure to 100 mbar for 5 min.)

V.II Additional Ergosterol Information

V.II.1 Ergosterol Stock Solution

100.33 mg Ergosterol in 200 ml MeOH $\rightarrow$ 500 μg ml^{-1}
10.04 μg ml^{-1} $\rightarrow$ 10'040 ng ml^{-1}

V.II.2 1st Dilution of Ergosterol

1.000 g Ergosterol stock solution on 100 g with MeOH $\rightarrow$ 5000 ng ml^{-1}

V.II.3 2nd Dilution of Ergosterol

5.000 g 1st dilution Ergosterol on 50 g with MeOH $\rightarrow$ 500 ng ml^{-1}

Table 6.8: Point calibration for blank determination (K samples), calibration standard and check Ergosterol (Erg) standard values.

Point calibration for blank determination (K samples)		
Std. 1(K)	1 g 2nd Erg dilution on 10 g with MeOH	50 ng ml^{-1}
Std. 2(K)	2 g 2nd Erg dilution on 10 g with MeOH	100 ng ml^{-1}
Calibration standard		
Std. 1	1 g 1st Erg dilution on 10 g with MeOH	500 ng ml^{-1}
Std. 2	2 g 1st Erg dilution on 10 g with MeOH	1000 ng ml^{-1}
Std. 3	3 g 1st Erg dilution on 10 g with MeOH	1500 ng ml^{-1}
Std. 4	4 g 1st Erg dilution on 10 g with MeOH	2000 ng ml^{-1}
Std. 5	5 g 1st Erg dilution on 10 g with MeOH	2500 ng ml^{-1}
Std. 6	1st Erg dilution	5000 ng ml^{-1}
Check Ergosterol standard		
K01	1.5 g 1st Ergl dilution on 10 g with MeOH	750 ng ml^{-1}
K02	2.5 g 1st Erg dilution on 10 g with MeOH	1250 ng ml^{-1}
K03	3.5 g 1st Erg dilution on 10 g with MeOH	1750 ng ml^{-1}
K04	4.5 g 1st Erg dilution on 10 g with MeOH	2250 ng ml^{-1}
K05	7 g 1st Erg dilution on 10 g with MeOH	3500 ng ml^{-1}
K06	8.5 g 1st Erg dilution on 10 g with MeOH	4250 ng ml^{-1}
K07	2.000 g Erg stock solution on 100 g with MeOH	10000 ng ml^{-1}
K08	3.000 g Erg stock solution on 100 g with MeOH	15000 ng ml^{-1}

V.III Chromatograms

V.III.1 Chromatogram of the Hexan Extraction

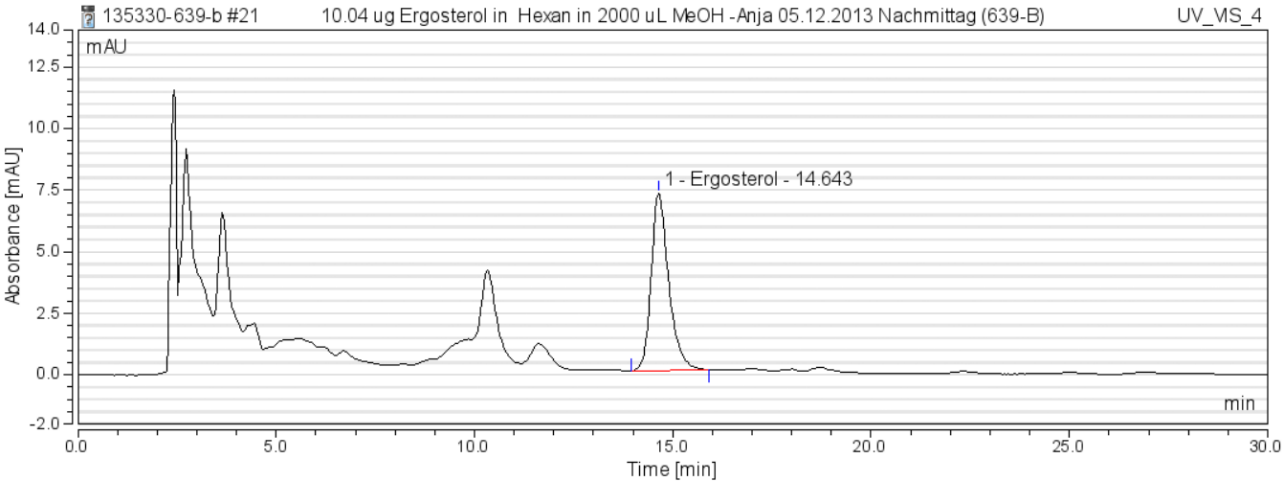


Figure 6.3: Chromatogram of the hexane extraction of the sample number 21. A recovery value of 10012.2 ng Ergosterol per g sample was revealed.

V.III.2 Chromatogram of the Liquid Phase Extraction

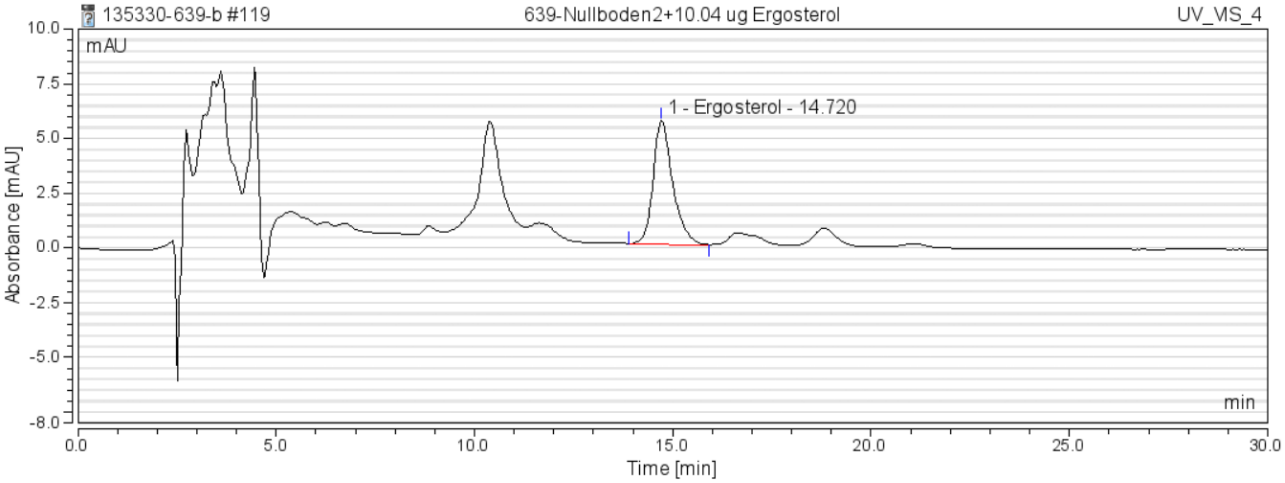


Figure 6.4: Chromatogram of the liquid phase extraction of the non-mycorrhized soil sample number 119. A recovery value of 8663.2 ng Ergosterol per g sample was found.

VI Plant Biomass

VI.I Mean and Median Values of the Root Length

Table 6.9: Mean and median values of the root length per soil volume [cm cm^{-3}]. Non-mycorrhized treatments are abbreviated by A (*Alnus incana*), P (*Poa pratensis*), AP (*Alnus incana* and *Poa pratensis*) and the corresponding mycorrhized (M) treatments by AM, PM and APM.

Treatment	**Mean**	**Median**
A	2.330	2.321
AM	1.805	1.697
P	18.048	17.768
PM	13.997	14.158
AP	15.934	14.509
APM	12.842	11.760

VI.II Mean and Median Values of the Aboveground Plant Dry Weight

Table 6.10: Mean and median values of the aboveground plant dry weight [g]. Non-mycorrhized treatments are abbreviated by A (*Alnus incana*), P (*Poa pratensis*), AP (*Alnus incana* and *Poa pratensis*) and the corresponding mycorrhized (M) treatments by AM, PM and APM.

Treatment	**Mean**	**Median**
A	0.144	0.14
AM	0.39	0.34
P	0.294	0.3
PM	0.182	0.16
AP	0.284	0.29
APM	0.481	0.52

VI.III Mean and Median Values of the Root Dry Weight

Table 6.11: Mean and median values of the root dry weight [g]. Non-mycorrhized treatments are abbreviated by A (*Alnus incana*), P (*Poa pratensis*), AP (*Alnus incana* and *Poa pratensis*) and the corresponding mycorrhized (M) treatments by AM, PM, APM.

Treatment	**Mean**	**Median**
A	0.134	0.136
AM	0.138	0.141
P	0.352	0.343
PM	0.223	0.22
AP	0.313	0.313
APM	0.242	0.24

VI.IV Mean and Median Values of the Total Plant Biomass

Table 6.12: Mean and median values of the total plant biomass dry weight [g]. Non-mycorrhized treatments are abbreviated by A (*Alnus incana*), P (*Poa pratensis*), AP (*Alnus incana* and *Poa pratensis*) and the corresponding mycorrhized (M) treatments by AM, PM and APM.

Treatment	**Mean**	**Median**
A	0.278	0.282
AM	0.528	0.485
P	0.646	0.633
PM	0.404	0.38
AP	0.596	0.601
APM	0.723	0.763

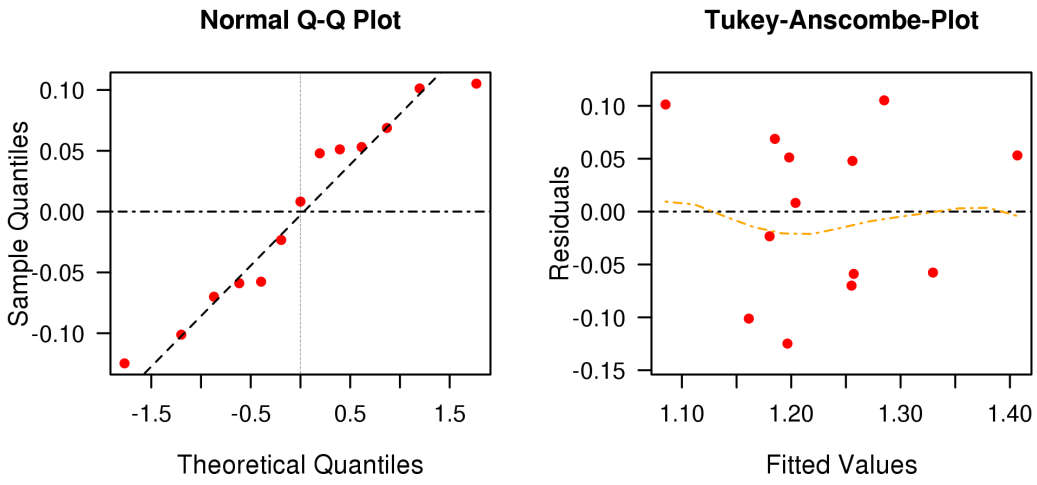
VII Linear Regression Models of Soil Aggregate Stability and Root Length

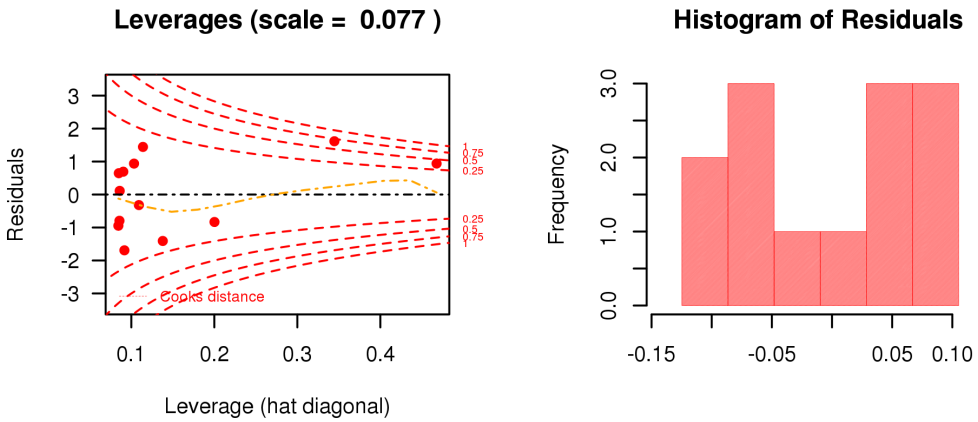
VII.I Treatment AP: Simple Linear Regression Model

Table 6.13: Summary of the linear regression model of treatment AP lm(asin(sqrt(Soil aggregate stability AP)) ~ $\log_{10}$(Root length AP)).

Coefficients	**Estimate**	**Std. Error**	**t value**	**Pr(>|t|)**
Intercept	0.199	0.297	0.670	0.517
$\log_{10}$(Root length AP)	0.865	0.248	3.484	0.005 **
Residual standard error	0.081 on 11 degrees of freedom			
Multiple R-squared	0.525	Adjusted R-squared		0.481
F-statistic	12.14 on 1 and 11 DF		P-value	0.005

VII.II Treatment AP: Normal Q-Q Plot, Tukey-Anscombe-Plot, Leverages and Histogramm of Residuals



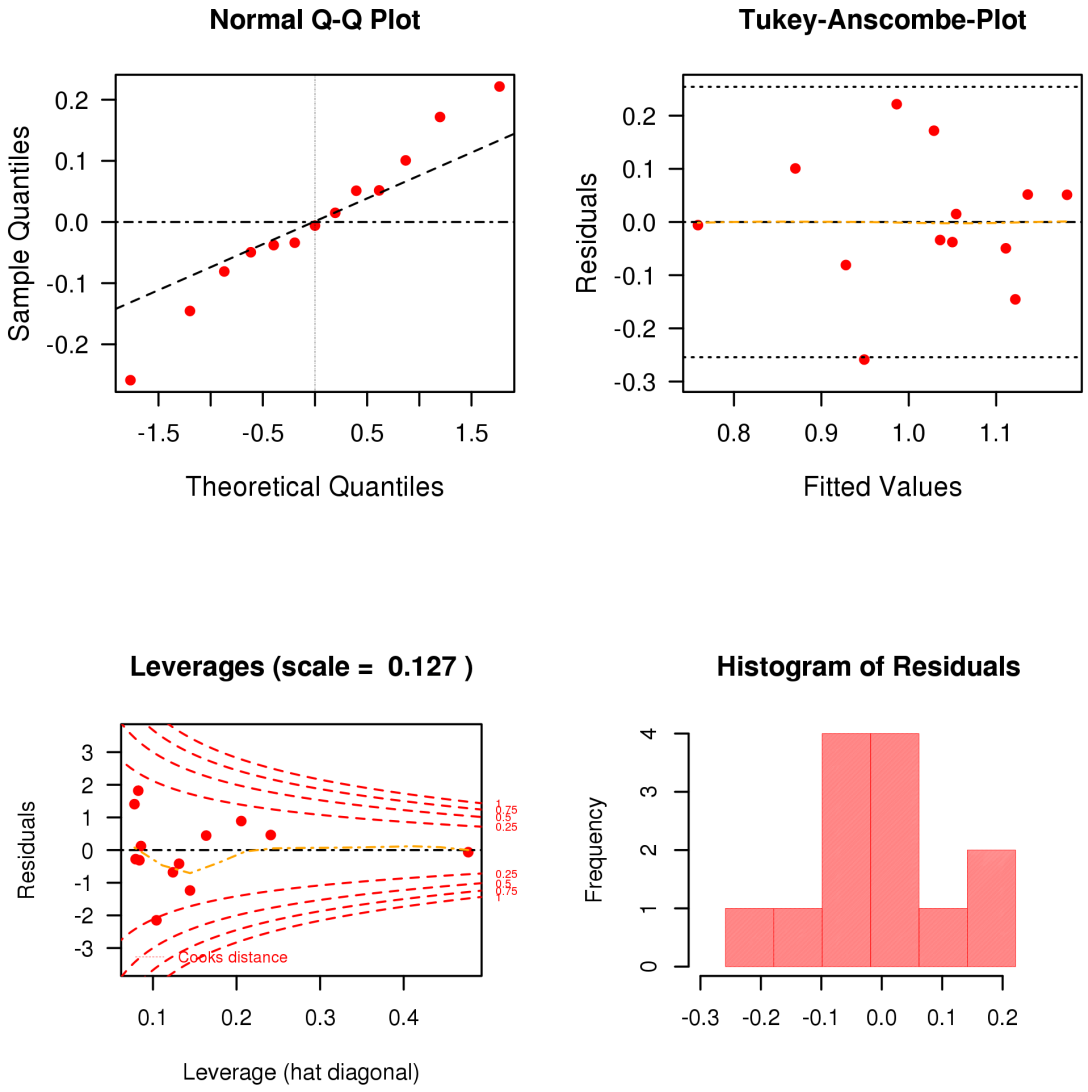


VII.III Treatment PM: Simple Linear Regression Model

Table 6.14: Summary of the linear regression model of treatment PM lm(asin(sqrt(Soil aggregate stability PM)) ~ $\log_{10}$(Root length PM)).

Coefficients	**Estimate**	**Std. Error**	**t value**	**Pr(>|t|)**
Intercept	-0.007	0.336	-0.021	0.984
$\log_{10}$(Root length PM)	0.906	0.296	3.065	0.011 *
Residual standard error	0.133 on 11 degrees of freedom			
Multiple R-squared	0.461	Adjusted R-squared		0.412
F-statistic	9.393 on 1 and 11 DF		P-value	0.011

VII.IV Treatment PM: Normal Q-Q Plot, Tukey-Anscombe-Plot, Leverages and Histogramm of Residuals



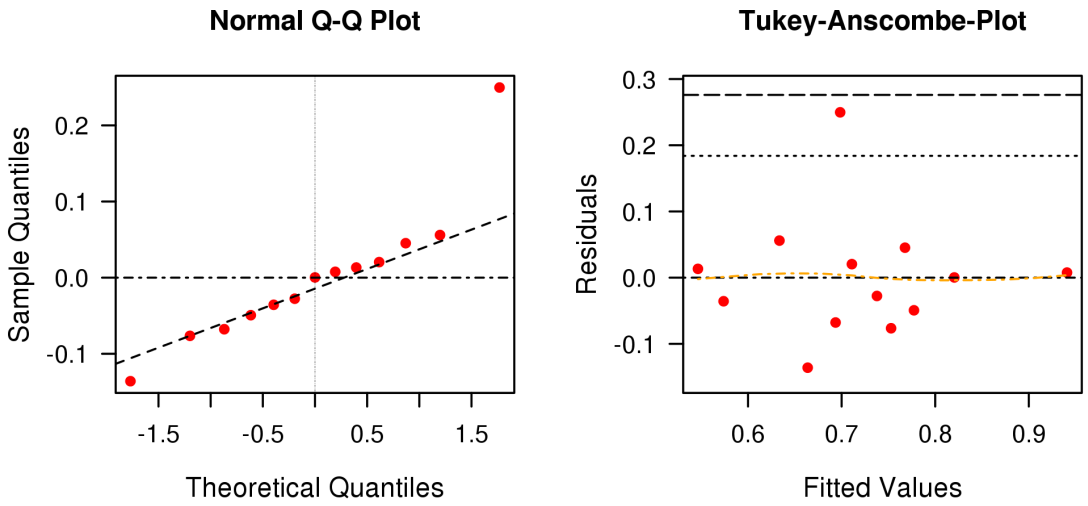
VIII Linear Regression Models of Soil Aggregate Stability and Water Content

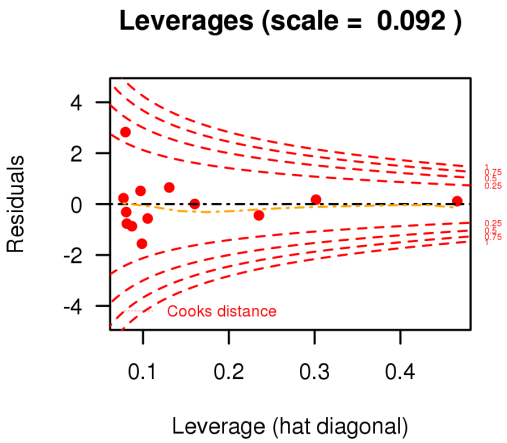
VIII.I Treatment A: Simple Linear Regression Model

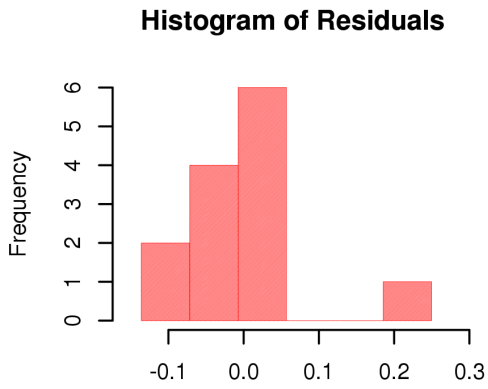
Table 6.15: Summary of the linear regression model of treatment A lm(asin(sqrt(Soil aggregate stability A)) ~ Water content A).

Coefficients	**Estimate**	**Std. Error**	**t value**	**Pr(>|t|)**
Intercept	1.470	0.203	7.232	1.7e-05 ***
$\log_{10}$(Water content A)	-17.992	4.814	-3.737	0.003 **
Residual standard error	0.096 on 11 degrees of freedom			
Multiple R-squared	0.559	Adjusted R-squared		0.519
F-statistic	13.97 on 1 and 11 DF		P-value	0.003

VIII.II Treatment A: Normal Q-Q Plot, Tukey-Anscombe-Plot, Leverages and Histogramm of Residuals





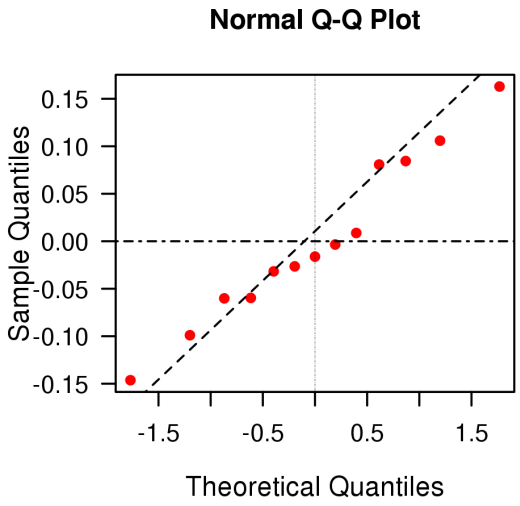


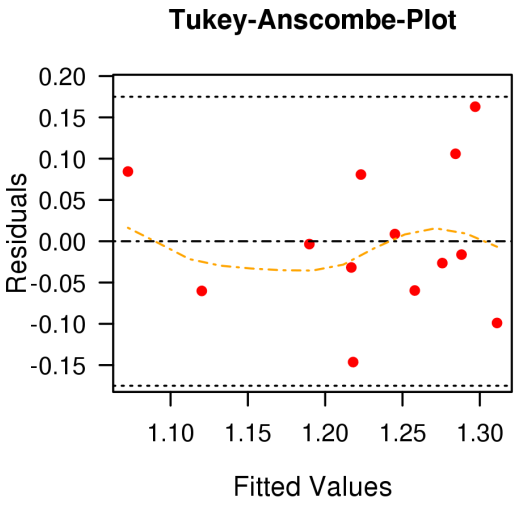
VIII.III Treatment AP: Simple Linear Regression Model

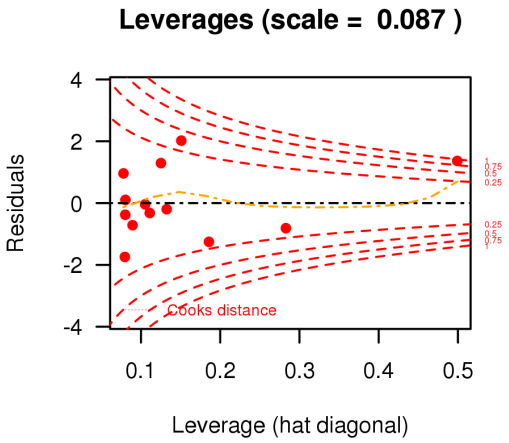
Table 6.16: Summary of the linear regression model of treatment AP lm(asin(sqrt(Soil aggregate stability AP)) ~ Water content AP).

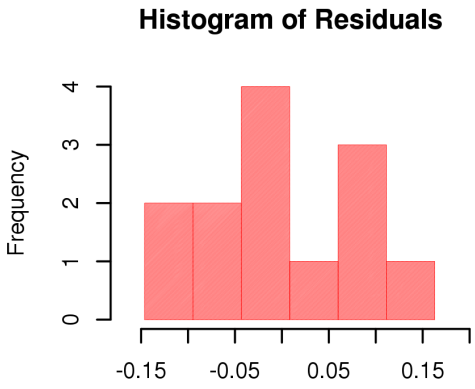
Coefficients	**Estimate**	**Std. Error**	**t value**	**Pr(>|t|)**
Intercept	1.519	0.111	13.685	3.0e-08 ***
$\log_{10}$(Water content AP)	-10.018	3.757	-2.667	0.022 *
Residual standard error	0.091 on 11 degrees of freedom			
Multiple R-squared	0.393	Adjusted R-squared		0.337
F-statistic	7.111 on 1 and 11 DF		P-value	0.022

VIII.IV Treatment AP: Normal Q-Q Plot, Tukey-Anscombe-Plot, Leverages and Histogramm of Residuals







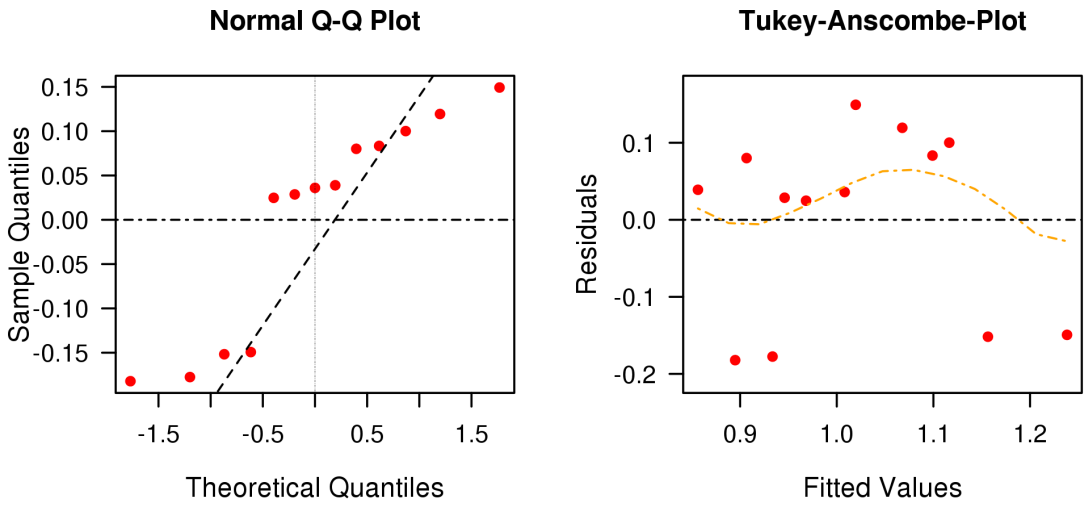


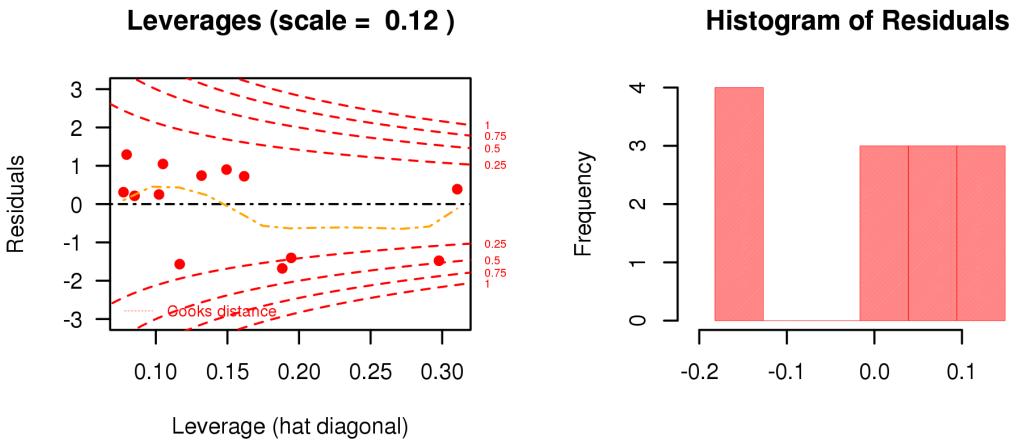
VIII.V Treatment PM: Simple Linear Regression Model

Table 6.17: Summary of the linear regression model of treatment PM lm(asin(sqrt(Soil aggregate stability PM)) ∼ Water content PM).

Coefficients	**Estimate**	**Std. Error**	**t value**	**Pr(>|t|)**
Intercept	1.426	0.125	11.426	1.9e-07 ***
$\log_{10}$(Water content PM)	-16.110	4.710	-3.421	0.006 **
Residual standard error	0.126 on 11 degrees of freedom			
Multiple R-squared	0.515	Adjusted R-squared		0.471
F-statistic	11.7 on 1 and 11 DF		P-value	0.006

VIII.VI Treatment PM: Normal Q-Q Plot, Tukey-Anscombe-Plot, Leverages and Histogramm of Residuals





IX Linear Regression Models of Plant Dry Weight and Root Length

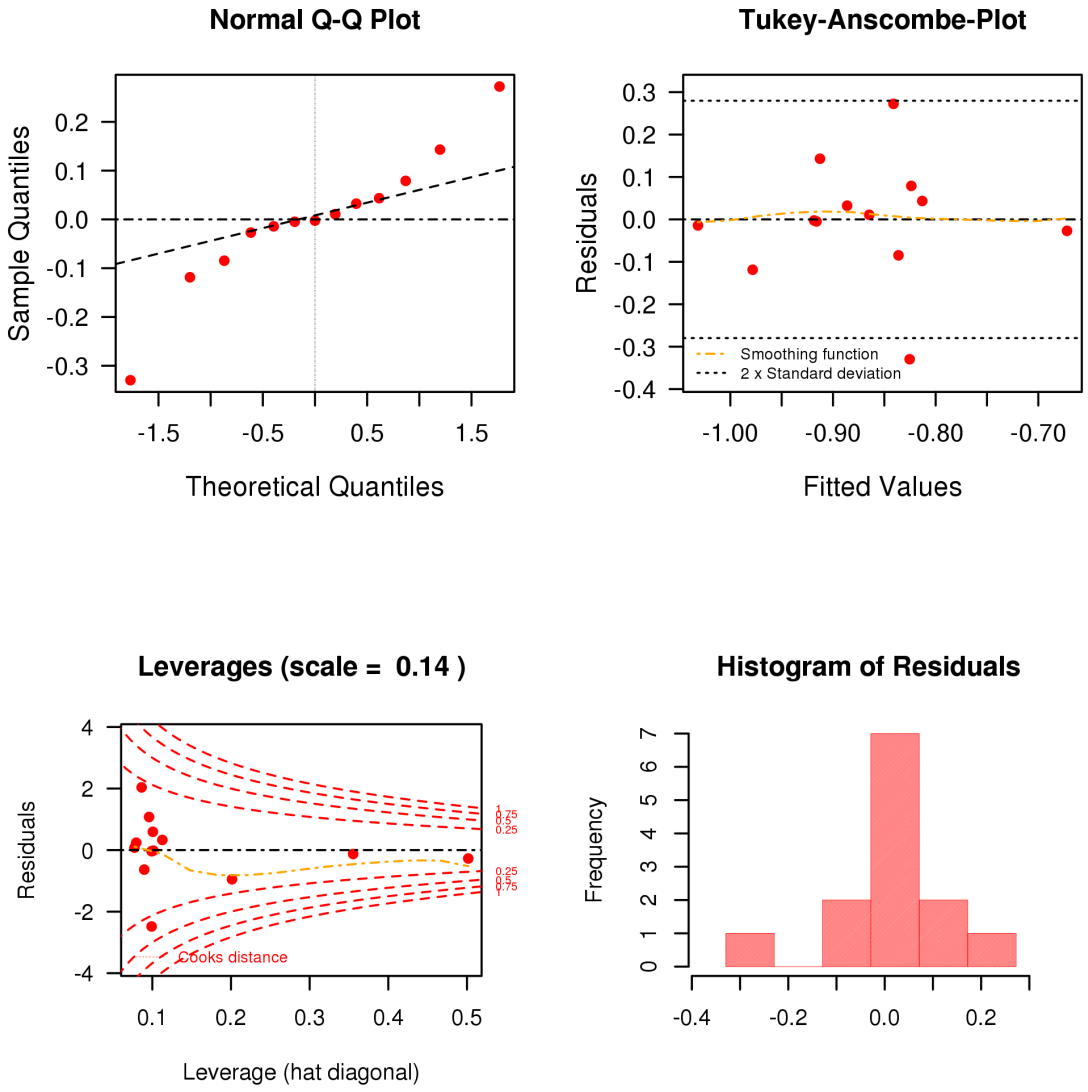
IX.I Treatment A: Simple Linear Regression Model

Table 6.18: Summary of the linear regression model of treatment A lm($\log_{10}$(Plant dry weight A) $\sim$ $\log_{10}$(Root length A)).

Coefficients	**Estimate**	**Std. Error**	**t value**	**Pr(>|t|)**
Intercept	-1.260	0.191	-6.597	3.9e-05 ***
$\log_{10}$(Root length A)	1.082	0.518	2.087	0.061
Residual standard error	0.1461 on 11 degrees of freedom			
Multiple R-squared	0.284	Adjusted R-squared		0.219
F-statistic	4.354 on 1 and 11 DF		P-value	0.061

The linear regression model lm($\log_{10}$(Plant dry weight A) $\sim$ $\log_{10}$(Root length A)) was not significant considering the Pearson p-value but the Spearman p-value was 0.0495.

IX.II Treatment A: Normal Q-Q Plot, Tukey-Anscombe-Plot, Leverages and Histogramm of Residuals

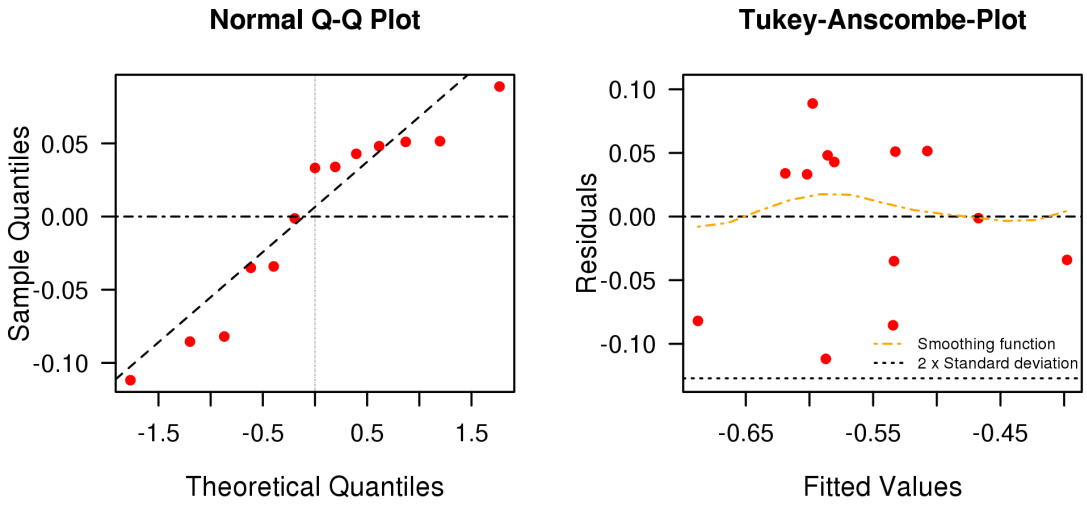


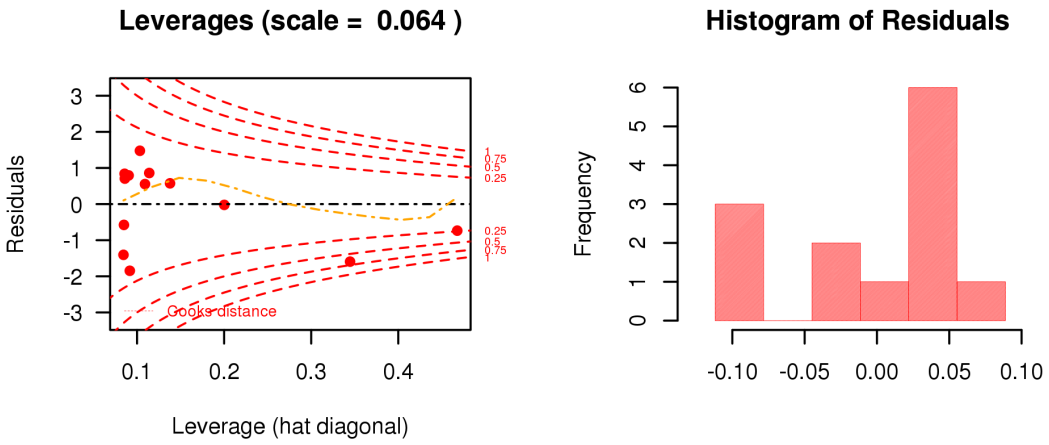
IX.III Treatment AP: Simple Linear Regression Model

Table 6.19: Summary of the linear regression model of treatment AP $\text{lm}(\log_{10}(\text{Plant dry weight AP}) \sim \log_{10}(\text{Root length AP}))$.

Coefficients	**Estimate**	**Std. Error**	**t value**	**Pr(>|t|)**
Intercept	-1.486	0.244	-6.092	7.8e-05 ***
$\log_{10}$(Root length AP)	0.779	0.204	3.821	0.003 **
Residual standard error	0.066 on 11 degrees of freedom			
Multiple R-squared	0.570	Adjusted R-squared		0.531
F-statistic	14.6 on 1 and 11 DF		P-value	0.003

IX.IV Treatment AP: Normal Q-Q Plot, Tukey-Anscombe-Plot, Leverages and Histogramm of Residuals



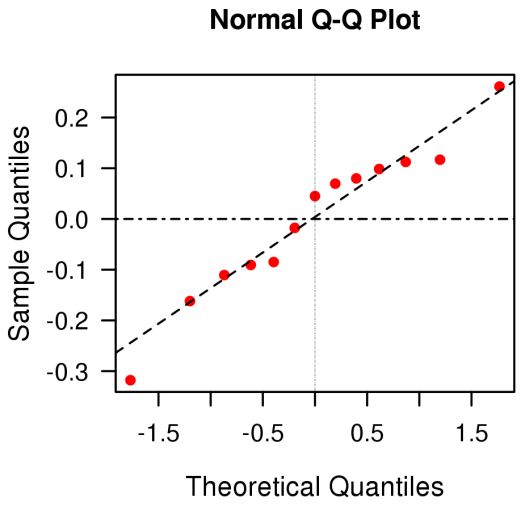


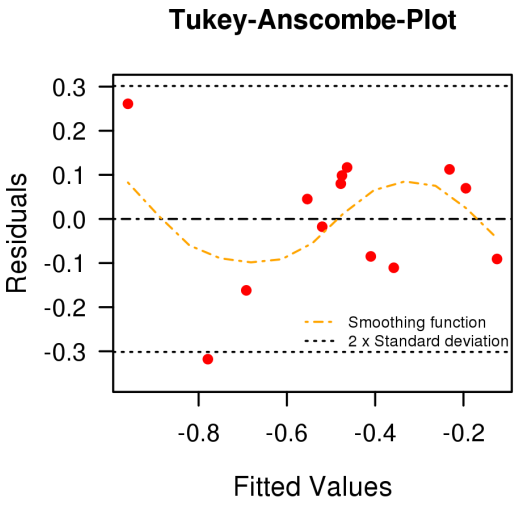
IX.V Treatment AM: Simple Linear Regression Model

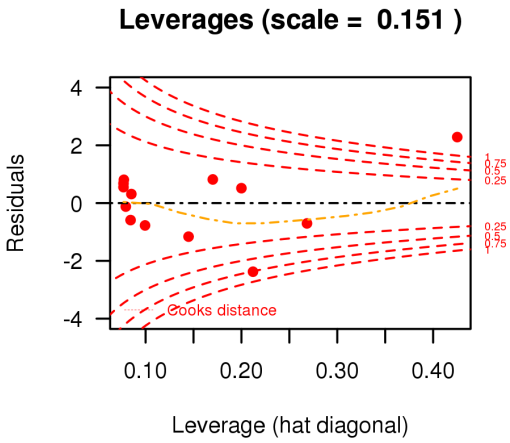
Table 6.20: Summary of the linear regression model of treatment AM $\text{lm}(\log_{10}(\text{Plant dry weight AM}) \sim \log_{10}(\text{Root length AM}))$.

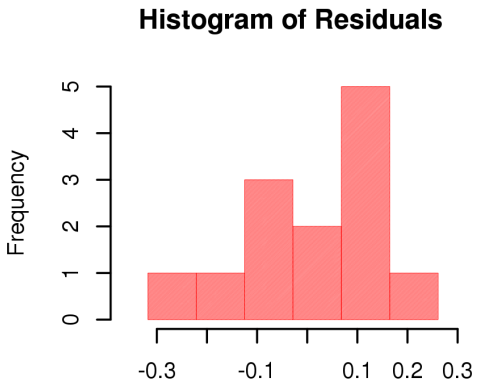
Coefficients	**Estimate**	**Std. Error**	**t value**	**Pr(>|t|)**
Intercept	-0.785	0.073	-10.691	3.8e-07 ***
$\log_{10}$(Root length AM)	1.350	0.261	5.168	3.1e-04 ***
Residual standard error	0.157 on 11 degrees of freedom			
Multiple R-squared	0.708	Adjusted R-squared		0.682
F-statistic	26.7 on 1 and 11 DF		P-value	3.1e-04

IX.VI Treatment AM: Normal Q-Q Plot, Tukey-Anscombe-Plot, Leverages and Histogramm of Residuals







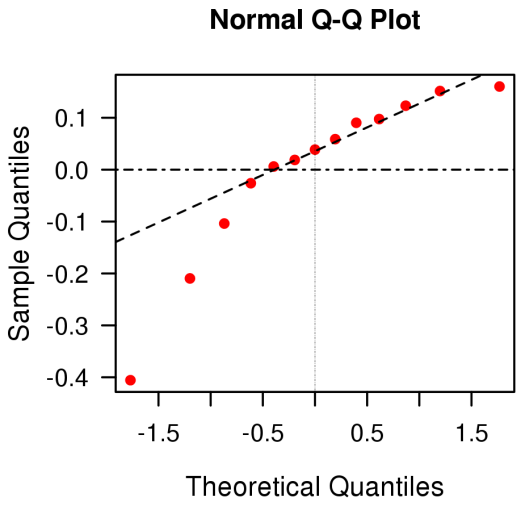


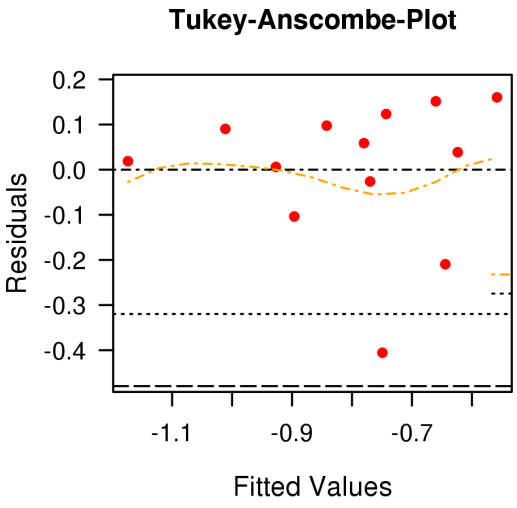
IX.VII Treatment PM: Simple Linear Regression Model

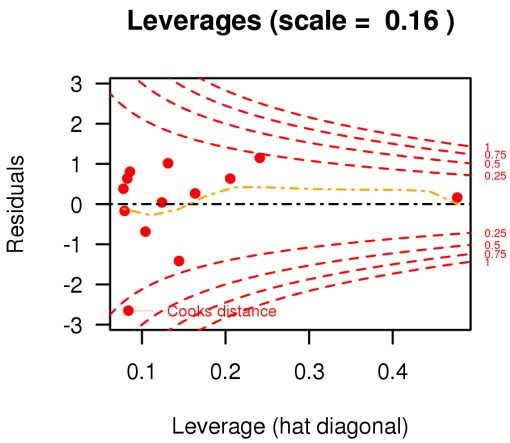
Table 6.21: Summary of the linear regression model of treatment PM $\text{lm}(\log_{10}(\text{Plant dry weight PM}) \sim \log_{10}(\text{Root length PM}))$.

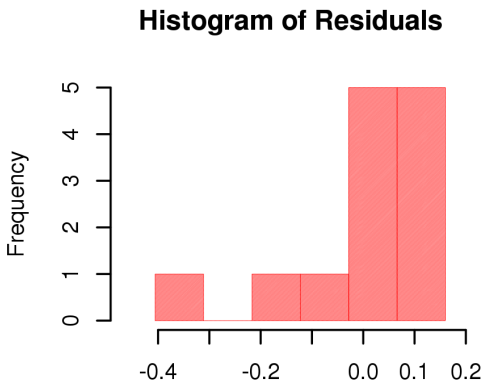
Coefficients	**Estimate**	**Std. Error**	**t value**	**Pr(>|t|)**
Intercept	-2.290	0.422	-5.421	2.1e-04 ***
$\log_{10}$(Root length PM)	1.321	0.372	3.552	0.005 **
Residual standard error	0.167 on 11 degrees of freedom			
Multiple R-squared	0.534	Adjusted R-squared		0.492
F-statistic	12.62 on 1 and 11 DF		P-value	0.005

IX.VII.1 Treatment PM: Normal Q-Q Plot, Tukey-Anscombe-Plot, Leverages and Histogramm of Residuals






Leverages (scale = 0.16)
Residuals
Leverage (hat diagonal)
Cooks distance
1
0.75
0.5
0.25
0.25
0.5
0.75
1


Histogram of Residuals
Frequency

Eigenständigkeitserklärung

Die unterzeichnete Eigenständigkeitserklärung ist Bestandteil jeder während des Studiums Umweltnaturwissenschaften verfassten schriftlichen Arbeit (auch der elektronischen Version). Im Falle von Bachelor- und Masterarbeiten ist eine Kopie dieses Formulars dem Diplomantrag beizulegen.

Ich bestätige, die vorliegende Arbeit selbständig und in eigenen Worten - ausgenommen Korrekturvorschläge - verfasst zu haben.

Titel der Arbeit:

Verfasst von:

Name Vorname

Ich bestätige mit meiner Unterschrift:

- Ich habe keine im Merkblatt www.ethz.ch/students/exams/plagiarism_s_de.pdf beschriebene Form des Plagiats begangen.
- Ich habe alle Methoden, Daten und Arbeitsabläufe wahrheitsgetreu dokumentiert.
- Ich habe keine Daten manipuliert.
- Ich habe in der Danksagung alle Personen erwähnt, welche die Arbeit wesentlich unterstützt haben.
- Ich verstehe die oben genannten Regeln.

Ich nehme zur Kenntnis, dass die Arbeit eventuell mit elektronischen Hilfsmitteln auf Plagiate überprüft wird.

Ort, Datum Unterschrift*

* Bei Gruppenarbeiten sind die Unterschriften aller Verfasser und Verfasserinnen erforderlich. Durch die Unterschrift bürgen sie gemeinsam für den gesamten Inhalt dieser schriftlichen Arbeit.