The potential use of waste wool pellets as a substrate amendment affecting arbuscular mycorrhizal development in container-grown *Allium porrum*

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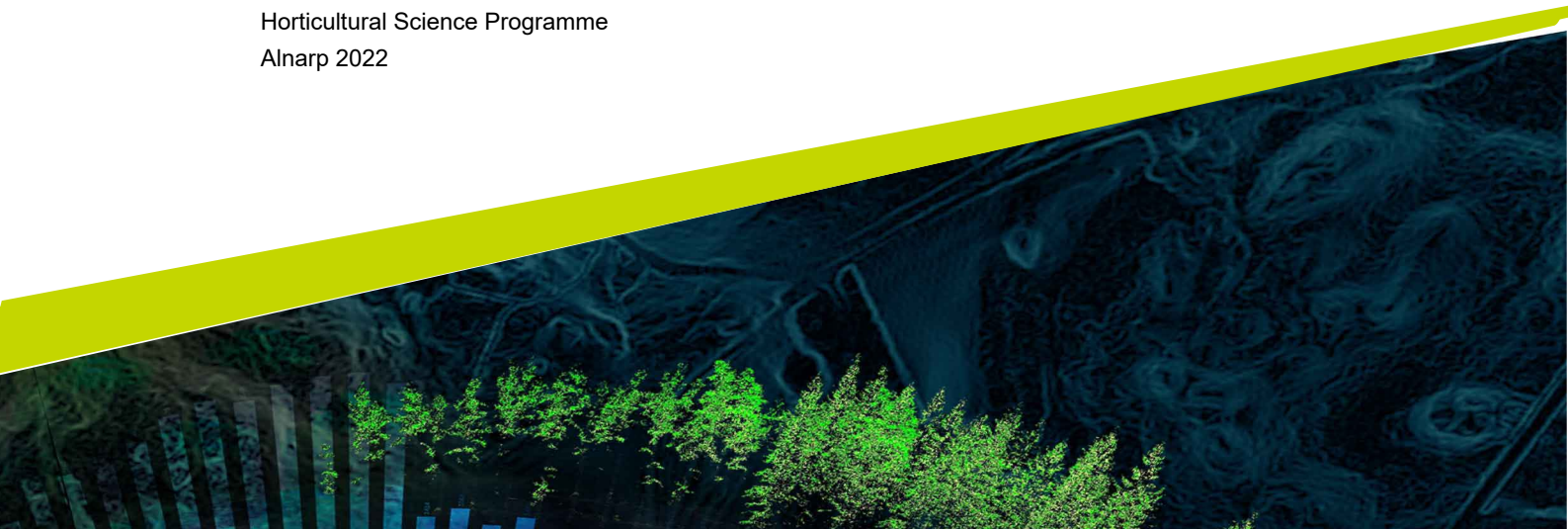

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Abstract

Based on future scenarios, agricultural areas will be dramatically influenced by climatic patterns, and sustainable measures such as reduced applications of inorganic fertilizers and practices to increase carbon sequestration are needed in order to reduce carbon dioxide from the atmosphere. Decreased soil fertility is a considerable stress factor limiting the productivity of agriculture. Soil fertility is based on chemical, biological, and physical components. Soil biology is a prime indicator of soil health and comprises the presence of micro-and macroorganisms. Arbuscular mycorrhizal fungi (AMF) are manufactured and sold as commercial products and are one important tool used as a microorganism-based strategy to support long-term sustainable agricultural practices.

EU was in 2020 the second-largest sheep producer globally, and the sheep are primarily reared for meat production. Sheep wool is, due to low market prices, often not considered a commercially viable product and a significant part of the wool is either dumped, burnt, or sent to landfill sites. Sheep wool is by the European Commission classified as special waste and there is ongoing research in finding environmentally friendly ways of waste wool management. Sheep wool has during the last decade gained interest within the horticultural sector due to its nutrient content and physical properties. The suitability of sheep wool as a long-term organic fertilizer is established in several studies. However, less is known about its interaction and combined effects with AMF.

For this trial, the main aim was to examine the plant performance of leek (*A. porrum*) in the combined presence of AMF and sheep wool pellets (SWP). Prior to the experiment, nutrient analyses and physical measurements were performed on each substrate and SWP. The main trial was performed in a greenhouse for 56 days. The treatments included mycorrhizal inoculation by *R. irregularis,* and sheep wool pellets (5 g/L) and the growth was then evaluated by measurements of plant biomass and tissue N concentration. Also, root colonization by AM fungal structures was examined.

The results showed that SWP addition had a significant effect on plant growth, the tissue N concentration and biomass increased correspondingly. The combined effects of SWP and AMF inoculation could not be fully determined. However, sheep wool has most certain the potential of being an organic fertilizer, but also the ability to promote soil microbiota due to its biochemical properties. Long-term trials would be needed to fully evaluate sheep wool as a plant biostimulant and as a substrate amendment.

Keywords: arbuscular mycorrhizal fungi, sheep wool, soil fertility, organic fertilizer

Table of contents

List of tables

List of figures

Abbreviations

AMF	Arbuscular mycorrhizal fungi
FAS I	Fatty acid synthase type I
MF	Mycorrhizal fungi
PGPR	Plant growth-promoting rhizobacteria
PHs	Protein hydrolysates
PBs	Plant biostimulants
PSB	Phosphate solubilizing bacteria

1. Introduction

Agricultural areas are dramatically influenced by altered climate patterns (Corwin 2021). Based on future scenarios, adaption and mitigation are essential to raise resilience capacity in agricultural systems (Bulgari et al. 2019) and the sector is urged to take strong measures to increase carbon dioxide removal from the atmosphere, such as reduced amounts of inorganic fertilizer along with practices that increase carbon sequestration (Abdallah et al. 2019b). The most important abiotic stresses limiting the productivity of agriculture are drought, salinity, non-optimal temperatures, and low soil fertility (Bulgari et al. 2019).

Soil fertility is a comprehensive term used in order to describe the ability of soil to sustain plant growth (Panpette & Yogeshvari K., 2019). There are three main components of soil fertility: chemical, biological, and physical. To determine the productivity of all agricultural systems, soil fertility is a steppingstone, and the three above-mentioned components need to be in balance for well-functioning soil. The prime indicator of soil health is soil biology, namely micro-and macroorganisms (Panpette & Yogeshvari K., 2019). Microorganism-based strategies are suggested as long-term sustainable agricultural practices and according to Golubkina et al. (2020), arbuscular mycorrhizal fungi (AMF) is one important tool in the development of more ecologically oriented production systems. It has been shown that there is a decreased presence and diversity of AMF in cultivated soils (Golubkina et al. 2020). The reason may be connected to hyphal network disruption caused by tilling, the inclusion of non-mycorrhizal species in crop rotation, the use of agrochemicals, and fallow periods (Golubkina et al. 2020).

1.1 Arbuscular Mycorrhizal Fungi (AMF)

Mycorrhizal fungi are specialized members of the vast population of microorganisms that colonize the plant rhizosphere. Arbuscular mycorrhizal fungi (AMF) are the most common type of mycorrhizal fungi and are classified into a separate phylum, the Glomeromycota (Smith & Read, 2008). AMF are recognized as obligate symbionts of a very wide range of plant species and key components of the soil microbial community. The symbioses are biotrophic, normally mutualistic, and are mainly based on bidirectional nutrient transfer, sometimes also supplemented by other benefits such as soil aggregation and stabilization, disease resistance, and drought tolerance (Smith & Read, 2008).

Mycorrhizal colonization takes place within the root cortex, where tree-like branched hyphae structures are formed, so-called arbuscules (Salmeron-Santiago et al. 2022). AMF facilitates plant uptake of mineral nutrients (Jiang et al. 2017). In return, the plant gives up part of its photoassimilates to the fungus (Salmeron-Santiago et al. 2022). It is estimated that around 20% of the photosynthetically produced carbon is destined for the maintenance of the symbiosis (Bravo et al. 2017). Obtaining carbon for the production of energy and the skeleton of fungal cell components is a key step in AMF growth and reproduction (Sugiura et al. 2020).

Early studies have suggested carbohydrates as the primary source of carbon (C) transferred from the plants to the fungi (Jiang et al. 2017). However, later discoveries have shown that AMF are fatty acid auxotrophs and therefore supplied with fatty acids by the plant (Jiang et al. 2017; Sugiura et al. 2020; Salmeron-Santiago et al. 2022). The auxotrophy is caused by the absence of the multi-domain enzyme fatty acid synthase type I (FAS I), which controls de novo lipid biosynthesis in fungi (Salmeron-Santiago et al. 2022). Jiang et al. (2017) showed that the AM fungi *R. irregularis* were unable to synthesize palmitic acid ($C_{16}H_{32}O_2$) due to the lack of genes encoding FAS I. Nevertheless, studies have shown results suggesting that AMF may be cultured independently from host plants under artificial conditions (Kameoka et al. 2019; Sugiura et al. 2020). Sugiura et al. (2020) tested eight saturated and unsaturated fatty acids (C:12-C:18) and two β-monoacylglycerols. It was shown that myristate ($C_{17}H_{34}O_2$) (Figure 1) led to an increase in the biomass of *R. irregularis*. In trials conducted by Kameoka et al. (2019), hyphae branching from mother spores and formation of secondary spores were stimulated in *R. irregularis* by the bacterial isolate 12-methyltetradecanoic acid ($C_{15}H_{30}O_2$) and palmitoleic acid ($C_{16}H_{30}O_2$) (Figure 1).

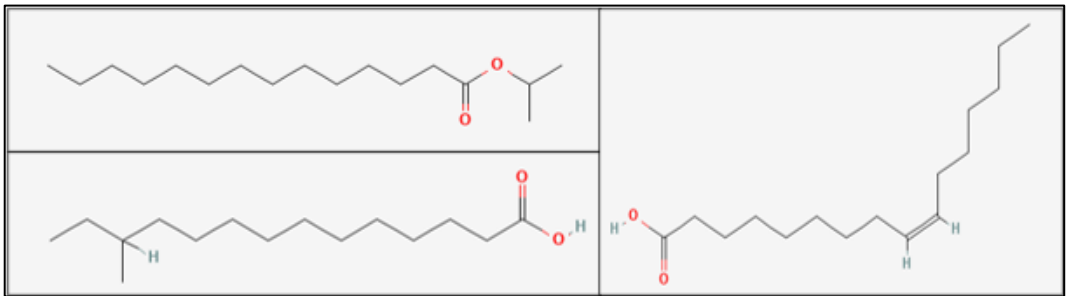


Figure 1. Chemical structures of myristate (top left), 12-methyltetradecanoic acid (bottom left) and palmitoleic acid (right) (PubChem n.d.b, n.d.a, n.d.c).

1.1.1 Commercial AMF production

Over the last decades, companies all over the world have produced and manufactured commercial AMF products (Gianinazzi & Vosátka 2004; Faye et al. 2013). The efficiency of AMF, when applied to cultivated soil, is dependent on several factors such as genetic peculiarities of plant and AMF species, soil characteristics, and environmental factors, including biotic and abiotic stresses, temperature, and precipitation (Golubkina et al. 2020). Therefore, a pre-evaluation of the products is necessary before launching large-scale use. The products are sold as either single species, or in combination with other microbes, for added beneficial effects. A group of bacteria, referred to as plant growth promoting rhizobacteria (PGPR), are present in the mycorrhizosphere and have a synergistic relationship with the AMF (Nadeem et al. 2014). It has been shown that the presence of PGPR supports the establishment of mycorrhizae and improves their ability to perform various functions adequately (ibid). For example, Jiang et al. (2021) demonstrated the AMF incapability to acquire organic P due to the lack of genes for phosphatase. Consequently, AMF are interacting with phosphate solubilizing bacteria (PSB) in order to mineralize organic nutrients (ibid).

1.2 Sheep wool

1.2.1 Wool contaminants and processing

In 2020, the EU had the second-largest sheep production in the world, the majority found in the United Kingdom (26.8 %) (Petek & Marinšek Logar 2021). Sheep in the EU are primarily reared for meat production. Due to low market prices, wool is not a commercially viable product and therefore often presented as a sheep farm by-product (Petek & Marinšek Logar 2021). In Europe, the sector produces more than 200 000 tonnes of coarse wool per year. Coarse wool is the category of wool with a fibre diameter of <50 micrometer (Holkar et al. 2016). Coarse wool does not meet the required quality standards for further processing and is either dumped, burnt, or sent to landfill sites (Abdallah et al. 2019b). Wool kept for further processing, raw wool, needs to follow a procedure of steps in order to remove impurities on the surface of the wool fibres (Salem Allafi et al. 2021). Petek & Marinšek Logar (2021) estimate at the number of impurities of raw wool may reach 40%. The three categories of raw wool contaminants come from natural, acquired, and applied sources (Zakaria El-Sayed et al. 2018). The natural contaminants are produced by the sheep and consist of wool grease and suint.

The acquired and applied contaminants are picked up by the sheeps' surroundings (Zakaria El-Sayed et al. 2018). Acquired contaminant can be stones, dirt, sand, grass straw, and twigs (ibid). The applied contaminants are pesticide or fertilizer residues and generally occur in trace quantities (Petek & Marinšek Logar 2021). The natural contaminants represents a significant part of the raw wool impurities (Zakaria El-Sayed et al. 2018). Suint is water-soluble and secreted by sweat glands. It comprises a mixture of primarily potassium-based salts (ibid), and has been used as a fertilizer since its concentration of potassium (K) could be as high as 187,5 mg kg^{-1} (Zheljazkov et al. 2009). Wool grease is excreted from the sebaceous root of each wool fiber and its main components are fats and oils (Zakaria El-Sayed et al. 2018).

When raw wool is processed for use within the textile industry, the removal of impurities are crucial since they are vectors of microbial contamination (Allafi et al. 2022). The standard method of raw wool fibre processing is based on a series of treatments such as scouring, washing, bleaching, carbonization and functional finishing (Salem Allafi et al. 2021). Scouring is the main step and refers to washing and drying of wool. Carbonization involves the action of high temperatures and strong acids (Allafi et al. 2022), where the aim is to remove vegetable matter (Czaplicki & Strzelecki 2020). Lastly, functional finishing is performed for the fibres to attain their required properties. It is customary to expose the fibres to different chemical and physical treatments (Gulrajani 2013).

1.2.2 The wool fibre structure

The main component of the wool fiber is keratin (Petek & Marinšek Logar 2021). It is one of the most abundant animal proteins and is recovered in hair, nails, feathers hooves, and horns. Keratins from sheep wool fiber can be classified into different groups. The predominant group are α-keratins. They represent 50-60% of the wool fiber and are found in the wool fiber cortex (Allafi et al. 2021). The chains of amino acids are compactly packed and stabilized with a high degree of inter-and intra- molecular disulphide bonds (Kornilłowicz-Kowalska & Bohacz 2011). The non-protein fraction of the wool fiber is the outer hydrophobic lipid layer, known as lanolin (Salem Allafi et al. 2021). Due to the complex fiber structures, the wool is quite resistant to degradation (Petek & Marinšek Logar 2021). Therefore, waste wool represents a serious environmental problem. Recent studies have been conducted in order to review ways of waste wool management, focused on environmentally friendly biotechnological approaches, and the aim is to explore the possibility of producing value-added products.

One alternative solution is to convert waste wool products into different hydrolysates (Petek & Marinšek Logar 2021). Protein hydrolysates (PHs) are a mixture of peptides and amino acids and considered an important group of plant biostimulants (PBs) (Colla et al. 2015). Plant biostimulants (PBs) are defined by du Jardin (2015), as 'any substance or microorganism applied to plants with the aim to enhance nutrition efficiency, abiotic stress tolerance and/or crop quality traits, regardless of its nutrients content'. Protein hydrolysates (PHs) have gained a growing interest due to their positive effects on crop performance (Colla et al. 2015). Protein hydrolysates (PHs) applied in combination with other PBs, such as AMF, has also been investigated. Rouphael et al. (2017) found that plant derived PHs and fungal strains of *Rhizophagus* and *Trichoderma* worked in synergy and increased the marketable fresh yield and root and shoots dry weight in greenhouse lettuce (*Lactuca sativa*) exposed to salt and alkali stress.

1.2.3 The potential use of waste wool in the horticultural sector

As previously mentioned, there is ongoing research in waste wool management. Sheep wool has a rich content of nutrients, especially C, N, and K (Zheljazkov et al. 2009), and has during the last decade gained more attention as a potential alternative of an eco-friendly slow-release fertilizer in modern agriculture (Chen et al. 2021). Several studies have performed trials in a wide range of experimental set-ups and wool products (Table 1).

Waste wool has also been examined as an amendment to peat-based growing substrates (Górecki & Górecki 2010). Special attention is drawn to the high hygroscopic properties of wool (Abdallah et al. 2019a), and according to (Allafi et al. 2021), wool can absorb 30% of its own weight in water and release it subsequently.

Table 1. Over-view of trials conducted with wool products

Examined product	*Added amount*	*Production system*	*Crop examined*	*Findings*	*Authors*
Carbonized wool (BW) and non-carbonized wool (WW)	0.5%, 1% and 2% (corresponds to 0.04%,0.08% and 0.16% N)	Outdoor pot cultivation	Ornamental sunflower and maize	BW increased plant growth and biomass production. Supplementary N fertilization was not needed.	(Abdallah et al. 2019b)
Sheep wool (W) and leather shavings (L)	1, 2 and 4 t/ha (corresponds to 140, 280 and 560 kg N/ha).	Open field trial	Asparagus	Highest yield obtained by W application rate at 2 t/ha.	(Vončina & Mihelic 2013)
Sheep wool pellets combined with added cellulose, starch or casein.	1,2,5 and 10 g pellets/l, (corresponds to a 10-11% total N content.)	Open field, greenhouse cultivation and pot plant cultivation	Iceberg lettuce kohlrabi, tomatoes, and poinsettias	Sheep wool pellets are viable long-term fertilizers.	(Böhme et al. 2012)
Unprocessed and low-grade raw wool	20, 40, 80 and 120 g per 0,851 l.	Container experiment	Swiss chard and basil	Raw wool is suggested as soil amendment, growth medium constituent, and nutrient source.	(Zheljazkov et al. 2009)

Soil organic matter (OM) plays an important role in promoting microorganisms (Abdallah et al. 2019a), and long-term organic fertilization will increase soil microbial diversity and heterogeneity (Sun et al. 2020). Earlier publications have studied sheep wool products as viable fertilizers and/or growing substrates (Zheljazkov et al. 2009; Böhme et al. 2012; Vončina & Mihelic 2013; Abdallah et al. 2019b). Less is known about how sheep wool with its physical and/or chemical properties, affects the soil microbiota, and more particularly, the AM fungi development. The aim of this study was to examine the combined effect of mycorrhizal development and leek (*A. porrum*) growth performance in the presence of sheep wool. To reach the aim, following research questions were stated.

1.3 Research questions

- Is AMF development enhanced by the presence of sheep wool as a substrate component?

- Is there any relation between AMF structures and leek (*A. porrum*) biomass?

- Is leek (*A. porrum*) growth performance enhanced by the presence of sheep wool in combination with AMF inoculation?

2. Material and methods

2.1 Materials

2.1.1 Sheep wool pellets

Waste sheep wool (SWP) was provided by Ullkontoret, situated in Endre (Gotland, Sweden). The waste wool used in the trial had undergone a pelleting process. First, the wool fibers were cut to a length of 5-7 mm, followed by a drying procedure at 80 °C for 1,5 hours until the matter had a moisture content of approx. 15%. The wool was then pelleted with a pressure of 400-650 bar at 120 °C followed by cooling to 20-30 °C before packaging and storing (appendix 1).

2.1.2 Substrates

Field soil (LP), used as a clean substrate, was obtained from Lilla Böslid farm (Halland, Sweden). Peat and compost (PC) were mixed to a 40:60 ratio, and the proportions were set in regard to a suitable pH value for leek (*A. porrum*) growth. The peat was pure sphagnum without additives, a humification rate of H 2-4 and a pH level of 3.5-4.5 (Hasselfors 'Solmull'). The compost used was manufactured by SYSAV waste facility and produced from park- and garden waste (SYSAV grönkompost.

2.1.3 Plant material

Leek (*Allium porrum,* L. Lancaster F1) was used as a model plant. *Allium porrum* along with other *Allium* species are highly dependent on mycorrhiza for survival since they lack expanded root systems, compared to most other species (Golubkina et al. 2020). Leek has previously been successfully used in studies on the examination of AMF development (Hamel et al. 1997; Akpinar et al. 2019).

2.1.4 AMF product

Mycorrhizal spores of the fungal strain *R. irregularis DAOM197198* was used as a substrate inoculum (26040 spores/l). Regarding the control treatments, a corresponding amount of carrier medium was added. The products are research-graded, manufactured and sold by Symplanta® (Darmstadt, Germany).

Figure 2. AMF R.irregularis DAOM197198 inoculum (left) and a corresponding amount of carrier medium (right).

2.2 Methods

2.2.1 Physical properties of sheep wool pellets (SWP) and substrates

Measurements of physical properties were performed according to SS EN13040:2007, comprising sample preparation for chemical and physical tests, determination of dry matter content, moisture content and laboratory compacted bulk density on each substrate. The procedure was conducted according to a lab manual from course material of Hydroponic Systems in Horticultural Production and Public Environment, Bl1233 (appendix 2).

Bulk density refers to the substrate density with air-filled pores included. An iron cylinder was filled with each substrate. It was extended over the edges with an extension ring. A standard weight was added for three minutes and then removed along with the extension ring. The excess substrate was gently wiped off and the cylinder content was emptied into a foil mold and weighed. Each measurement was conducted three times.

Figure 3. Bulk density measurements: filling (a) and excess removal (b) from iron cylinder.

Compact density refers to the density of the substrate where the pores are not included. It is therefore the relationship between the true weight and the volume of the substrate. A volumetric flask of 50 mL was weighed and then filled to half the volume with the substrate.

The flask with substrate content was weighed again. In order to push out the air from the substrate, 25 mL of alcohol was added from a burette. The flask was then sealed with a plastic film and shaken for 30 minutes. The amount of alcohol and water was recorded as a total additive to the flask. The volume that was not absorbed by liquid was the volume of the substrate. Each measurement was conducted three times.

substrate (g) / alcohol (L) = compact density, (g/L) *Eq. 1*

When both values for bulk density and compact density were obtained it was possible to determine the porosity of the substrate.

1- (bulk density/compact density) = % porosity *Eq.2*

Water holding capacity measurements were performed on the pot tray volume for each treatment. The pot tray was filled with substrate and then put in a sink. Water was filled to the edge of the tray. Water was also carefully poured on top with a watering can to soak the entire pot tray. It was left for 1 h, the sink was drained, and the pot trays were left for 30 minutes. Then the pot trays were removed from the sink and left freely draining for 24 hours. After 24 hours the trays were emptied into foil molds and weighed, then put in a drying cabinet at 105°C for 72 hours. The dry substrate was then weighed. Measurements were conducted four times for each treatment. Calculations were performed in order to establish the pot capacity at 40,50,60,70,80 and 100%.

pH, and EC (mS/cm) were determined (pH; EN13037, and EC; EN13038). The substrate and de-ionized water ratio were 1:5. 30 mL of substrate were measured and transferred to a sample jar. 150 mL of room-temperature de-ionized water was added. The sample jar was placed in an "end over end" shaker for 1 hour. The jars were then removed and shook by hand again before the measurements. Four replicates were performed for each treatment.

2.2.2 Nutrient analyses

Prior to the trial start, a complete nutrient analysis of SWP was performed by a commercial accredited laboratory (Eurofins Environment Testing Sweden AB (Lidköping, Sweden). The total N was extracted according to the Kjeldahl method (SS 028101). Based on the results, the amount of NH_4^+ was calculated. The remaining nutrients were extracted by aqua regia according to SS-ISO 11466:1996mod/SS-EN ISO 11885:2009.

A modified Spurway analysis was performed by Lennart Månsson International AB, (Helsingborg, Sweden) on LP and PC with and without the addition of SWP (LPSWP and PCSWP). The nutrients were extracted in a gentle acetic acid solution and the results of the analysis present the plant-available nutrient content for the upcoming weeks and/or months.

A quantification of the total biomass N was performed at SLU (Alnarp, Sweden) by the Elemental Partical Analyzer (EPA) method. The preparations were conducted in steps described as follows: dried biomass of roots and shoots obtained from harvest were milled by either IKA® A10 basic (15 s) or Retsch MM400 (60 s), depending on the sample amount. The milled matter was transferred to Eppendorf tubes and left in a drying cabinet for 2 hours at a temperature set to 60 °C. Then, 5 ± 0.2 mg were weighed on a microbalance (Mettler Toledo® XP6) and transferred to aluminum caps and then left in a desiccator until next step of the procedure.

The samples then underwent a burning at high temperature (900-1000°C) inside the combustion chamber. After combustion, the produced gases were conveyed by a helium flow to a second reactor filled with copper, then swept through CO_2 and H_2O traps and onto a gas chromatography (GC) column equipped with a thermal conductivity detector (TCD). Interpretation of the results were performed using the software EagerSmart (Appendix 3).

a b

Figure 4: Dried (a) and milled (b) A. porrum *biomass by IKA® A10 basic.*

2.2.3 Trial set-up

The trial was initiated in February and lasted for 8 weeks to allow *A. porrum* germination and early plant growth. The trial consisted of 8 treatments and 7 replicates in a randomized set-up (Table 2). Each replicate consisted of a pot tray of 12 cells (total n=672).

Table 2. Set-up treatment abbreviations and treatment descriptions

Treatment	Description
LP -	Field soil, no mycorrhizal inoculation
LP +	Field soil, mycorrhizal inoculation
LPSWP-	Field soil + SWP 5 g/L, no mycorrhizal inoculation
LPSWP+	Field soil + SWP 5 g/L, mycorrhizal inoculation
PC-	Peat: compost 40:60, no mycorrhizal inoculation
PC+	Peat: compost 40:60, mycorrhizal inoculation
PCSWP-	Peat: compost 40:60 + SWP 5 g/l, no mycorrhizal inoculation
PCSWP+	Peat: compost 40:60 + SWP 5 g/l, mycorrhizal inoculation

The substrates for each treatment were thoroughly mixed and the proper volume weight for each pot tray was determined. The substrates were first stored in plastic bags in order to keep humidity at a stable level. Spores of *R. irregularis DAOM197198* or carrier medium without spores were added to each treatment. Pot trays were filled (0,2 L/cell) and three seeds of leek were sown, 10 days later to be reduced to two plants per cell.

The trays were placed in a greenhouse at SLU (Alnarp, Sweden). The climate conditions were set to a minimum temperature of 20 ℃ during the day and 18 ℃ during the night, the ventilation temperature was set to 22℃ during the day and 20 ℃ during the night. Added artificial light (HPS) were turned on between 06.00 am and 10.00 pm.

The pot trays were positioned with spacing of 10 cm in order to avoid mycorrhizal contamination. Watering was performed every third day and initially set to a pot capacity of 65%. Throughout the trial, it was observed that the water usage differed between treatments and a new watering strategy was formed, where treatments only were watered if they deceeded the limit of a 50% pot capacity.

2.2.4 Harvest

Harvest was performed after a cultivation period of 8 weeks and lasted for 7 days. Pot trays were picked randomly, and plant shoots and roots were separated. Roots were then carefully washed and sieved in order to remove substrate particles. Shoots and roots were weighed and put in marked paper bags. The obtained harvest was divided. All above ground material and half the amount of roots were dried at 65 ℃ for 72 hours and later used for a total N tissue determination. The remaining roots were kept for the upcoming root colonization examination.

The roots were first cut in 1-2 cm pieces, (Rajapakse & Miller 1992), and then transferred to sample jars in a solution of water and alcohol (50:50).

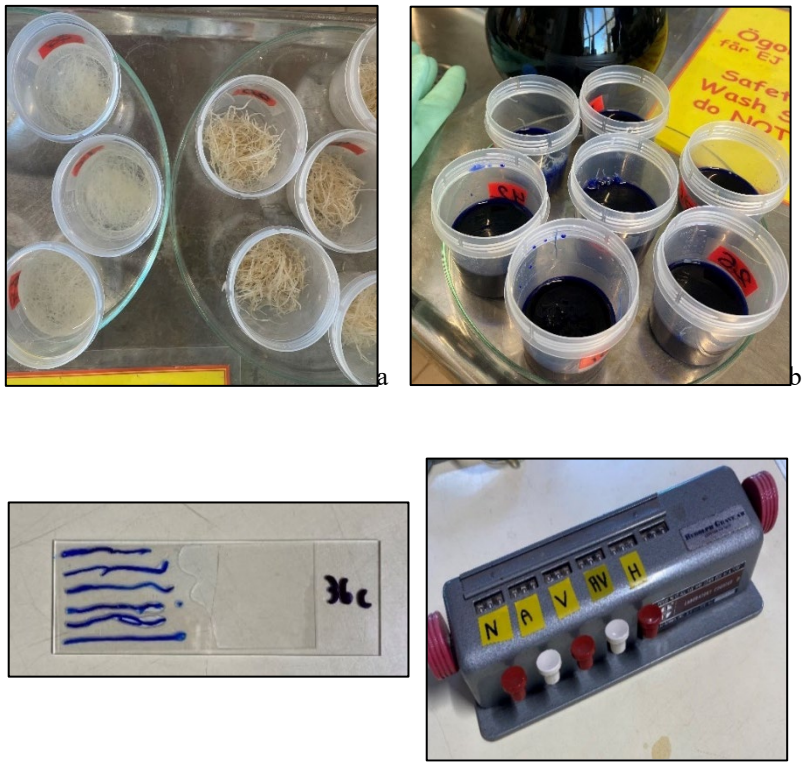
a b

Figure 5. Handling of roots and shoots: (a) Cut-off and rinsed roots of leek and (b) A. porrum *left in a drying cabinet*

2.2.5 Root colonization examination

The procedure continued with clearing and staining of the roots. For the clarification part, the roots were put in a 2.5% KOH solution and then heated in a microwave oven at 900 watts for approximately 2 minutes. It was important to observe the sample throughout the heating and not let it start boiling. The samples were left for 24 hours. The roots were then rinsed in a sieve before an acidic treatment of 1% HCl solution. The samples were left for 24 hours. The staining was performed by *Pelikan 400l Royal Blue* and soaked the roots for 24 hours. Destaining was conducted by an acid glycerol solution. Destaining was performed two times. Each destaining step lasted for 24 hours.

Root segments were picked from the sample jars and were then aligned parallel to the long axis of slides and covered with slips. There were four slides per sample, and they were marked a-d. The colonization measurement followed the 'magnified intersections method' (McGONIGLE et al. 1990), and the degree of colonization was determined by a quantification model where the AMF structures were categorised into groups of arbuscules, vesicles and hyphae. The magnification was set to 10-40x, and each slide included 50 observation points (total per sample=200).



a b c d

Figure 6. Sample preparation for quantification of AMF structures: (a) leek roots after and before 2.5 % KOH treatment, (b) during staining treatment (upper right), (c) placed on slides for microscopic examination, and (d) quantification device for AMF structures categorization.

2.2.6 Statistical analyses

The collected data for each part of the experiment were run through RStudio (Version 1.4.1106, © 2009-2022 RStudio, PBC). For all statistical analyses, a confidence level of 95% was used. For data obtained from measurements of the substrates physical and chemical properties, a one-way ANOVA was initially performed followed by a t-test to detect the significant differences between the treatments (p-value <0.05). For data obtained from measurements of the harvested biomass and root colonization, sample normality for the parameters was tested using the Shapiro test, followed by a two-way ANOVA. Tukey's test (p-value <0.05) was performed in order to detect significant differences between the treatments. In order to see the correlations between harvested biomass and tissue N, and harvested biomass and AMF total structures respectively, a linear regression analysis at a significant level of 95% was performed.

3. Results

3.1 Substrate physical properties

Prior to the trial start, measurements of the substrate physical properties were performed and compared within each group of substrates. Significant differences were observed between LP and LPSWP in the bulk density measurement (Figure 7) and water holding capacity test (Figure 10). No significant differences were recorded between the PC treatments.

Results from bulk density measurements are shown in figure 7. A significant difference was recorded between LP and LPSWP where the mean value decreased from 886.3±19.69 to 866.53±12.73 (g/L) when SWP was added to the substrate (p-value: 0.022). There was no significant difference between PC and PCSWP (p-value: 0.893).

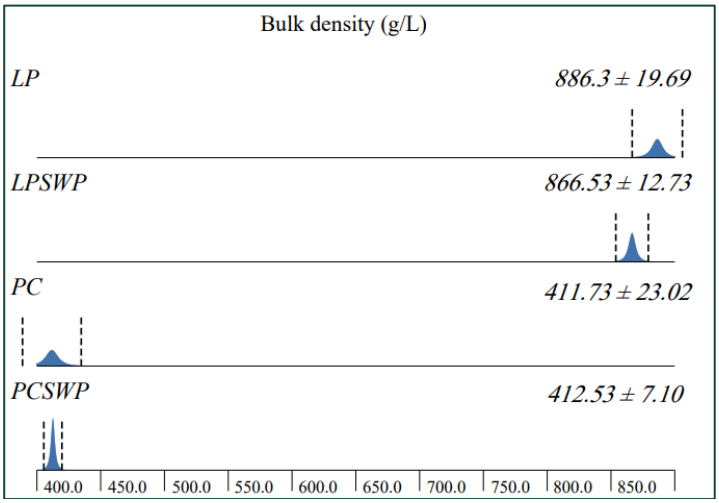


Figure 7. Bulk density measurements, mean±SE, (g/L). LP= field soil, LPSWP= field soil + sheep wool pellets, 5 g/L, PC= peat: compost 40:60, PCSWP= peat: compost 40:60 + sheep wool pellets, 5 g/L. (n=4).

Results from compact density measurements are shown in Figure 8. No significant differences were recorded between LP and LPSWP or PC and PCSWP (p-values: 0.444, 0.952).

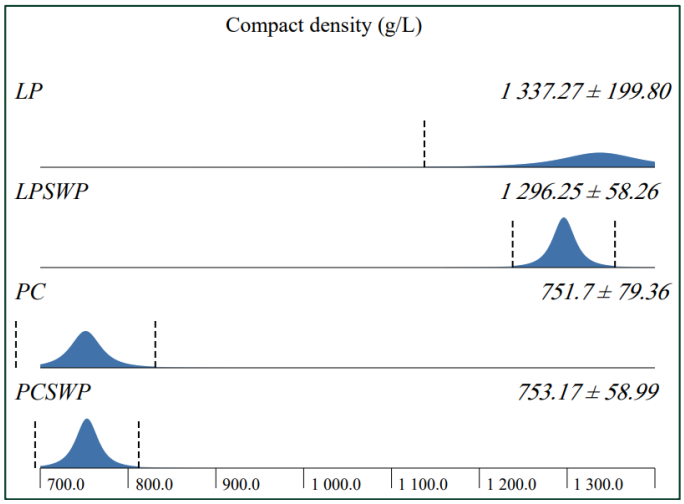


Figure 8. Compact density measurements, mean±SE, (g/L). LP= field soil, LPSWP= field soil + sheep wool pellets, 5 g/L, PC= peat: compost 40:60, PCSWP= peat: compost 40:60 + sheep wool pellets, 5 g/L. (n=4).

Results from the porosity calculations are shown in Figure 9. No significant differences were recorded between LP and LPSWP or PC and PCSWP (p-values: 0.865, 0.916).

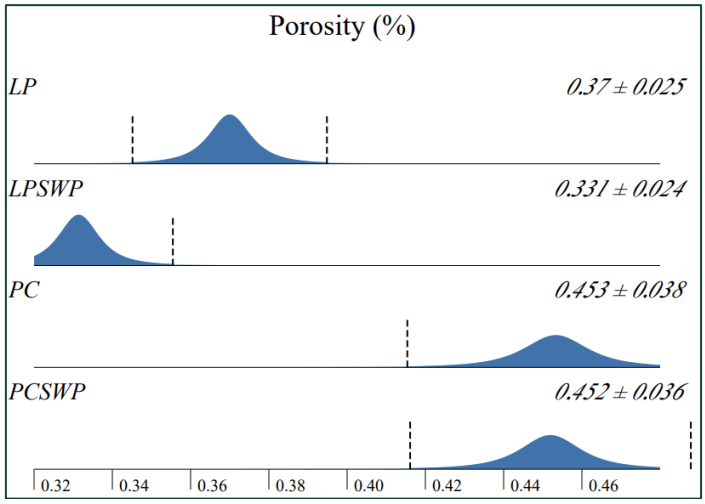


Figure 9. Figure showing calculations of porosity, mean±SE, (%). LP= field soil, LPSWP= field soil + sheep wool pellets, 5 g/L, PC= peat: compost 40:60, PCSWP= peat: compost 40:60 + sheep wool pellets, 5 g/L.

Results from water holding capacity measurements are shown in Figure 10. It was shown that there was a significant difference between LP and LPSWP (p-value: 0.024). When SWP was added to LP the substrate value increased from 46.0±2.0 to 49.0±2.0 (%). The difference between PC and PCSWP was not significant (p-value: 0.173).

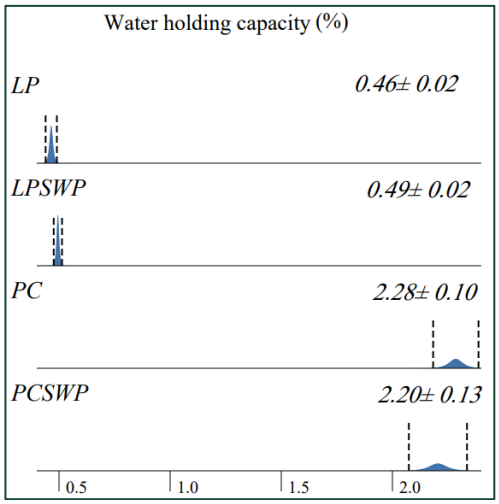


Figure 10. Water holding capacity measurements, mean±SE, (g/L). LP= field soil, LPSWP= field soil + sheep wool pellets, 5 g/L, PC= peat: compost 40:60, PCSWP= peat: compost 40:60 + sheep wool pellets, 5 g/L. (n=4).

3.2 Substrate chemical properties

3.2.1 pH and EC measurements

Results of the pH values are shown in Figure 11. When SWP was added to the substrate, the pH value increased significantly from 5.7±0.3 to 6.3±0.2 (p-value: 0.002). The difference between PC and PCSWP was not significant (p-value: 0.575).

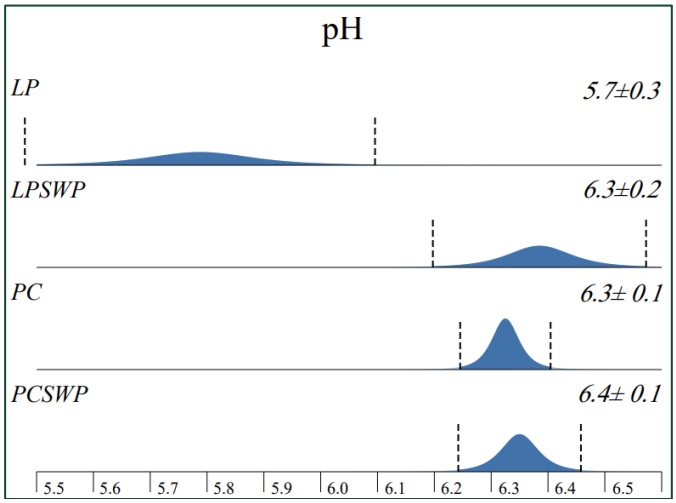


Figure 11. pH value measurements, mean±SE. LP= field soil, LPSWP= field soil + sheep wool pellets, 5 g/L, PC= peat: compost 40:60, PCSWP= peat: compost 40:60 + sheep wool pellets, 5 g/L. (n=3).

Results from the EC measurements are shown in Figure 12, and significant differences for both treatment comparisons (p-values: <0.001, <0.001) were recorded. When SWP was added to LP, the EC value increased from 49±8 to 520±152 (µS/cm). The addition of SWP to PC led to an increase from 535±38 to 860±46 (µS/cm).

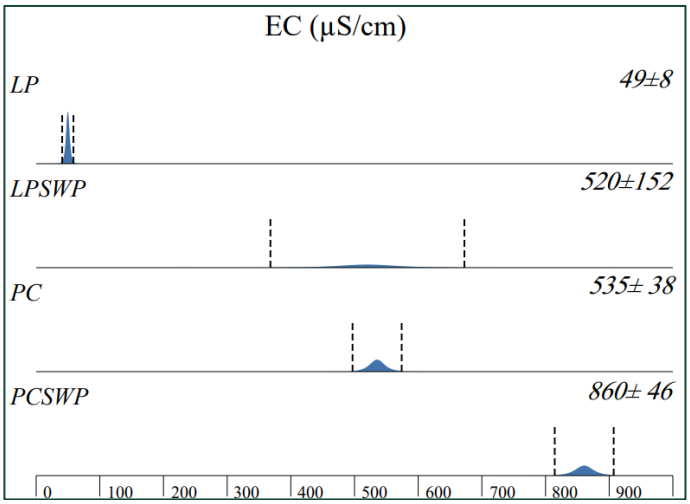


Figure 12. EC value measurements mean±SE, (µS/cm). LP= field soil, LPSWP= field soil + sheep wool pellets, 5 g/L, PC= peat: compost 40:60, PCSWP= peat: compost 40:60 + sheep wool pellets, 5 g/L. (n=3).

3.2.2 Macronutrient analysis of SWP

Results from the macronutrient analysis performed on SWP are shown in Figure 13. The total amount of N was 102.5 kg/t, where plant available NH_4^+ stands for approx. 15%. SWP is also shown to be rich in K and S.

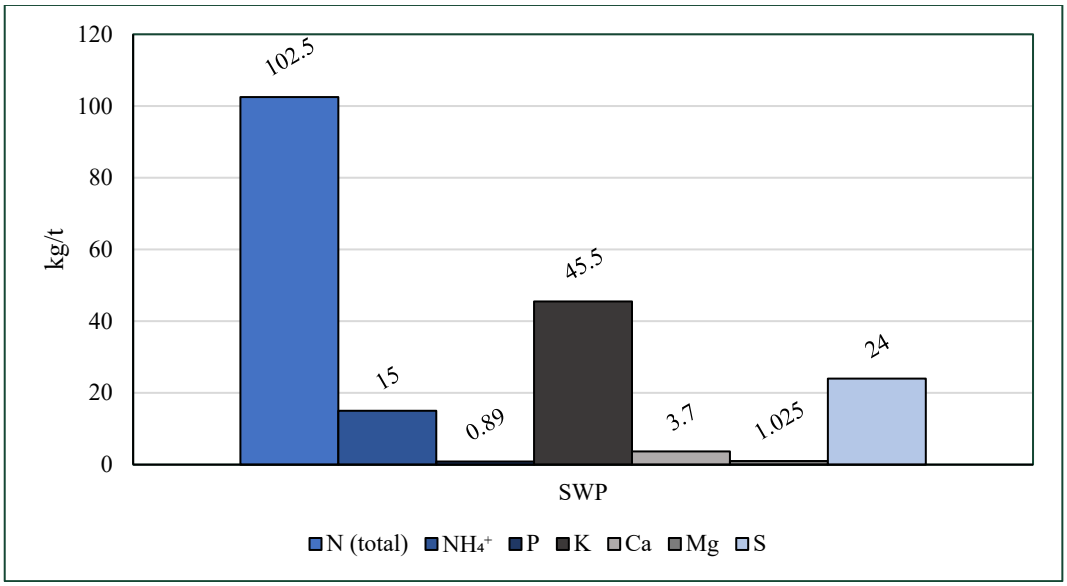


Figure 13. Results shown from the complete nutrient analysis (kg/t).
LP= field soil, LPSWP= field soil + sheep wool pellets, 5 g/L, PC= peat: compost 40:60, PCSWP= peat: compost 40:60 + sheep wool pellets, 5 g/L (n=2).

3.2.3 Spurway analysis of substrates

The Spurway analysis is shown in Figure 14. The results show the plant-available nutrient content present for the upcoming weeks and/or months. Total N in LP increased from 19 to 32 mg/L when SWP was added. A noticeable increase of P and K was also observed, from 2 to 5.5 mg/L and 64 to 340 mg/L respectively. In PCSWP NH_4^+ and K increased, from 2.5 to 4.5 mg/l and 1450 to 1550 mg/L respectively. Contradictory to the result of LPSWP, total N and P decreased in PCSWP, that is, when SWP was added to PC.

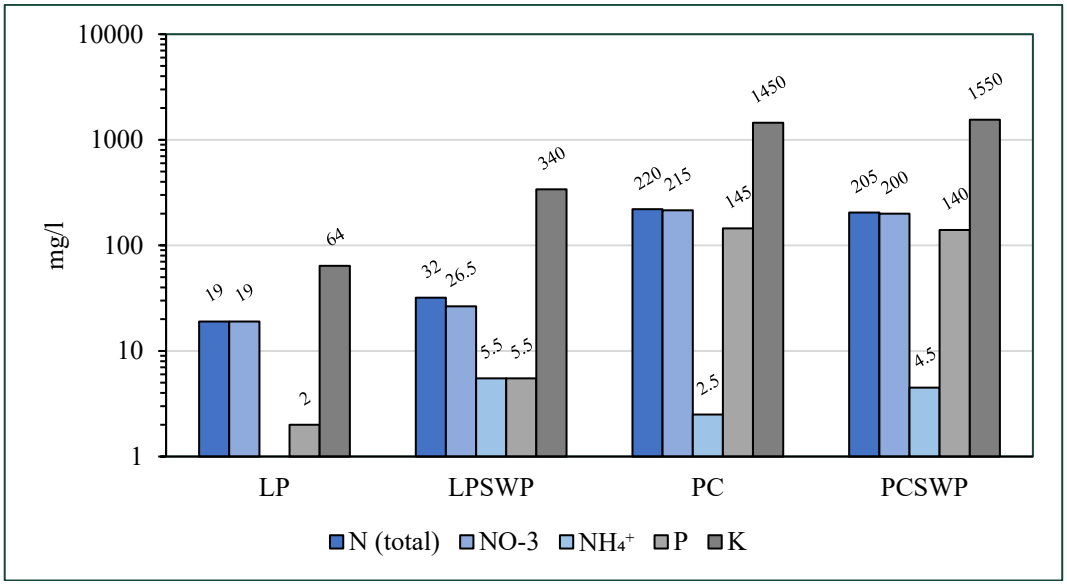


Figure 14. Nutrient content of the substrates with and without SWP addition (mg/L). LP= field soil, LPSWP= field soil + sheep wool pellets, 5 g/L, PC= peat: compost 40:60, PCSWP= peat: compost 40:60 + sheep wool pellets, 5 g/L (n=2).

3.3 Harvest

The harvest comprised visual observations along with plant growth measurements of the dry weight matter of shoots and roots. Additionally, the tissue N (%) was measured.

3.3.1 Visual observations

Before and during harvest, visual observations were performed, shown in Figure 15 and Figure 16. It was possible to spot differences between all treatments. Observation of the leek shoots prior to harvest (Figure 15) showed green and vital plants in PCSWP+ (the far right), whereas the shoots in LP+ (the far left) seemed wilted with slightly yellow tips.

Figure 15. Pre-harvest observations.
LP+= field soil, AMF inoculation, LPSWP+= field soil, sheep wool pellets 5 g/L, AMF inoculation, PC+= peat: compost 40:60, AMF inoculation, PCSWP+= peat:compost 40:60, sheep wool pellets 5 g/L, AMF inoculation, (n=7).

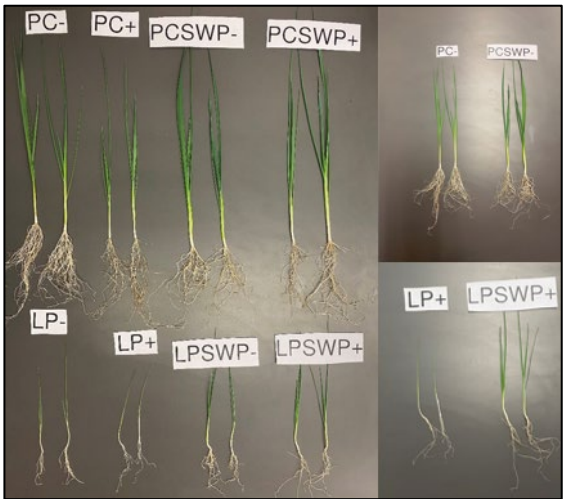


Figure 16. Observation of leek shoots and roots during harvest.
LP-=field soil LP+= field soil, AMF inoculation, LPSWP-= field soil, sheep wool pellets 5 g/L, LPSWP+= field soil, sheep wool pellets 5 g/L, AMF inoculation, PC-= peat:compost 40:60, PC+= peat:compost 40:60, AMF inoculation, PCSWP-=peat:compost 40:60, sheep wool pellets 5 g/L, PCSWP+=peat: compost 40:60, sheep wool pellets 5 g/L, AMF inoculation, (n=7).

3.3.2 Biomass

Results from the total biomass in LP treatments and PC treatments are presented in Figure 17 and Figure 18, respectively. LPSWP+ differed significantly from the other treatments and had a mean dry weight of 0.62 g, which suggests a combined effect of SWP and AMF inoculation. There was no recorded difference between LP+ and LP-. Between PC treatments, PCSWP- had a significantly higher obtained yield with a mean dry weight of 3.32 g.
The biomass of PC+ was significantly lower (mean dry weight 0.87 g). The results suggest a negative effect of AMF inoculation in PC substrates, with and without SWP addition.

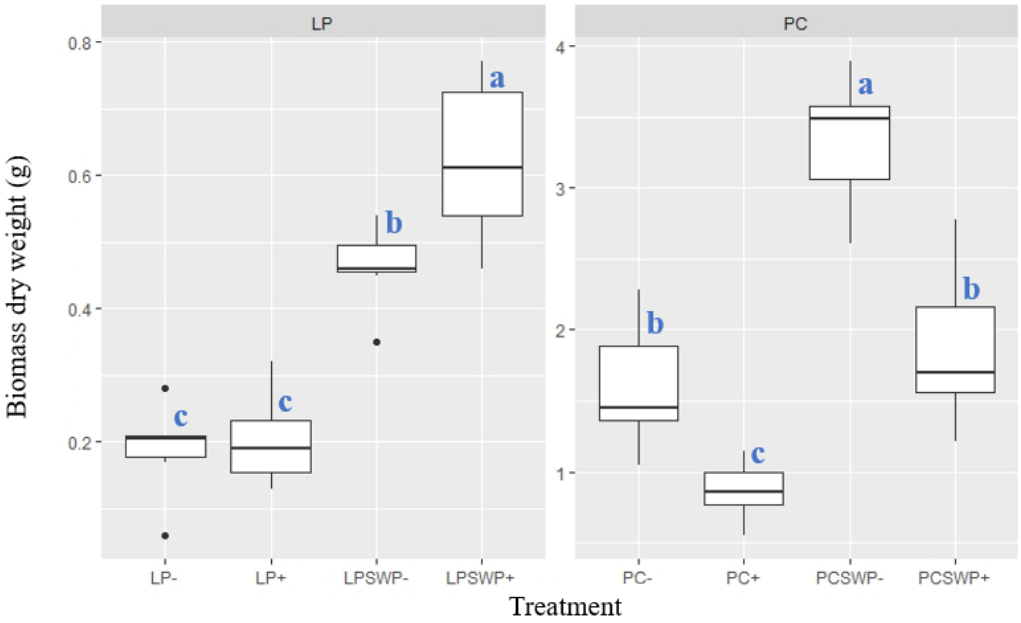


Figure 17. Yielded biomass in the LP and PC treatments respecitvely. LP+= field soil, AMF inoculation, LPSWP+=field soil, sheep wool pellets 5 g/L, AMF inoculation, LP-= field soil, LPSWP-=field soil, sheep wool pellets 5 g/L, PC+=peat:compost 40:60, AMF inoculation, PCSWP+=peat:compost 40:60, sheep wool pellets 5 g/L, AMF inoculation, PC-=peat:compost 40:60, PCSWP-= peat:compost 40:60, sheep wool pellets 5 g/L (n=7). Different letters indicate the significant differences between treatments.

*Table 3. p-values obtained from yielded total biomass analysis of LP and PC treatments. *=p<0.05, **=p<0.01, ***=p<0.001, ns= not significant*

Treatment	p-value	Level of sign.
LP+: LP-	0.988	ns
LPSWP-: LP-	0.000	***
LPSWP+: LP-	0.000	***
LPSWP-: LP+	0.000	***
LPSWP+: LP+	0.000	***
LPSWP+: LPSWP-	0.006	***
PC+: PC-	0.023	*
PCSWP-: PC-	0.000	***
PCSWP+: PC-	0.692	ns
PCSWP-: PC+	0.000	***
PCSWP+: PC+	0.001	**
PCSWP+: PCSWP-	0.000	***

A regression analysis was performed in order to observe the relation between leek growth as biomass dry weight (g) and the tissue N concentration (%). The coefficient of determination was 0.46.

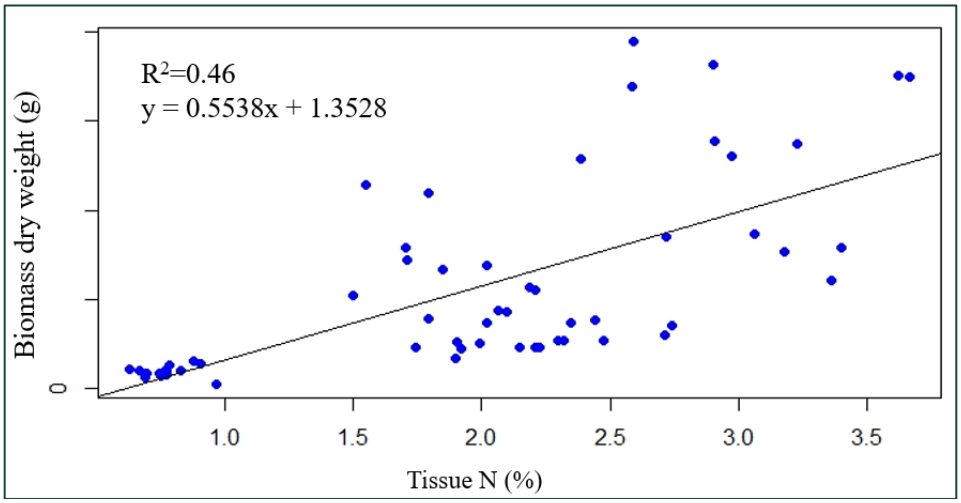
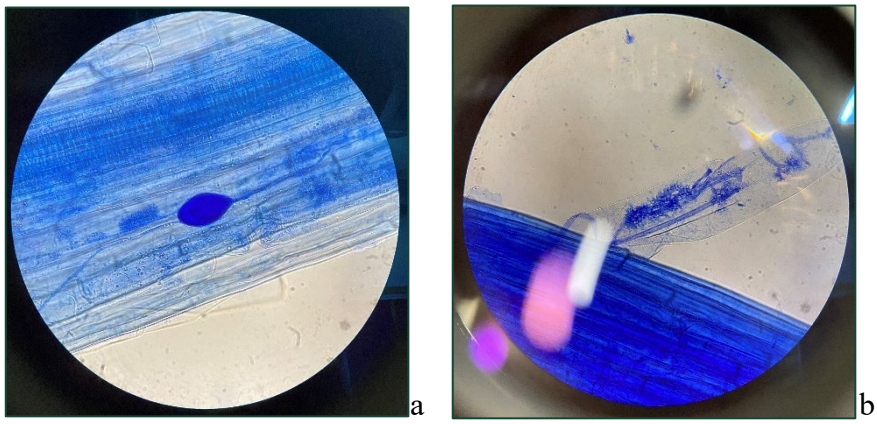


*Figure 18. Regression analysis of the correlation between leek (A. porrum) growth as biomass dry weight (g) and the tissue N concentration (%) R^2=0.46, n=56 (p-value:0.000***).*

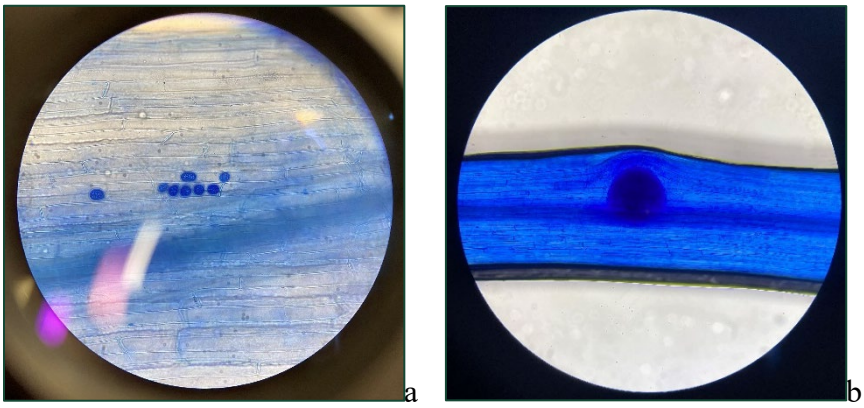
3.4 AMF root colonization

3.4.1 Visual observations



a b

Figure 19. (a) Vesicle and attached hyphae. Identified as AMF. (b) Damaged root tissue with clear arbuscular structure. Identified as AMF.



a b

Figure 20. (a) Unidentified fungal spore structure. (b)*: Emerging side root of* A. porrum

3.4.2 AMF root colonization

Results of the presence of AMF structures are shown in Figure 21 and Figure 22. The highest proportion of fungal structures were found in PC+. In PC- and PCSWP- no AMF structures were found and the treatments are therefore not showed in Figure 22.

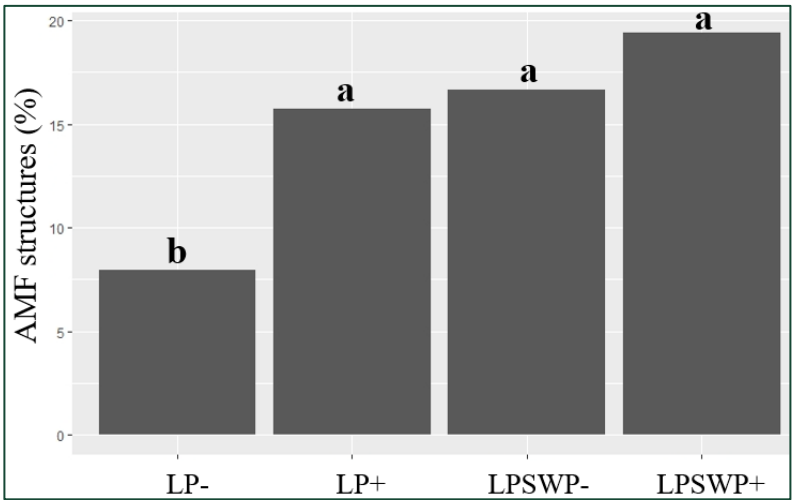


Figure 21. Presence of AMF structures in LP treatments (%).
LP+= field soil, AMF inoculation, LPSWP+=field soil, sheep wool pellets 5 g/L, AMF inoculation, LP-= field soil, LPSWP-=field soil, sheep wool pellets 5 g/L. Different letters indicate the significant differences between treatments (n=7).

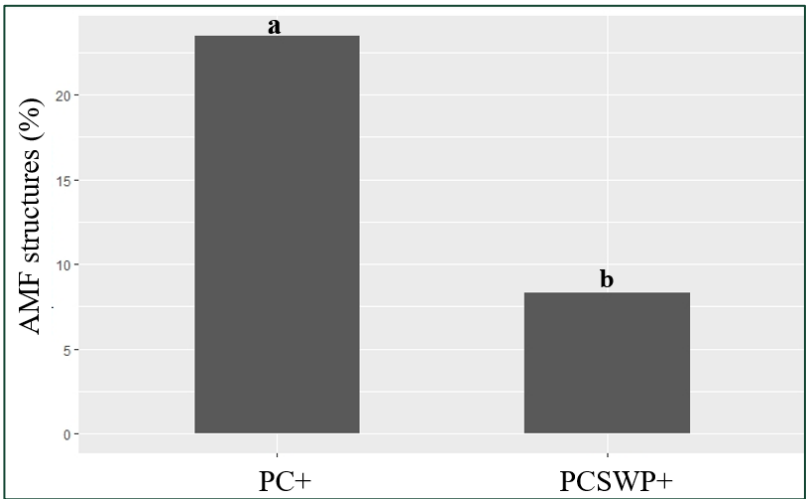


Figure 22. Presence of total AMF structures in PC treatments.
PC+=peat: compost 40:60, AMF inoculation, PCSWP+=peat:compost 40:60, sheep wool pellets 5 g/L, AMF inoculation, PC-=peat:compost 40:60, PCSWP-= peat:compost 40:60, sheep wool pellets 5 g/L. Different letters indicate the significant differences between treatments,(n=7).

*Table 4. p-values obtained from analysis of root colonization rates of total AMF in LP and PC treatments. *=p<0.05, **=p<0.01, ***=p<0.001, ns= not significant*

Treatment	p-value	Level of sign.
LP+: LP-	0.004	**
LPSWP-: LP-	0.001	**
LPSWP+: LP-	0.000	***
LPSWP-: LP+	0.977	ns
LPSWP+: LP+	0.367	ns
LPSWP+: LPSWP-	0.615	ns
PC+: PCSWP+	0.000	***

Regression analyses were performed within each substrate type in order to see the relation between rate of AMF colonization and leek (*A. porrum*) growth. A positive trend was reported in the LP treatments (R^2=0.24) (Figure 23), whereas a negative trend was observed in the PC treatments (R^2=0.32) (Figure 24).

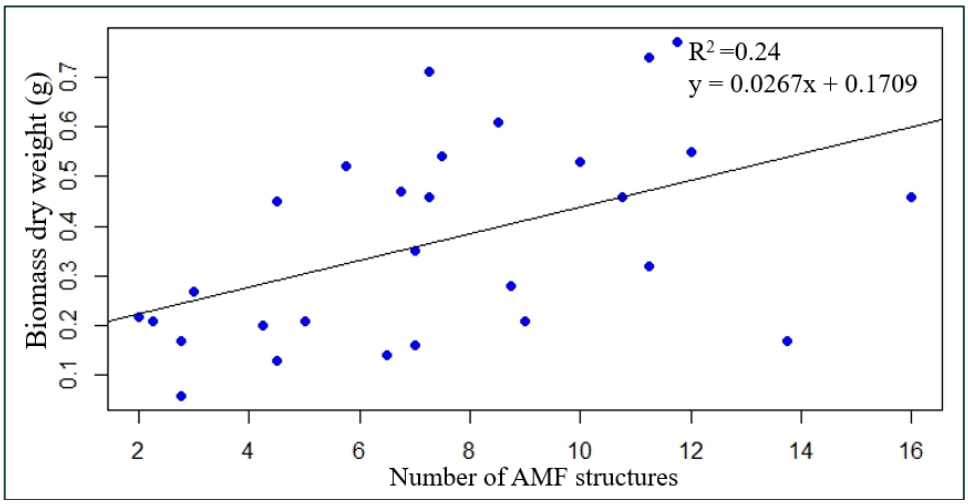


Figure 23. Regression analysis within LP treatments between obtained biomass in dry weight (g) and number of AMF structures. AMF structures comprise arbuscules, vesicles, arbuscules + vesicles and hyphae. R^2=0.24, n=28 (p-value: 0.009).

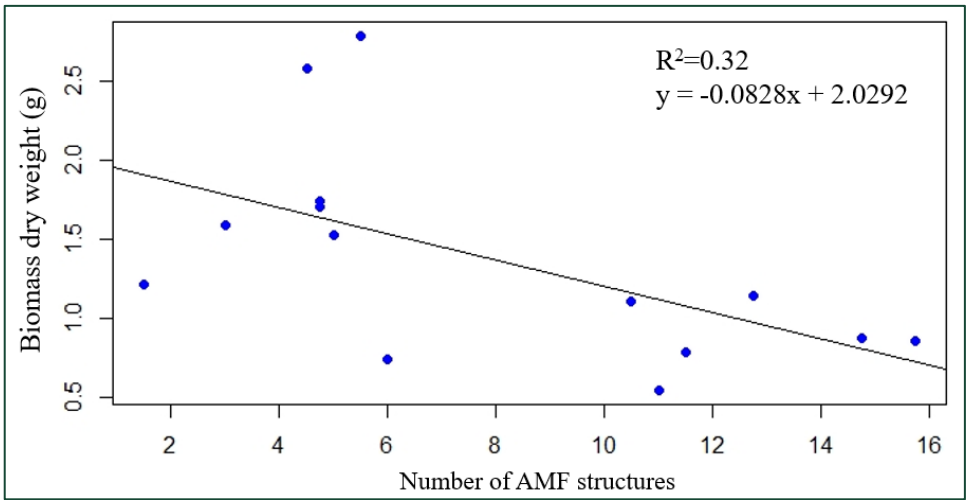


Figure 24. Regression analysis within PC treatments between obtained biomass in dry weight (g) and number of AMF structures. AMF structures comprise arbuscules, vesicles, arbuscules + vesicles and hyphae. R^2=0.32, n=14 (p-value: 0.03).

4. Discussion

4.1 Substrate properties

4.1.1 Physical properties

The bulk density significantly decreased when SWP was added to the pure substrate (figure 7). In a mixed substrate, the characteristics of each component contribute to the total bulk density (Raviv et al. 2002), and in compacted soils the bulk density increases and the porosity decreases correspondingly (Keller & Håkansson 2010). The trial results indicate that the substrate obtained a looser state when SWP was added. Similar observations were made by Abdallah et al., (2019a) where the addition of 2% of white wool residues significantly decreased bulk density and increased total porosity. However, the compact density and porosity did not significantly change by the SWP addition. In order to evaluate the addition of SWP as a suitable amendment for heavier soils such as LP, trials including larger amounts and different dosages might be required.

There was a significant increase in water holding capacity of approx. 3% when SWP was added to LP (figure 10). As mentioned earlier, the wool fibres has, due to their unique chemical structure, the ability to retain water (Allafi et al. 2022). Also as suggested by Abdallah et al. (2019), higher amounts of wool may improve water retention. However, unprocessed wool fibres still keep their hydrophobic surface, and the amount of adsorbed water is smaller (Górecki & Górecki 2010). As shown in table 1, sheep wool products have been exposed to different treatments. Chemicals and high temperatures used during processing may alter the wool fibre characteristics (Allafi et al. 2021), and the water holding capacities may change. Each wool product needs to be evaluated separately.

4.1.2 Chemical properties

pH governs many chemical processes in a substrate, and consequently its nutrient bioavailability (Liu & Hanlon 2012). The initial pH values recorded were held at a suitable level for *A. porrum* growth (figure 11). General recommendations for vegetable crops ranges between 5.5 and 7.0 (Liu & Hanlon 2012). However, a significant increase in pH was noted in the LPSWP treatment and Abdallah et al. (2019), suggested that sheep wool may cause substrate pH modifications and a cascade effect on nutrient availability. Substrate components naturally low in pH, such as peat (3.5-4.5), may in this context, gain from SWP addition.

As mentioned earlier, suint is a mixture of potassium-based salts (Zakaria El-Sayed et al. 2018) and is most likely the cause of the significantly higher EC levels in all treatments where SWP was an added substrate component (figure 12). Böhme et al. (2012) noticed that problems may arise when EC levels are elevated, especially in salt-sensitive plants. Detrimental effects of salinity not only affect plant growth but also AMF symbiosis (Rouphael et al. 2017) and adverse effects of salt stress on germination of spores, hyphal development and the production of arbuscules was reported by Miransari (2010). In PCSWP the mean EC value reached approx. 860 µS/cm which was the highest recording between all treatments (figure 12). Soil salinity is generally defined by EC levels more than 4 dS/mL (corresponding to 4000 µS/cm) (Keren 2005), and PCSWP is therefore not considered as saline substrate. However, as shown in previous mentioned articles (Miransari 2010; Böhme et al. 2012; Rouphael et al. 2017), EC is an important factor to consider when determining the proper amount of coarse wool as a substrate component.

Spurway analyses were performed and slightly increased levels of $NH4^+$ were a common observation for both treatments that contained SWP (figure 14). Plants predominately take up N in the form of NO_3^- and NH_4^+ (de Bang et al. 2021), and as shown by the harvested biomass (figure 17), SWP contributed to an significant increase in produced biomass. Organic N in the form of peptides and amino acids may also be absorbed by plants (de Bang et al. 2021). However, the analysis of SWP macronutrients showed the total N but did not specify further in which form, it was limited to only present its NH_4^+ content. Further examination of organic compounds in sheep wool would be an interesting complement to cover the full SWP profile.
Another aspect to take into consideration is the degradation rate of the wool fibres in the substrate. With the aim of assessing the rate of wool-waste degradation in soil, Zheljazkov et al. (2009) performed SEM/EDX microanalyses. After 250 days, the wool-waste showed variable levels of decomposition (ibid).

This experiment lasted for 56 days and most likely the wool was not fully degraded in the substrate, which also was confirmed by visual observations. However, it was clearly shown that the sheep wool had an effect on leek (*A. porrum*) performance in terms of biomass, and the added sheep wool would most certain last as a nutrient source for a longer cultivation period, as suggested by Górecki & Górecki (2010).

4.2 Cultivation trial

4.2.1 Harvest

The plants in the LP treatments appeared smaller compared to the plants in the PC treatments (figure 16). Possible explanations may be the initial nutrient status of the soil (figure 14) and the soil compaction. The compact density varied greatly between 751.7±79.4 and 1337.3±199.8 in the pure substrates of LP and PC respectively (figure 8). Compacted soil results in high penetration resistance for roots and poor soil aeration (Keller & Håkansson 2010), and leek (*A. porrum*) is particularly sensitive to anaerobic soil conditions (Brunström n.d.).

An overall trend for all treatments were differences for the addition of SWP which significantly increased the produced biomass (figure 17) and correlated with the tissue N concentration (figure 18). These findings are supported by observations in trials conducted by Zheljazkov et al. (2009), where the total yield of basil and Swiss were 1.6-5 and 2-5 times greater when wool waste was added as a substrate amendment, compared to the control. Meanwhile, the tissue N increased (ibid). These findings are supported by earlier studies (Górecki & Górecki 2010; Abdallah et al. 2019b). In the LP treatments it was also possible to observe a synergistic effect of SWP and AMF inoculation (figure 17). On the other hand, the produced biomass was significantly lower in the inoculated PC treatments (figure 17). One possible explanation might be an unequal balance between benefits and costs of the symbiosis, where the fungal demands of C causes growth depressions (Li et al. 2008).

The harvest turned out to be more time-consuming than first expected and did most probably affect the end result since some trays were left in the greenhouse for up to six days longer than the first harvested trays. The trays were picked randomly, therefore some treatments might have been favoured. Complementary physiological measurements on the plant performance would probably yield more accurate results. According to Long et al. (1996), measurements of the photosynthetic CO_2 provide additional information that cannot be provided by dry matter measurements. Additionally, photosynthetic performance of plants are considered incomplete without fluorescence data (Maxwell & Johnson 2000) and chlorophyll content measurements (Bergstrand, 2022). However, the leek plants were in some cases small, and the plant needs to reach a certain size in order to be examined (ibid).

4.2.2 AMF root colonization

Roots of leek were examined according to the 'magnified intersections method', a method widely used to assess the colonization status of AMF (Hu et al. 2020). AMF colonization takes place in the fine roots of the plants (Zangaro et al. 2012) and in the LP treatments, a lack of fine roots was observed. The roots were also hard to obtain due to its heavy soil characteristics. These observations did probably affect the total number of roots and therefore recorded AMF structures in the samples.

The rate of colonization varied greatly between substrates and treatments. In the PC treatments without AMF inoculation, no fungal structures were found. Indeed, peat and compost contain no or very low amounts of mycorrhizal material, and the reason may be certain types of green material low in density of infectious propagules, and high temperatures during the composting process which further reduces the number of AMF (Perner et al. 2006).

The regression analysis of the PC treatments reported a negative correlation between the obtained total biomass and number of AMF structures (figure 28) and as earlier mentioned, in the non-inoculated PC treatments, no AMF structures were found. This suggests no initial presence of fungal propagules in the PC substrates. The reduced abundance and diversity of AMF propagules can limit AMF-derived benefits if either different AMF species of genotypes provide complementary benefits (Verbruggen et al. 2013).

In contrast, the regression analysis performed on LP substrates indicated a positive trend on the correlation between the obtained total biomass and number of AMF structures (figure 27). However, the measurements performed were limited to the presence of fungal structures. As mentioned earlier, groups of bacteria such as PSB works in synergy with AMF and may increase plant growth and vitality (Nadeem et al. 2014; Jiang et al. 2021). Whole bacterial and fungal communities can be investigated by DNA analyses (Xu et al. 2018) and whether mycorrhizas should be defined as tripartite interactions or not are discussed by Bonfante & Anca (2009). An entire examination of the mycorrhizosphere and the inclusion of bacterial communities would most likely show stronger trends in the correlation between microbial presence and total obtained biomass (figure 27). Additionally, AMF structures were present in the non-inoculated treatments, LP- and LPSWP- (figure 27) which suggests an indigenous abundance of AMF in the LP soil.

The Spurway analyses performed on each substrate showed differences in initial nutrient content between the treatments (figure 18). The total N content was present in plant-available forms as NO_3^- and NH_4^+ and its abundance varied greatly between LP and PC treatments (ibid). In nitrogen-limited settings, the plant is to a larger extent, more dependent on AMF for soil nutrient absorption (Lu et al. 2020). However, in nitrogen-rich environments, the plants rely on their own roots for nutrient acquisition and uses their gained photosynthetic products for aboveground growth instead of AMF relationship maintenance (ibid). This phenomenon might be the reason for the negative trend recorded in the PC regression analysis (figure 28) and even though, the coefficient of determination was considered low in the LP treatments (figure 27), the positive trend may predict a stronger long-term correlation. In a study conducted by Eulenstein et al. (2016) it was clearly shown that the degree of colonization was directly correlated to the plant dry weight. These findings were also supported by Akpinar et al. (2017) where different compost types were evaluated in combination with AMF performance. The trials performed by Eulenstein et al. (2016) and Akpinar et al. (2017) were relatively longer than the present experiment of 56 days. It would be interesting to follow the effects of AMF establishment and development during an extended cultivation period.

The biochemical content of SWP is, as earlier suggested, of interest in order to evaluate its ability to promote plant growth and AMF development. Waste sheep wool is according to the European Commission classified as special waste (Abdallah et al. 2019b) and Petek & Marinšek Logar (2021) have published alternative ways of turning wool into protein hydrolysates viable as fertilizers

and/or plant biostimulants (PBs). The authors were able to identify strains of bacteria, actinomycetes and fungi capable of sheep wool degradation.
These keratinolytic microbes have been isolated from a wide range of environments, in aerobic and anaerobic settings and from hot springs to Antarctic soils (ibid). Plant-derived protein hydrolysates have been shown to work in synergy with AMF to promote plant growth (Rouphael et al. 2017). Based on the trial results, a synergistic effect of SWP and AMF inoculation on leek (*A. porrum*) biomass were shown in the LP treatments (figure 23). However, it was not possible to see a significance regarding synergistic effects of SWP presence and AMF development in the PC treatments (figure 23) which may suggest the absence of keratinolytic microbes. Additionally, the findings of Rouphael et al. (2017) refers to plant-based protein hydrolysates. An interesting topic for further research would be to examine sheep wool degradation rates in difference types of substrate and environmental settings, and how the obtained derivates would interact with plants and microbes in its surrounding, AMF in particular.

Another group of organic compounds abundant in sheep wool are fatty acids (Burger et al. 2011). As earlier mentioned has AMF been considered fatty acid auxotrophs (Jiang et al. 2017; Sugiura et al. 2020; Salmeron-Santiago et al. 2022). However, studies have shown the ability of AMF to obtain fatty acids independently from its host plant (Kameoka et al. 2019; Sugiura et al. 2020). The fatty acids that led to an increase in fungal biomass and spore formation in *R. irregularis* were myristate ($C_{17}H_{34}O_2$), the bacterial isolate 12-methyltetradecanoic acid ($C_{15}H_{30}O_2$) and palmitoleic acid ($C_{16}H_{30}O_2$) (ibid, figure 1). Wool grease is categorized as a natural contaminant of sheep wool and consists mostly of fats and oils (Zakaria El-Sayed et al. 2018). Burger et al. (2011) performed a comprehensive analysis of volatile organic compounds of cranial lamb wool. Interestingly, the presence of myristate ($C_{17}H_{34}O_2$) and palmitoleic acid ($C_{16}H_{30}O_2$) were confirmed (ibid). These findings would imply that the addition of sheep wool can enhance AMF development based on its fatty acid content. However, the root colonization readings of the present study gave contradictory results, where colonization rates among the PC treatments were significantly reduced in PCSWP+, compared to PC+ (figure 26). Among the LP treatments there were not significant difference by the addition or absence of SWP (figure 25). Therefore, it is not possible to draw any conclusions based on the findings by Kameoka et al. (2019), Sugiura et al. (2020) and Burger et al. (2011).

4.3 Conclusions

To support a future of sustainable agricultural practices, soil fertility is an important factor that can be enhanced by amendments of organic origin, and it is commonly known that organic matter supports the abundance and diversity of soil microbiota. Waste sheep wool poses an environmental risk and sustainable ways of handling this by-product is crucial. Earlier studies have shown waste wool as a viable fertilizer in a range of horticultural production systems. The present study supported earlier findings. It was concluded that the sheep wool addition increased leek (*A. porrum*) total biomass.

Indeed, sheep wool is a product with a complex yet interesting and rich chemical profile, and reports in the literature about its content of organic compounds suggest sheep wool to be considered as not just a fertilizer, but also a source of plant biostimulants (PBs).

Sheep wool in combination with AMF inoculation had a promoting effect on the leek (*A. porrum*) growth performance, however, it was only shown in LPSWP+. Regarding the other treatments, it was not possible, based on the result, to draw any conclusions about a combined effect of AMF inoculation and sheep wool on plant performance.

Interestingly, the correlations between yielded biomass and total AMF presence, both positive and negative trends were recorded. In general, microorganism-based strategies are considered as long-term strategies, and a trial of 56 days is probably not enough time to evaluate all the potential beneficial effects of sheep wool. Additionally, analyses of the total mycorrhizosphere microbiome were not performed and important groups of bacteria that enhance AMF performance were not counted for. For further research, longer trials with various amounts of sheep wool additions and substrate types would be interesting to conduct, along with a more comprehensive analyses of the mycorrhizosphere microbial community and sheep wool biochemical properties.

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Appendix 1

Arbeitsablauf der Schafwollpelletierung

Die Schafwolle wird vom Landesschafzuchtverband OÖ angeliefert, oder direkt vom Landwirt, nach einer telefonischen Terminvereinbarung, gebracht. Die Wolle wird gewogen, und in einer Halle als Rundballen, in Big Bags oder lose gelagert.

Anschließend wird sie mit einer Schneidemaschine auf 5 bis 7mm geschnitten, gleichzeitig wird die Wolle mit einem Windwurfgebläse in einen Lagerraum transportiert und zwischengelagert.

Von dort kommt sie händisch in den Trocknungsraum. Dort wird die Wolle mit einer Vorlauftemperatur von 80°C und einer Dauer von 1 bis 1.5 Stunden auf ca. 15% Feuchtigkeit getrocknet. Die Trocknungsanlage wird mit einer Hackschnitzelheitzung (32 KW) beheizt.

Anschließend wird die Wolle mit einer Absauganlage in die Pelletsmaschine befördert. Wo sie mit einer Temperatur von 80 bis 120°C (Matrize) und einen Pressdruck von ca. 400 bis 650 bar (abhängig von der Stärke der Matrize) pelletiert wird.

Die fertigen Pellets, die einen Durchmesser von 6 mm aufweisen, werden 1 Stunde auf 80°C erhitzt.

Nach der Erhitzung werden die Pellets auf 20 bis 30 °C abgekühlt.

Anschließend wird er in einer Halle bis zur Abfüllung zwischengelagert.

Danach werden die Pellets entweder in 1 kg oder 3 kg Graskarton bzw. in 20 kg Säcke abgefüllt. Etikettiert mit einer Chargennummer versehen und in Kartons verpackt. Auf Paletten geschlichtet und in der Halle bis zur Abholung gelagert.

Die Vermarktung erfolgt übers Internet oder direkt ab Werk. Je nach Bestellung und Menge werden die Pellets zur Post gebracht oder von einer Spedition abgeholt.

Appendix 2

BI 1233 Hydroponic Systems in Horticultural Production and Public Environment, 2021/2022
Laboratory manual:
Water and growing media lab
Introduction
In many hydroponic/soilless systems substrates are used as a mean to store and distribute water and nutrients to the plants. An example is for instance drip irrigation to pots with peat. In order to determine whether a substrate is suitable for plant production, there are some physical and chemical characteristics that one should understand and know how to measure them. It is mainly about the density, porosity and ability of the substrate to hold water and air.

The aim with the lab is to compare chemical and physical properties of some substrates and mixes.
The substrates to be compared is: peat, anaerobic digestate, perlite, pumice and leca. Each group use peat, one of the substrates and a mix according to:

Group a	peat	perlite	peat:perlite	1:1 (volume)
Group b	peat	vermiculite	peat:vermiculite	1:1(volume)
Group c	peat	digestate	peat;digestate	1:1 (volume)
Group d	peat	pumice	peat: pumice	1:1 (volume)

1. Determination of bulk density (Sv. skrymdencitet)
The bulk density refer to the density of the substrate with the air filled pores (g/dm3) compacted in a standardized way to resemble the compaction of the substrate when in use.
Implementation
Fill the iron cylinder (with extension ring) with the substrate so that it extends over the edge of the extra ring. Make sure the entire cylinder volume is filled without compacting the substrate. Wipe off the abundance with a ruler. Add the weight. After 3 minutes, remove the weight and extension ring. Gently wipe off excess substrate along the edge of the iron cylinder. Empty the content in a foil mold and determine the weight of the substrate.

Calculate the bulk density in g/dm3
Remember to determine the volume of the measuring cylinder (it is unfortunately not one dm3)
2. Determination of the compact density /Sv. Kompaktdensitet, matrialitet)
Compact density refers to the density of the substrate without the pores, i.e. the relationship between the dry substrate´s true weight and its volume.
Implementation
Make two measurements and use the mean value in your calculations.
A dry 50 mL (or 100 mL) volumetric flask is weighed and filled to about half the volume with your substrate. Weigh the flask again, with the substrate. To push out air from the substrate, add exactly 25 (or 50 if the larger flask) mL of alcohol from a burette. Seal the flask with plastic film and shake for 30 min. Then add water from a burette to the mark on the flask. Record the amount of alcohol and water as a total additive to the flask. The volume that is not absorbed by liquid is the volume of the substrate.
Calculate the compact density of the substrate (g/dm3).
Now that you know the bulk density (density with pores) and compact density (density without pores), you can calculate the porosity (volume percent of pores) in the substrate. Try to figure out how, and make a general formula for this.
For all substrates, specify bulk density (g/dm3), compact density (g/dm3) and porosity (the amount of pores (%) of the total volume).

3. Water holding capacity (water retention) at pot capacity
Implementation
Assemble a plastic bottom cylinder with a cylinder extension and, if necessary, place gauze in the bottom. Fill the cylinders with substrate up to about 2 cm from the upper edge of the extension. Place the cylinders in an empty water container (tub). Water saturate for 2 days by slowly filling the tub to about 2 cm below the upper edge of the cylinder extensions (some weights may be needed to hold the cylinders in place). After 1 day carefully lift the cylinders and allow them to drain vertically for 24 hours covered with plastic foil. Remove the extensions and wipe of the excess substrate. Dig out all contents from the bottom cylinder and place in a weighted and labelled foil mold. Weigh the mold with the wet substrate. Dry in a drying cabinet (105 °C) for at least 1 day (Monday). Weigh the dry material in the mold.
Calculate how much water the substrate contained in % of the total volume and what percentage of the pores that were water-filled after 24 hours of drainage.

4. Electric conductivity (Sv. ledningsförmåga) and pH of the substrate
Determination of pH and conductivity EC in substrates is done according to a European agreed standard (pH; EN13037, EC; EN13038 (with some

modification)). Over the years, there have been many ways to measure these parameters, all of which give slightly varying answers. This method is agreed to apply to substrates and soil improvers. The same method is used for both parameters except the final measurement.

Implementation
pH and conductivity (EC) should be measured in distilled water in substrate to water ratio: 1:5 (v / v). Measure 30 mL of substrate with a measuring glass and transfer to a sample jar and add 150 mL of room-temperature clean (osmosis water from light green tap) water. Shake in an "end over end" shaker for 1 hour. Take out the jars shake each jar by hand just before the measurement and measure pH and EC directly in the jar or decant over a little of the solution in another vessel (e.g. the jar lid). Read the value when it has stabilized so that it does not change more than 0.1 (pH) in about 15 seconds. The EC number is normally quite stable. Rinse the electrodes (pH and EC) between measurements.
In the report, state the pH and EC for the various substrates and mixtures

To the report
The report is made in your group.
No long introduction or method part is needed. In principle only tables with measured values, calculations and results, but arranged in such a way that I understand what is what, and how the calculations are made.
Enter all the measurement values (average when you have made two measurements) and calculations, as above, for your substrates. Compare the substrates and discuss why they differ and how suitable you consider them to be as plant substrates.
What would you like to know more about the substrates, in this context, in order to determine their suitability as a culture medium?
What major sources of error can be found in the different methods?

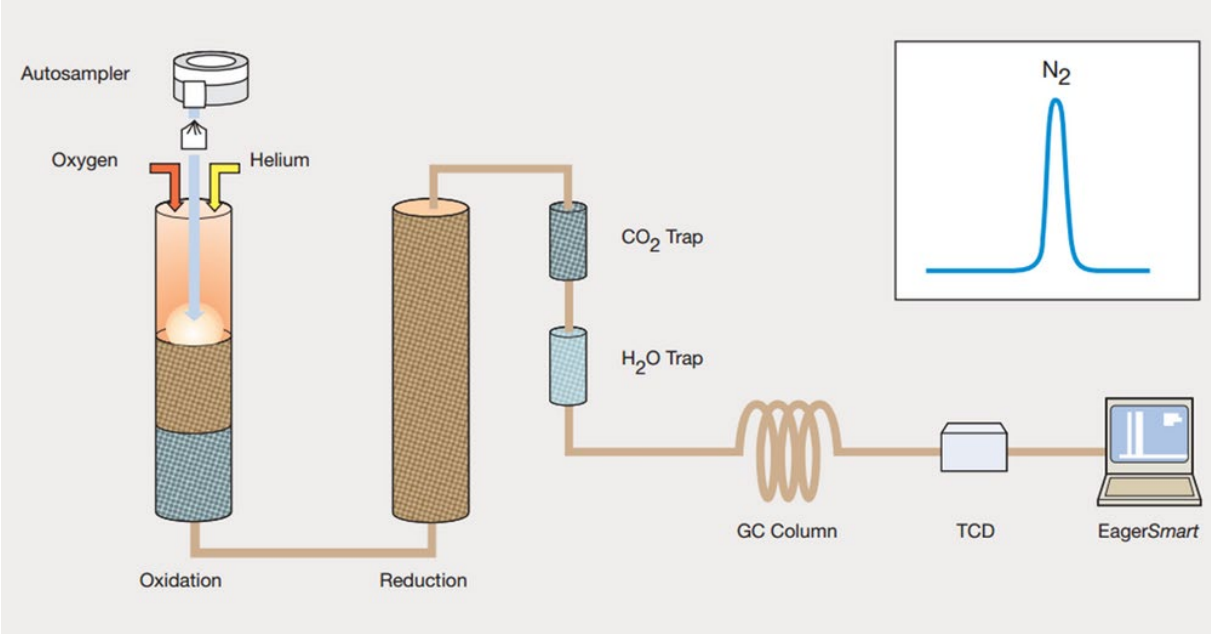
Good luck
Håkan

Appendix 3

Nitrogen and carbon quantification using the Elemental Particle Analyser (EPA) method

The elemental analyser (Flash 2000 Elemental Analyzer, Thermo Scientific), also known as the Dumas Nitrogen Analyser, is an automated system operating according to the dynamic flash combustion method. The principle is that an accurately weighed sample undergoes burning at high temperature (900-1000°C) inside the combustion chamber. After combustion, the produced gases are conveyed by a helium flow to a second reactor filled with copper, then swept through CO2 and H2O traps and onto a gas chromatography (GC) column equipped with thermal conductivity detection. GC provides separation and higher sensitivity advantages over purge and trap techniques. Figure 1 provides a diagram of the process with soil analysis application example. Aspartic acid & acetanilide is used as the standard for calibration.

Measurements of N as a proxy for total protein can be calculated from the total nitrogen require that a nitrogen-to-protein conversion factor (NPCF) be used to calculate the total (crude) protein in food/plant samples. A schematic figure describing the processes in the EPA nitrogen analyser from sample preparation to interpretation of results using software EagerSmart.


Autosampler
Oxygen
Helium
CO_2 Trap
H_2O Trap
N_2
Oxidation
Reduction
GC Column
TCD
EagerSmart

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