WAGENINGEN
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Wool for crop resilience

Final wildcard project report

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TRANSFORMATIVE Bioeconomies

This project has been funded by the investment theme Transformative Bioeconomies: Towards a materials transition that phases out fossil feedstock

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1. A short accessible document following the headers below (max. 600 words to be published on the website)

Innovative idea and objective

In Europe, about 87 million sheep are kept and they generate lots of wool (as a side product) that, although being a valuable material stream, cannot be used as a source for textile as fibers are often too coarse, . Because of this, the wool is currently burned, which costs money to farmers and increases CO_2 emissions and valuable materials are waisted. On the other hand, growing media for crops in greenhouse horticulture depend on materials such as stone wool and peat. Stone wool is difficult to fully re-use and its production energy-demanding. Furthermore, peatlands are natural protected areas which can store CO_2 from the atmosphere. Interestingly, keratin-rich soil amendments are able to reduce disease spread in crop plants and they harbor a very specific microbiome. Previous work has shown the positive effect on yield in wool-grown cucumber plants, compared to other substrates. Wool, consisting of keratin (a natural protein polymer), could make a good alternative as growing medium supplement to cultivate crops in the greenhouse. Thus, **how could we re-introduce Dutch wool as a valued side stream into the food production chain**? With this project we aim to answer:
(1) which wool treatment is needed for its application to support a good plant performance?
(2) does wool allow plants to grow and help to reduce disease caused by soil-borne pathogens?
(3) which microorganisms dominate in wool-based media?
(4) look for partners that might be interested to further develop the use of wool in horticulture.

Relevance to the materials transition in textiles and/or building materials?

This project is relevant to go a step forward into the reuse of carbon-based materials i.e. wool and reduce the environmental footprint, i.e. CO_2 emissions, by avoiding the burning of the wool. The potential benefits of the use of Dutch wool in crop production are:

- ✓ Reducing the need for non-recyclable rockwool and natural peat in horticulture, which also reduces the CO_2 emissions.
- ✓ Creating a value chain for wool, through which , sheep farmers and landscape managers could benefit from the whole product (wool).
- ✓ Wool could reduce the need for chemical additives (i.e. pesticides) in greenhouses

This project contributes to the textiles domain, as raw coarse wool cannot be reintroduced in the textile industry could potentially be revalued in horticulture as growing media or soil amendment.

What did you do?

We performed three types of tests in order to answer the following questions:
(1) which wool treatment is needed for its application to support a good plant performance?
(2) does wool allow plants to grow and help to reduce disease caused by soil-borne pathogens?
(3) what microorganisms are dominating in the wool-based media?

Wool was purchased via a collective center for wool in the Netherlands (https://www.hollandswolcollectief.nl/). The wool material was a mix of wool from diverse origins (coming from different sheep breeds). We got two types of wool: one treated, which was washed following a specific protocol (unknown) and one unwashed, raw wool that was neither washed nor treated. First we characterized both types of wool (washed and unwashed) for fatty acid and protein-derived nitrogen content. This generated data that could support question (1). Secondly we performed a greenhouse experiment with strawberry plants, where seedlings were grown in growing media made out with washed wool (at different percentages) mixed with potting soil. This data helped to answer question (1). Secondly we performed an pathogen bioassay, where different wool concentrations and types of wool (washed and unwashed) were amended into soil with garden cress seedlings, with and without *Pythium* (soil borne pathogen). This bioassay gathered data to support question (2). Soil samples from the bioassay were analyzed for the fungal and bacterial communities, together with some physico-chemical characteristics. These data would help answering question (3).

Main results, achievements and highlights

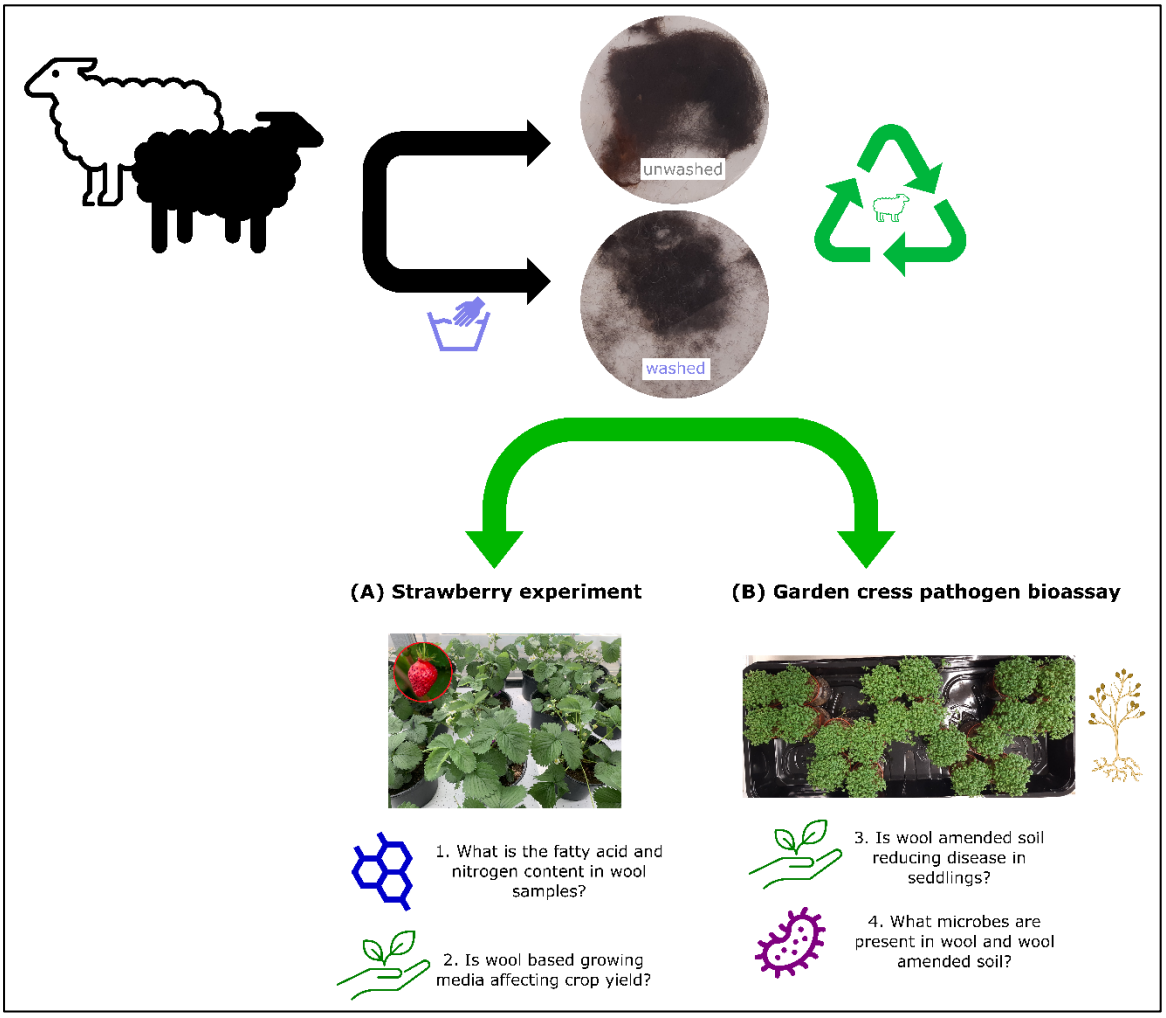
Our project supports the possibility of using Dutch wool in horticulture and agriculture to increase plant performance. This application is in line with a circular agricultural system and revalue of a carbon-based material from the textile domain. The project highlights are:

- ✓ washing the wool removed most of the fatty acids, and 25% w/w wool soil samples still contained detectable wool grease.
- ✓ wool is rich in nitrogen from proteins (keratin).Nitrogen content in washed wool is even higher as the wool fat (lanolin) was removed.
- ✓ Washed wool at 10% v/v was beneficial in many cases where fruits seemed to ripe faster, while 25% v/v wool seemed more harmful to the plants.
- ✓ Washed wool improves photosynthesis efficiency in plants, and at 0.1% w/w seems to delay the disease spread.

Key message

In this project we investigated how wool from Dutch breeds may be applied in horticulture and agriculture as a growing medium material and soil amendment. Results support the idea of re-using this carbon-based material in crop production to boost plant performance, where the type of treatment that is applied to the raw wool is relevant.

Visual abstract



Wool for crop resilience project working pipeline and research questions.

2. Additional questions about progress and 'readiness' (max 200 word, not for the website)

Where you started

Wool is already being applied and sold in pellets as slow-release fertilizer. However, its application as a growing medium has been tested only in few research studies, where they

tested plant growth parameters, and the majority of the studies are more focused on soil quality. On the other hand, the effect of wool on plant fitness and its effect against disease has not been studied yet. There are studies performed with other side streams rich in keratin such as pig hairs, however wool has never been tested before in plant disease suppression assays.

Where are you now

We showed there is a potential of wool as plant biostimulant to reduce disease symptoms in crops. Furthermore, we have new data supporting the effect of wool in plant performance in terms of photosynthesis efficacy. Still, experiments need to be replicated to have more robust conclusions and complementary data on what type of wool treatment is increasing or decreasing those effects need to be collected. Also to finetune the wool dosage is needed, together with testing other types of wool. Finally, we need to discuss this project output and new ideas with potential stakeholders from farming and agricultural/horticultural sectors.

Potential and next steps

This project is relevant to go a step further into the valorization of Dutch wool where sheep farmers could benefit from, and to reduce the environmental footprint i.e. CO_2 emissions by avoiding the burning of the wool. Raw coarse wool could potentially be re-valorized in horticulture as a growing medium or a soil amendment.

To continue with the material transition of wool, we would need to:

- Finalize gathering all the data needed
- Design new experiments to test the effect of different treatments or wool composition
- Discuss with stakeholders about our findings and new ideas

Innovation readiness

The project innovation readiness could be set at a TRL of around 6 (based on the innovation readiness levels by Sartas *et al*, 2020). We are testing the capacity within a controlled environment that reflects the spatial-temporal context (greenhouse). However, some questions remain unanswered before we could move to a higher readiness level. For example, we need to:

- dive into what microorganisms are associated to the wool, for safety and as biostimulants source, and which of them could be potentially co-applied with the wool to enhance the positive effects we observe in plant performance

- to test what type of wool treatment is the most appropriate to enhance plant productivity and/or reduce disease
- how is soil composition modulating these effects?

3. Learning Journey (max 300 words)

1. Did your Wildcard project involve new collaboration with disciplines or people? If so, briefly explain what was new.

Yes, this project involved people with different background and expertise: greenhouse horticulture technology, soil health, microbiology, crop protection and phytopathology, biobased materials and organic chemistry, textiles industry , all of them from different teams within WUR: WPR-GTB, WPR-Biointeractions, WFBR-BBP-SCT, BBP-BSV-. Most of the people involved in this project have not earlier worked together in a project. Furthermore, collaboration between the Business Unit Greenhouse Horticulture (WPR-GTB) and Unifarm was new, if not atypical.

2. If applicable, did the new collaboration alter your original thinking about the topic? Did it change research directions or courses of action? If so, briefly characterize how.

The discussion with other experts opened up new ways of thinking about modes of action and on how to set-up and design new experiments, i.e. how to apply the wool (v/v or w/w) or to quantify other forms for nitrogen available for plants. We had quite some discussions about set up of the experiments, sample exchange and relevant analysis methods. We also learned that re-using other type of textile materials is not always possible due to the presence of non-organic dyes which are not possible to remove and they are not allowed in agriculture for example.

3. Did interactions during community days and/or meetings organized by the investment theme alter your original thinking about the topic? Did such interactions change research directions or courses of action? If so, briefly characterize how.

No, during the community days we learned about other interesting projects, but it did not change our thinking about our own project.

4. Did you meet any challenges during implementation of your wildcard project? If so, what kind of challenges where these?

We had one person who left the project due to a change in job position, which led to a adjustment of the budget and tasks. This caused a delay on the execution of the microbial community profile analysis. The first greenhouse experiment with strawberries failed due to a bad establishment of young seedlings leading them to die, however we managed to repeat it within the budget constraints. The initial pathogen bioassay also failed due to plant diseases present in the soil used, though a second trial was successfully performed.

5. If applicable, how were these challenges eventually addressed? Did activities organized by the investment theme contribute to overcoming challenges? If so, briefly indicate how.

The coordinators of the investment team were very supportive and helped with the practical issues needed to re-arrange the budget.

6. Has your involvement in the investment theme resulted in any new initiatives or spin-offs that would probably not have emerged if you had not participated? If so, briefly indicate how these new initiatives came about.

No, there are no new initiatives or spin-offs. However, we aim to look for stakeholders and to continue with the research and further implement the application of wool as biostimulant and as growing medium material in horticulture. We also built a new nice project team which is open to collaborate in future projects together.

4. Additional project specific deliverables

Promised deliverables (as formulated in the proposal):

1) Data of the physico-chemical properties and ideal treatment of wool based growing media
2) Data on plant performance in growing media with wool (yield and disease suppression)
3) Data on microorganisms that are enriched in wool-based media
4) Results dissemination during working theme meetings and other interesting scientific and/or stakeholders meetings i.e. growers, growing media companies
5) We will have collaboration with other projects working in a related topic, where we will exchange information: PPS Systematic Approach for Finding Alternatives to Peat Substrates, PPS Peat alternatives mushroom & horticulture sectors and KB-34 Microbiome connections in the circular production systems

Deliverable (1) has been done as shown in Figure 6 and Tables 4-5. Data for deliverable (2) has been collected as shown in Figures 1-4, 7-8 and Tables 1-3. Deliverable (3) has been performed as shown in Figures 9-11 and Table 7. Deliverable (4) has been/will be accomplished during the KB Community Day and other workshops i.e. Soil Ecology meeting in December 2023. Coming year we plan to share our findings during the KNPV Soil borne pathogen working group meeting. For deliverable (5) we are already discussing with colleagues involved in the PPS Systematic Approach for Finding Alternatives to Peat Substrates, PPS Peat alternatives mushroom & horticulture sectors and KB-34 Microbiome connections projects.

There is a delay in deliverables (4). Due to the budget arrangement because the change in the team, there was a delay on the microbial community profile data. We received the raw data the first week of December, and preliminary analysis could be included in this report, however they could not be presented during the Community Day. Furthermore, because all data could not be gathered on time, we could not organize a workshop with potential stakeholders. However we had a meeting the first week of December with one Wool collector in the Netherlands, the Hollands Wol Collectief, who are in contact and collect wool from several sheep farmers. They were highly interested in our results and are open to future collaborations in the future.

Status of each project specific deliverable

Please report the status of each deliverable.

Deliverable	Status	Where to find it/comments
1) Physico-chemical data	Done	Annex 1: Figure 6 and Tables 4-5
2) Plant performance data	Done	Annex 1: Figures 1-4, 7-8 and Tables 1-3
3) Microbial community data	Done	Figures 9-11 and Table 7
4) Dissemination with stakeholders	Ongoing	We shared results with the Hollands Wol Collectief in December 2023, and a presentation is planned in January 2024 for the KNPV Soil Borne Pathogens meeting. However still more partners need to be reached.
5) Collaboration with other projects	Ongoing	We are currently discussing with colleagues and collaborators about our results.
6) Posters during the Community Days	Done	Annex 2

Links to or copies of deliverables

Annex 1: Experimental procedures and results.

Strawberry greenhouse experiment – Deliverable 2

Due to working condition safety and Unifarm's greenhouse rules, only the washed wool was tested. Wool properties for crop growth were quantified previously, and later the strawberry trial was performed.

A) Crop trial

Strawberries ('Opera' variety, *Fragaria* x *ananassa*) were chosen, for several reasons. Firstly, in the Netherlands they are currently being grown in systems using potting soil, coconut coir or stone wool. For the first two substrates, there may be interest in adding wool to the mix. Secondly, a fruiting crop, the fruits could be counted and weighed, unlike for a crop like lettuce. Strawberry plants are also smaller than other fruiting crops grown in greenhouses, meaning they required far less care, compared to high-wire crops like tomatoes or cucumbers.

Three potting soil mixes were prepared for the strawberries, based on a volume percentage of wool: 0%, 10% and 25%. The rest of the potting soil was a mixture of coconut coir and peat, which had a lab bulk density of 325 g l^{-1}. As when measuring the wool's lab bulk density, the wool was torn into pieces between 5 and 10 cm long. Equal amounts of slow-release fertiliser (Osmocote® Pro, 19N-9P-10K+2MgO+TE) were added to each treatment, at 11 g per pot.

The strawberries were transferred from small pots to round 4.5 litre pots with the relevant mixture. These were kept at Wageningen University & Research's greenhouse facilities in Wageningen (Unifarm), on trays where they were regularly given water from above. The crop trial ran from 15 August to 12 September 2023. During this time, no fruits were harvested.

On 12 September, plants were harvested by cutting everything above ground and leaving the roots behind, along with fruits that had fallen off and rotten. The above-ground mass was weighed. Then, the number of leaves that either showed tipburn or were otherwise wilted was counted. All fruits on the plant were subsequently removed and weighed. Lastly, the fruits were counted: (1) fruits that had yet to ripen, (2) fruits that were ripe and (3) fruits that were no longer edible.

B) Results

Wool lab bulk density was around 24 g.l^{-1} (**Table 1**). Compared with other growing media used in the greenhouse this value is extremely low, since most materials have a density between 150 and 350 g l^{-1}. The dry matter content was around 88% (**Table 2**), which is on the higher end of substrate materials, which can range from 50% in extreme cases to 90%. Water retention was measured after draining, where the wool weighed 319 g. Since the wool weighed 23.3 g (the same wool as sample 4 in Table 1), this means that a litre of wool can hold 296 ml of water.

Table 1. The results of the lab bulk density measurements (g l^{-1}) for four samples.

Sample	**1**	**2**	**3**	**4**	**Average**
Lab bulk density (g l^{-1})	25.30	21.60	24.25	23.30	**23.61**

Table 2. The results of the dry matter content measurements, with masses before and after drying.

Mass before (g)	**Mass after (g)**	**Dry matter content (%)**
25.30	22.08	87.3
21.60	18.99	89.9
24.25	21.28	87.8
Average		
23.7	20.8	87.6

Strawberry experiment resulted and several data of plant traits (see **Table 3**).

Table 3. Strawberry plant traits measured with (10 and 25%) and without wool as growing media. Means and standard deviations are given, along with the two-tailed p-value compared to the control, between brackets.

Treatment (% wool)	**0 (control)**	**10**	**25**
Plant weight (g)	150±16	160±25 (0.30)	**126±12 (0.002)**
Proportion of damaged leaves (%)	19±8	**14±5 (0.07)**	23±7 (0.35)
Total fruit weight (g)	76±15	86±14 (0.17)	68±13 (0.24)
Fruit count (#)	18±3	**20±2 (0.07)**	17±4 (0.59)
Before ripe (%)	39±12	31±15 (0.18)	41±11 (0.75)
Ripe (%)	30±14	19±8 **(0.06)**	25±11 (0.36)
After ripe (%)	31±15	49±11 **(0.01)**	36±19 (0.57)

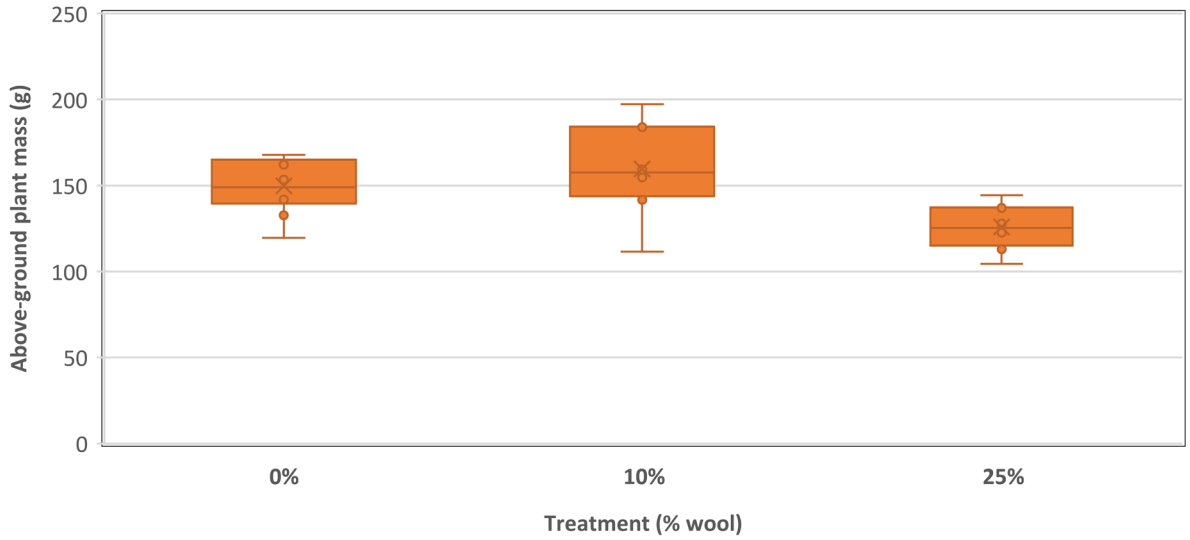


Figure 1. The above-ground plant biomass (g) of the three treatments. Bars represent the mean value; error bars represent the standard deviation.

The above ground biomass shows that 10% wool may be slightly beneficial – on average by just 7% – to the total weight of the plant (p=0.30) (**Figure 1**). The larger difference is seen in the 25% treatment, where plants weighed on average 16% less than in the control treatment (0%). Of all results seen here, this last one by far the most significant statistically (p=0.002).

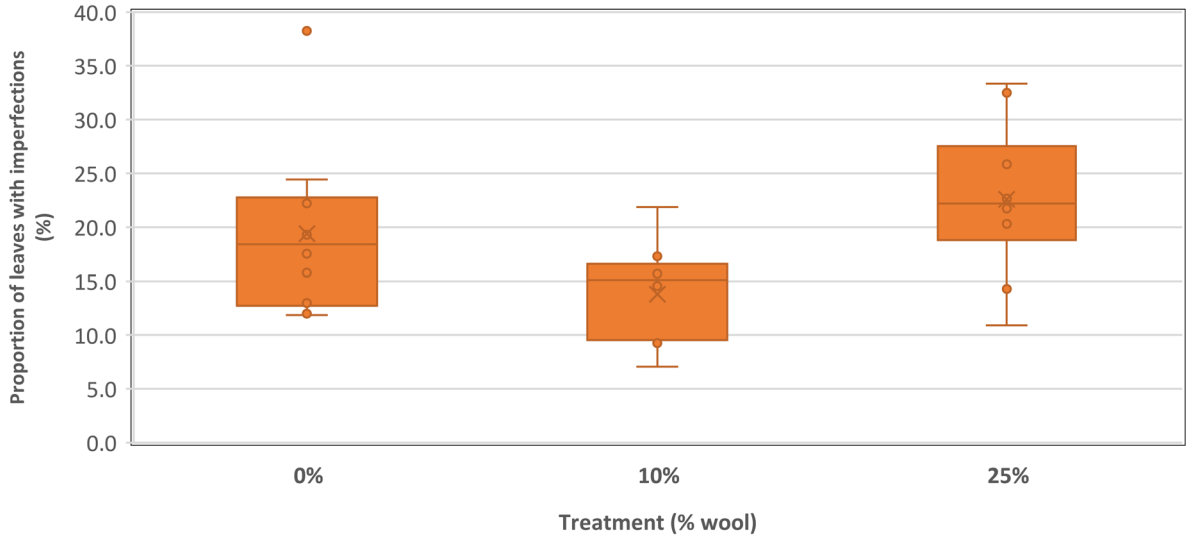


Figure 2. The proportion of leaves with tip burn, wilt or other imperfection (%) for the three treatments.

Similar effects are seen in **Figure 2** Compared to the control of 0% wool, 10% wool leads to a lower proportion of leaves with imperfections: 14% compared to 19%, or a 27% reduction (p=0.07). Here again, the plants grown with 25% wool do less well, with 23% of leaves showing an imperfection: 21% more than the control. That said, this increase in imperfections is statistically not very significant (p=0.35).

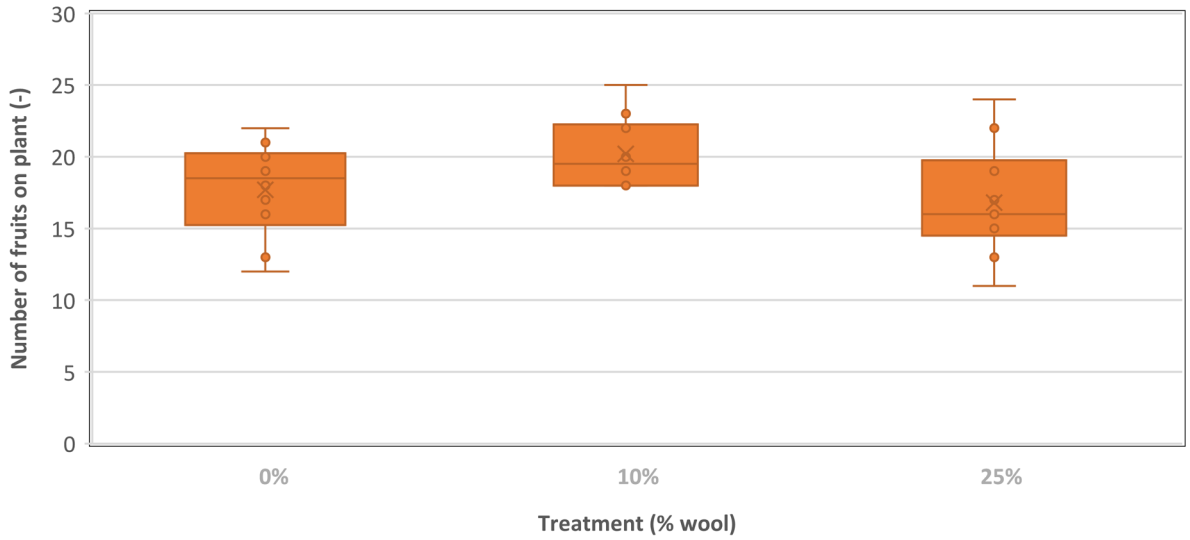


Figure 3. The number of fruits still on the plant (-) for the three treatments.

When visualised as in **Figure 3**, the number of fruits shows a similar pattern to **Figure 1**. 10% wool leads to 11% more fruits than the control (p=0.07), whereas 25% wool shows a slight reduction of fruits, at 6%. With a p-value of 0.59, this last difference is most likely due to chance than due to any effect of wool, however.

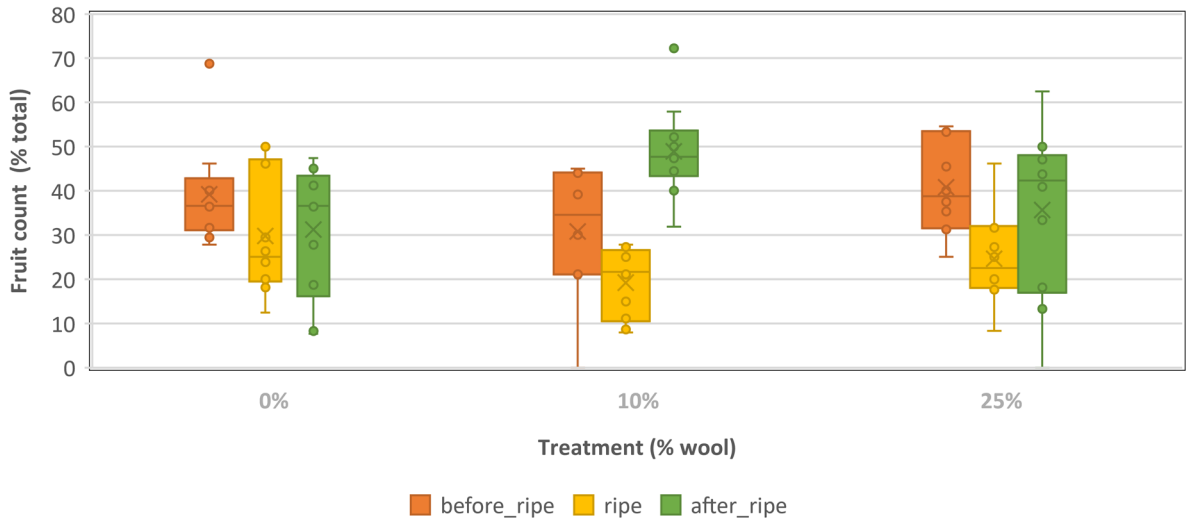


Figure 4. The proportion of fruits counted that were not ripe yet, ripe or beyond ripe (%). The size of the bars on this chart says nothing about the total number of fruits counted or their weight.

Lastly, the state of the fruits is visualised in **Figure 4**. These figures say nothing about the total number of fruits or their weight, but only their development stage. For the control and the 25% treatment, the largest proportion of fruits were not ripe yet, though all p-values related to the 25% treatment showed these differences to be statistically insignificant. In contrast, the 10% treatment showed most fruits to have already been ripe (p=0.01), and a lower proportion of fruits currently ripe (p=0.06). Some of these results may have to do with a difference in the absolute number of strawberries. Still, this suggests that the 10% treatment grew faster and developed fruits more quickly than the other two treatments.

C) Conclusions

Summarising the results presented in the previous section, an addition of 10% wool to the potting soil mix is beneficial to strawberries, but at 25% it is harmful. Although the statistical significance of the results vary, they all point in the same direction, with not one result suggesting the opposite. Most results were not statistically significant enough to be sure of this. Still, the most significant result ($p=0.002$) is that adding 25% wool leads to decreased plant growth. Furthermore, the 10% wool treatment showed an increase in the number of fruits and a decrease in the number of damaged leaves, both with a two-tailed p-value of 0.07. We recommend further research to confirm these results and their significance.
It is also important to determine the cause of these differences. The favourable results seen in the 10% treatment, combined with the unfavourable results in the 25% treatment, suggest that the wool may be releasing nitrogen into the substrate. Additional nitrogen may have been beneficial, but in the 25% treatment it may have been too much, hampering plant growth and leading to tip burn. In this study, an equal amount of slow-release fertiliser was added to all three treatments. Future research should adjust the fertilisation level to the expected nitrogen release from the wool. Not only would this confirm whether wool is releasing nitrogen; it would also give information on whether the observed benefits/harms come from just nitrogen or another property unique to the wool itself.
Future research should also determine how to mix wool into substrates in a standardised way. The size of the pieces of wool and the homogeneity of the mix are likely to have had an effect on the outcomes, and the optimal size is yet to be determined. This may also affect the lab bulk density (which this study showed was difficult to measure in the standard way). With the increasing use of new materials for growing media, this may be a challenge that needs to be overcome for other materials too.

Wool properties – Deliverable 1

Wool parameters as growing media

The following three measurements were done: lab bulk density, dry matter content and water holding capacity. Lab bulk density is used to calculate properties of mixtures using a standardised compaction, as density would otherwise vary. The dry matter content and water holding capacity are important to understand and predict how best to irrigate.

Lab bulk density

Initially, the wool was measured for its lab bulk density using the EN 13040 method, a European standard for measuring substrate volume under a standardised compaction with a weight for 3 minutes. This was done with wool torn into fragments of approximately 5 to 10 cm long. After compaction and the removal of the weight, it was found that the wool would spring back to its original volume, making it difficult to determine the lab bulk density the same way as is usually done. Still, as is done in the EN 13040 method, any wool that was outside of the cylinder (after removal of the weight) was removed.

Dry matter content

Three samples of wool were weighed and put into a drying oven for 38 hours at the Business Unit Greenhouse Horticulture's facilities in Bleiswijk. These were then weighed afterwards to determine the dry matter content.

Water holding capacity

A litre of wool – determined by the lab bulk density – was submerged in an Erlenmeyer flask overnight. The next day, it was removed from the flask and left to drain until dripping stopped. The remaining wool was weighed to determine the water holding capacity.

Fatty acid content in wool samples

In this project, the amount of extractable solids in wool and soil samples containing wool were determined using Soxhlet extraction with DCM (Allafi *et al.*, 2022). Next, the extracted compounds were characterized by gas chromatography coupled with mass spectrometry (GC-MS).
Soxhlet extraction was performed on a Behrotest® 4x250 mL extraction system with 100 mL extraction volume and 250 mL round bottom receiving flasks. Extraction thimbles were filled for approximately 3/4th with the wool and/or soil and the thimbles were closed with a cotton wool plug. Next, the system was filled with 150 mL DCM, and the receiving flasks were heated to reflux for 4 h. After, the extraction was stopped, and the solvent in the receiving flask was evaporated using a rotary evaporator. All samples containing soil were dried in a vacuum oven at 40 °C for 24 h prior to extraction. The wool samples were used without pre-treatment.
GC-MS analysis of the extracted solids dissolved in chloroform (approx. 5 mg/mL) was performed on a Interscience Trace 1300 with AS3000 II auto sampler (He-carrier gas, flow 1.2 mL/min, split flow 50 mL/min; Restek GC column Rxi-5ms 30 m x 0.25 mm x 0.25 µm; GC program: hold 2 min at 70 °C, ramp 10.0 °C/min, final temperature 300 °C) connected to an Interscience Trace ISQ 7000 (EI, mass range 35-800 Dalton, 200 Ms sample speed).
The samples that contained soil were dried in a vacuum oven at 40 °C overnight. **Figure 5** shows photographs of the samples after drying. It can be seen that only the potting soil was fully dry, and that the samples that were used in the strawberry experiment (0, 10, and 25% wool) still contained some moisture given the darker color of the soil. Furthermore, it can be seen that the wool containing samples were relatively inhomogeneous. The 25% wool sample shows large tufts of wool, whereas no wool was visually observed in the 10% wool sample.

Figure 5. Photographs of the soil containing samples after drying in a vacuum oven overnight. From left to right: potting soil, 0% wool, 10% wool, and 25% wool.

Nevertheless, the amount of extracted solids were determined for all samples using Soxhlet extraction in DCM. **Table 4** shows the extracted amounts in wt%. As expected, the unwashed wool contained significantly more extractable solids (mainly grease) as compared to the washed wool were during washing most of the grease was removed, yielding only 0.4 wt% of extractable solids. The blank potting soil was found to have 3.4 wt% of extractable solids indicating that there are significant amounts of compounds present in potting soil that are also soluble in DCM, but not necessarily have to be grease-like compounds. The high content of DCM soluble compounds will make the interpretation of the mixed wool/soil samples from the strawberry experiment less straightforward.

Table 4. The amount of extracted solids in wt% after 4 h of extraction with DCM. Note that the extraction of unwashed and washed wool was performed in duplicate.

Unwashed wool	Washed wool	Potting soil	0% wool	10% wool	25% wool
7.1 (1) 6.6 (2)*	0.4 (1) 0.4 (2)*	3.4	0.9	2.5	2.1

*duplicate values

The amount of extractable solids is lower for the soil samples from the strawberry experiment (0, 10, and 25% wool) than that of the blank potting soil. A slight drop in extractable solids is expected as wool was found to have only 0.4 wt% extractable solids, however the amount of solids found is lower than expected based on the extraction results of washed wool and potting soil. For example, for 25% wool a extractable amount of 3.2 wt% can be predicted, but 2.1 wt% is measured. Also the extremely low amount of extracted solids for the 0% wool sample (0.9 wt%) is very surprising, given the significant amount of extractable compounds in the potting soil.
There can be multiple explanations for the observed differences:

a) The extractions of the soil samples from the strawberry experiment were only performed once due to the limited size of the extraction set-up in combination with the budget. There might be some experimental errors in the value found. Nevertheless, the extractions of the wool samples were performed in duplicate, and were found to be relatively consistent.
b) The samples from the strawberry experiments (soil mixed with wool) were found to be very inhomogeneous. For example, barely any wool was visually observed in the 10% wool sample. Thus it might be that the samples in the extraction thimbles were not representative for the whole sample set.
c) The strawberry plants use nutrients from the soil during their growth. It is likely that some of the extractable compounds are taken up by the strawberry plants and thus that a lower amount of extractable solids is measured after growth.

The extracted solids were characterized by GC-MS. **Figure 6** shows the resulting GC spectra of all samples. The unwashed wool shows a broad signal from with a retention time of 23-27 min and some specific sharp signals on top of the broad signal. For example, the MS spectrum of the sharp signals at 23.58 and 25.06 correspond most likely to cholesterol and derivatives thereof which are present in wool grease. Washing the wool reduces the intensity of the broad signal, but the cholesterol signal at 25.06 min remains and a new signal at 25.35 min

(desmosterol) appears indicating that not all wool grease has been removed. This corresponds well to the 0.4 wt% extractable compounds that were found for the washed wool. Note that the concentration of the extractable compounds in all GC-MS measurements was kept constant at approximately 5 mg/mL.

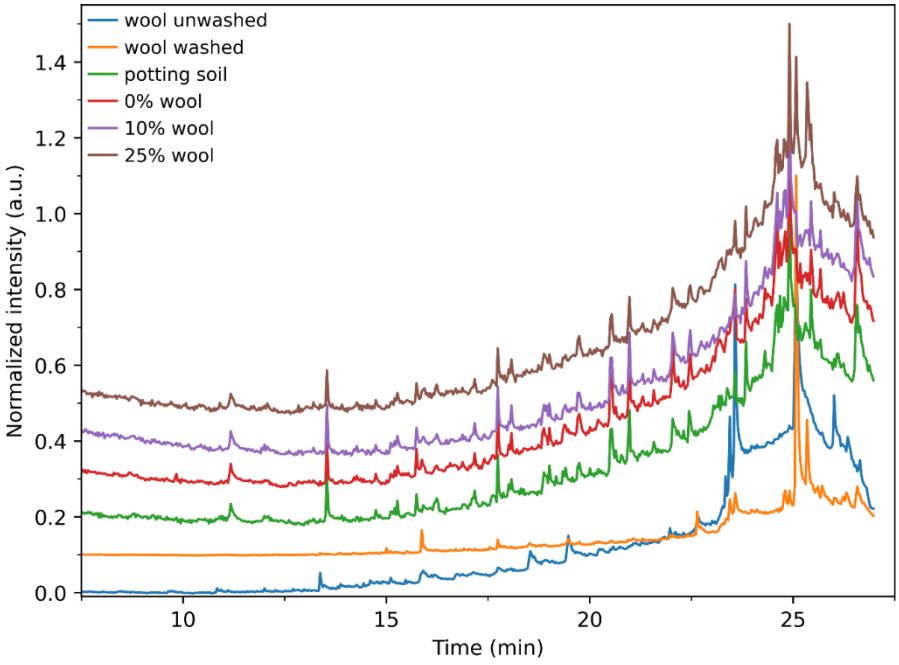


Figure 6. Normalized GC spectra of all samples measured at a concentration of approximately 5 mg/mL. All spectra were normalized to the highest peak.

The GC spectrum of the potting soil shows significantly more signals and thus a more variety in chemical nature of the extractable compounds. Characterization of all these signals is out of scope for this work. It can be concluded that the GC spectrum of the 0% wool sample is not different from the potting soil spectrum, indicating that the same compounds are still present in the sample. Note that the concentration can be different, but this cannot be distinguished from GC-MS experiments. Also the 10% wool sample shows no difference as compared to the potting soil sample, suggesting that little to no wool is present in that sample. This also corresponds to the visual observations of this specific sample. The GC spectrum of the 25% wool sample, on the other hand, does show signals at 25.06 (cholesterol) and 25.35 min (desmosterol) indicating that wool grease was present in that sample even after growth of the strawberry plants.

It can be concluded that the washing of the raw wool was successful as the amount of extractable solids decreased from 7% for unwashed wool to 0.4% for the washed wool. Also the potting soil contained significant amounts of extractable solids (3.4 wt%) making the characterization of the soil from the strawberry experiments complicated. Furthermore, the soil samples from the strawberry experiment were very inhomogeneous further complicating the characterization. Nevertheless, it was found that the 25% wool sample still contained wool grease after strawberry growth.

Nitrogen content in wool samples

The amount of nitrogen was determined by Kjeldahl method. Potting soil, soil 0% washed wool, soil 10% washed wool and soil 25% washed wool samples were analyzed, together with the raw and washed wool pure samples.
The Kjeldahl method can determine the fraction of reduced nitrogen, both organic and inorganic, in substances. The method is called kjaldahl nitrogen, which is the amount of nitrogen present in organic compounds and the amount of nitrogen in the form of ammonia and ammonium, but all nitrogen bound in the form of nitrates and nitrites is ignored. Phases of the analysis:

1) *Destruction*:

The sample is deconstructed with concentrated sulfuric acid at 420°C. The nitrogen is converted into ammonium sulphate.

$$\mathrm{a\,N} + \mathrm{H_2SO_4} \longrightarrow \mathrm{CO_2} + \mathrm{H_2O} + 1/2\,\mathrm{a\,(NH_4)_2SO_4}$$

2) *Distillation*:

After cooling, sodium hydroxide is then added to the liquid to a high pH, where the ammonium sulphate is converted into ammonia.

$$1/2\,\mathrm{a\,(NH_4)_2SO_4} + 2\,\mathrm{NaOH} \longrightarrow \mathrm{Na_2SO_4} + \mathrm{a\,NH_3\,(g)}$$

The ammonia is then steam distilled and distilled into a boric acid solution:

$$\mathrm{H_3BO_3} + \mathrm{a\,NH_3} \longrightarrow \mathrm{a\,NH_4^+ . H_2BO_3^-}$$

3) *Titration*:

The boric acid solution is titrated with 0.1 M HCl to pH 4.5.

$$\mathrm{a\,NH_4^+ . H_2BO_3^-} + \mathrm{HCl} \longrightarrow \mathrm{a\,NH_4Cl} + \mathrm{H_3BO_3}$$

Assuming that protein-containing substances contain on average 16 wt% nitrogen, the amount of protein can then be calculated by using a factor of 6.25. Measurements were performed in duplicate.
Results of the Kjeldahl analysis are shown in **Table 5**. The amount of nitrogen in the soil samples was comparable for the samples with 0 and 10% wool, whereas the sample with 25% wool had a much higher N-content.
The amount of protein was calculated with a factor 6.25. This factor is an average factor for protein-containing substances and can be different for the samples measured here, leading to miscalculation of the protein content. For the washed wool, the amount of proteins reached nearly 100%, as the washed wool is nearly 100% keratin (protein). The unwashed wool contained some non-protein components, and this was also reflected by the lower protein values.

Table 5. Nitrogen content in samples based on the Kjeldahl method.

code	sample	dm wt%	mg N	wt% N	mg protein	wt% protein
A	Wool unwashed	83.8	16.8	13.0	105.2	81.2
		83.3	15.4	9.9	96.3	62.1
B	Wool washed	87.3	16.9	15.6	105.4	97.8

		88.7	16.0	16.2	99.8	101.5
1	Soil unused	48.2	6.1	0.7	n.a.	n.a.
		48.2	6.9	0.7	n.a.	n.a.
2	Soil 0% wool	33.1	4.4	0.8	n.a.	n.a.
		33.1	4.2	0.8	n.a.	n.a.
3	Soil 10% wool	31.3	4.9	0.9	30.5	5.4
		31.3	4.2	0.9	26.3	5.5
4	Soil 25% wool	33.3	17.3	2.9	108.2	18.0
		33.3	17.1	2.8	106.8	17.7

What can be concluded is that the amount of nitrogen is only elevated in the sample with 25% wool. Of the 17 mg of N, part of it originates from the soil (4 mg), and then the other 13 mg then comes from the added wool.
Taking a representative sample was quite hard, and it is possible that some wool fibers were also present in the 25% wool sample taken, thereby elevating the amount of nitrogen in the sample. However, this does not elucidate the origin of the nitrogen (fertilizer added, wool), and if the plants had access to more free nitrogen due to the presence of wool. Given the short time of the cultivation trial, it is not likely that the wool is fully degraded to amino acids.

Plant pathogenicity bioassay with wool – Deliverable 2

We performed pathogenicity assays in greenhouse conditions with garden cress (*Lepidium sativum* L.). First, a mixture of potting soil and sand (1:3 v/v) was mixed with two types of wool separately (washed and unwashed wool) and it was stored at room temperature for two weeks. Later, this mixed soil was inoculated with 0.125 g of *Pythium* TK9 spores and left it at room temperature for two days. Finally, 0.5 g of garden cress seeds were sown in each pot, 4 replicates. .
One week after sowing, soil samples were taken for DNA extraction and soil chemical analyses. Disease score after 7 days resulted in almost 100% diseased plants for all treatments. However, five days after sowing, seedlings growing in the wool-amended soil seemed to present a slower disease spread, however no quantification was performed at this time point (no data available) (**Figure 7**).

Figure 7. Pathogenicity bioassay with *Pythium*. Signs of disease in garden cress seedlings 5 days after sowing (DAS). WW: washed wool; UW: unwashed wool; 0.5: 0.5% wool w/w; 0.1: 0.1% wool w/w.

Furthermore, seven days after sowing, non-infected plants (without *Pythium*) were analyzed with the PlantExplorerXS (PhenoVation Life Sciences), to quantify the photosynthesis efficiency. Results showed that seedlings growing in wool-amended soil presented higher photosynthesis efficiency values if compared with control plants (no wool), which is a sign of better performance and health (**Figure 8**). This efficiency was remarkable higher in seedlings growing in washed wool amended soil.

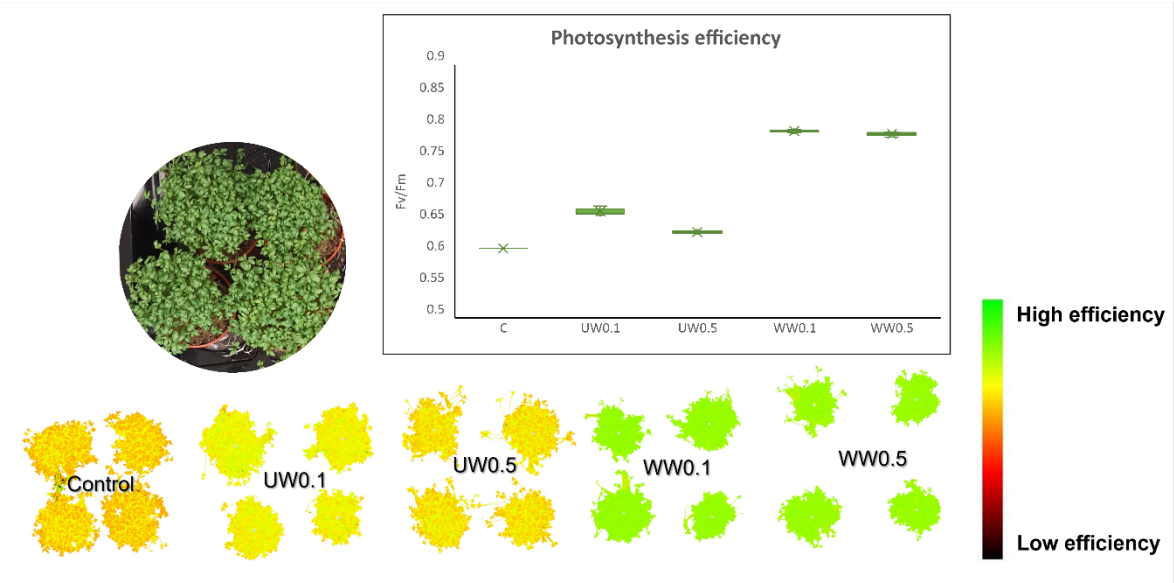


Figure 8. Pathogen bioassay with Pythium. Photosynthesis efficiency in plants without pathogen.

Microbiome profile – Deliverable 3

Soil bacterial and fungal communities were characterized by targeted sequencing of 16S (bacteria) and ITS (fungi) ribosomal DNA. We used Illumina MiSeq sequencing platform, and specific primers (**Table 6**) and adaptors (from sequencing provider) were added primer the sequencing. Results showed that wool present a unique microbiome, where different fungal and bacteria taxa were found between treated (washed) and untreated wool (**Figure 9** and **Figure 10**).

Beta diversity is not very different between wool treatments or pathogen treatments (**Figure 11**). PERMANOVA analysis supports that a very small percentage of the microbiome in soil samples is affected by wool treatment (washed vs unwashed wool), pathogen inoculation or wool concentration, being wool treatment the factor with the highest effect, where 9% and 12% of the fungal and bacterial communities respectively are significantly affected (**Table 7**). Remarkably the bacterial community is the most affected by the pathogen addition, affecting the 21% of the bacterial microbiome.

Table 6. Primers selected to profile the fungal and bacterial communities in soil samples amended with wool.

Primer name	Target	Sequence (5' → 3')
E341degNW-fwd	16S V3-V4	CCTACGGGNGGCWGCAG
E805degNVW-rev	16S V3-V4	GACTACNVGGGTWTCTAATCC
gITS7ngs-fwd	ITS2	CTTGGTCATTTAGAGGAAGTAA
ITS4ngsUni-rev	ITS2	GCTGCGTTCTTCATCGATGC

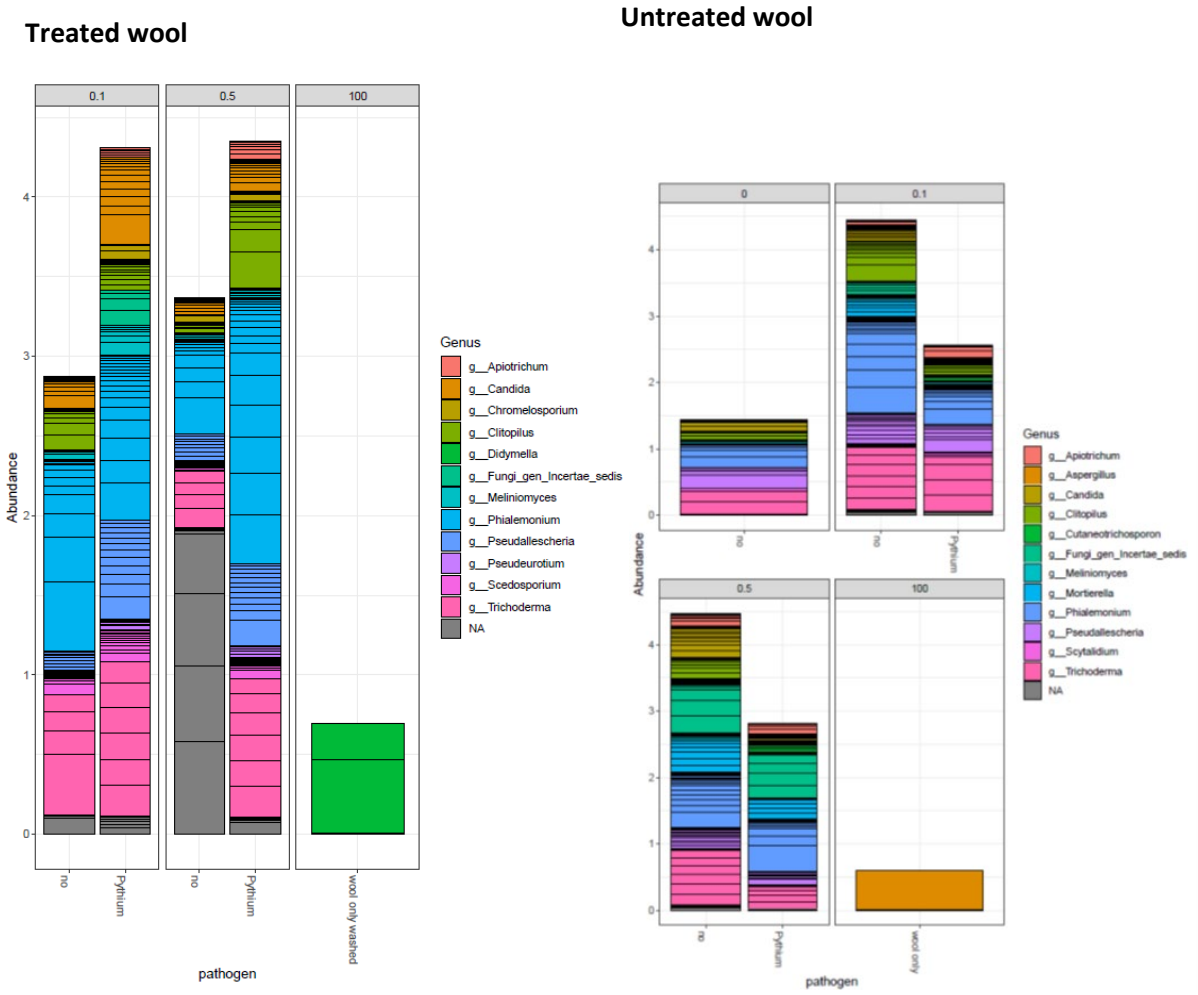


Figure 9. Top 20 Fungal ASVs from soil samples with (a) treated and (b) untreated wool. Note: the pure wool microbiome (no soil) is labeled as "100", and the pure soil microbiome (no wool added) is labeled as "0".

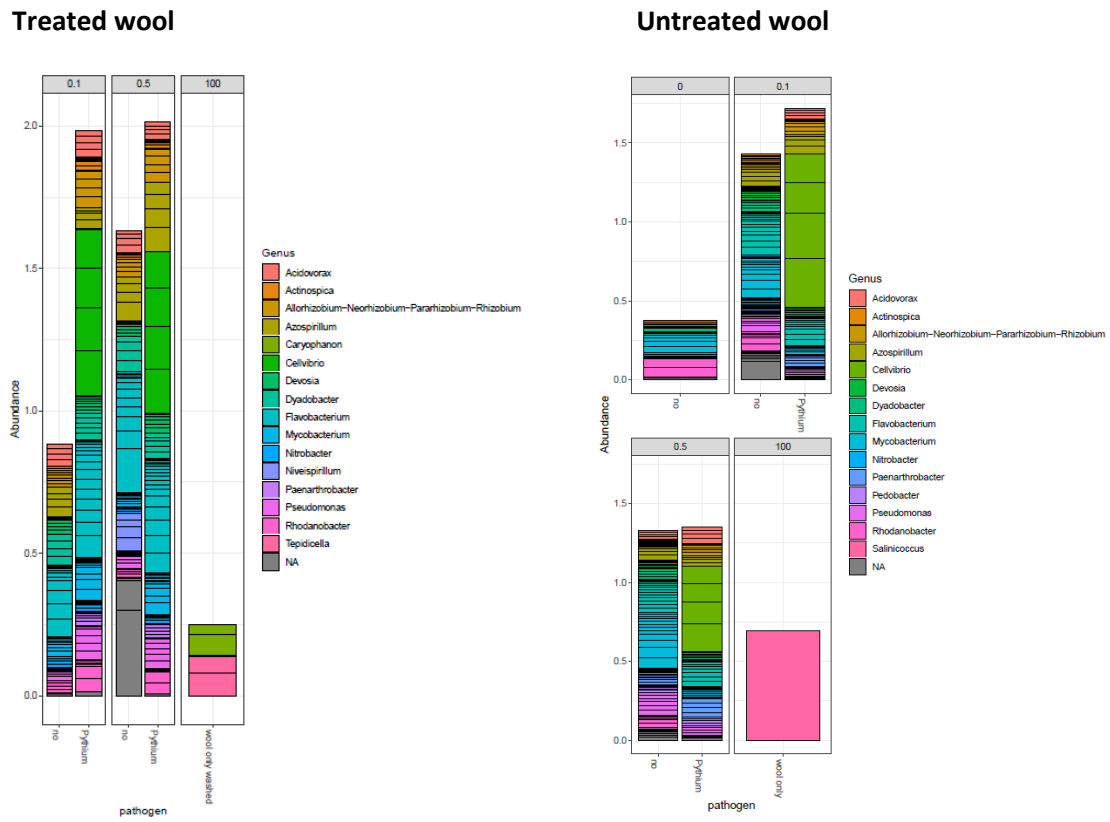


Figure 10. Top 20 bacterial ASVs from soil samples with (a) treated and (b) untreated wool. Note: the pure wool microbiome (no soil) is labeled as "100", and the pure soil microbiome (no wool added) is labeled as "0".

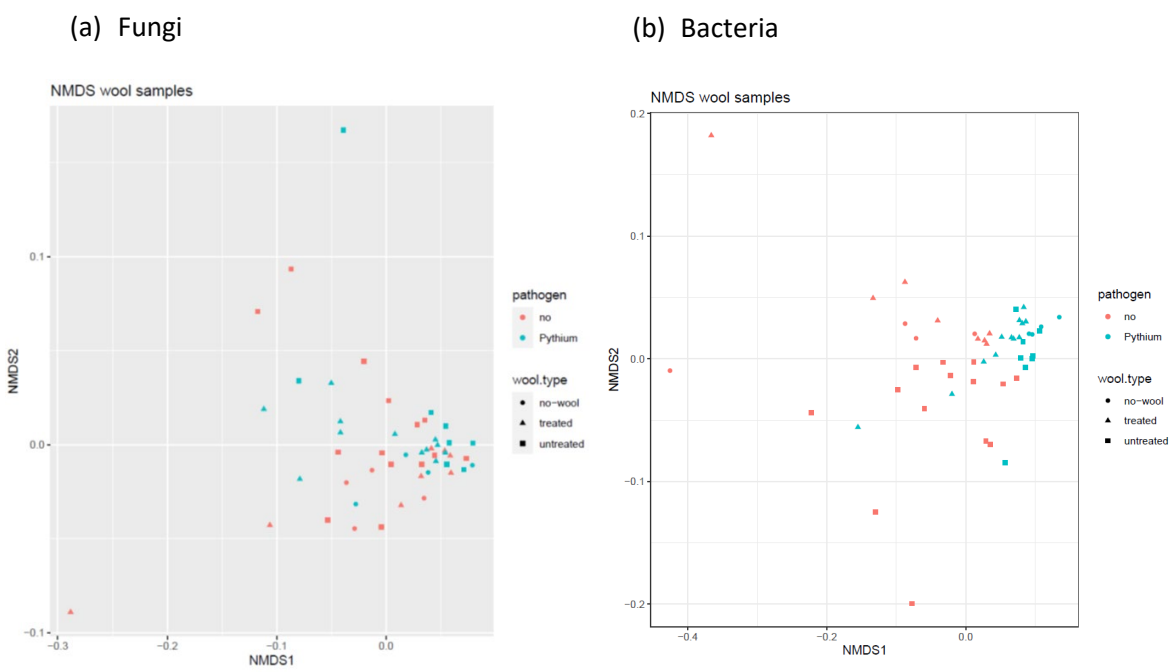


Figure 11. Beta diversity (a) fungal and (b) bacterial community.

Table 7. PERMANOVA analysis results on beta diversity in fungal and bacterial communities. Data from soil samples obtained from the pathogenicity bioassay.

Community	Factor	R^2	P-value
Fungi	Pathogen	0.04	0.003**
Fungi	Wool concentration	0.07	0.001***
Fungi	Wool treatment	0.09	0.001***
Bacteria	Pathogen	0.21	0.001***
Bacteria	Wool concentration	0.06	0.013*
Bacteria	Wool treatment	0.12	0.002**

Pure untreated wool is dominated by the genus *Aspergillus* sp. These fungi are very common and they are found in carbohydrate rich environments. Many species are know to cause diseases in animals and pathogens. Some *Aspergillus* species has been described to inoculate old textiles and they were able to degrade wool (Kavkler & Demšar, 2012). On the other hand, treated wool was dominated by *Didymella* sps. Species of this genus i.e. *Didymella bryoniae* are known as pathogens in Cucurbitacea plants such as the cucumber and in wheat, and some of the such i.e. *D. keratinophila* are able to degrade keratin and chitin (Ma *et al.*, 2022). When inoculated with the pathogen, soil samples with treated wool, which are the ones increasing plant performance, exhibit more species from *Clitopilus* sps., *Phialemonium* sps. and *Trichoderma* sps. (**Figure 9**), however with untreated wool we observe the opposite trend. *Trichoderma* sps. can be the causal agent of several plant diseases and rarely in humans, however many of them are plant endosymbiotic fungi and are well known biocontrol agents against crop diseases i.e. *Trichoderma harzianum* (Zin & Badaluddin, 2020). Compared to pure soil fungal microbiome, wool amended samples present more *Trichoderma*, *Clitopilus* and *Phialemonium* species.

Pure wool also presents a very specific bacterial community, where mainly *Caryophanon* sps. and *Tepicicella* sps. are found in treated wool, whereas only *Salinicoccus* sps. is found in untreated wool. *Tepidicella* sps. are found in waste water environments and they are able to degrade complex organic molecules (Huo *et al.*, 2023). *Salinicoccus* sps. are halophytic bacteria that belong to the Bacillaceae family. Another Bacillaceae specie, *Ornithinibacillus caprae*, present keratinolytic properties and is not degrading collagen, making it harmless to the skin (Li *et al.*, 2022). When inoculated with the pathogen, soil samples with treated wool present a huge increase on *Cellvibrio* sps., and a minor increase in *Rhodanobacter* sps., *Rhizobium* sps. and *Paenarthrobacter* sps. (**Figure 10**). *Paenarthrobacter* sps. are applied into soil for the bioaugmentation (removing pollutants) of herbicides in soils (Jia *et al.*, 2021). *Paenarthrobacter ureafaciens* is a commonly specie found in disease suppressive soils, which has antifungal effects (Nguyen *et al.*, 2023). *Rhizobium* sps. are known to form nodules in

leguminous plants and can fix nitrogen, but they can also reduce crop diseases and improve plant growth (Das *et al.*, 2017). *Rhodanobacter* sps. are antagonists of soil borne pathogens such as *Fusarium solani* among others (Huo *et al.*, 2018). *Cellvibrio* sps. are saprophytic bacteria able to degrade cellulose, xylan, starch, and chitin, and it produces chitinases among other enzymes (Nunes & Philipps-Wiemann, 2018). On the other hand, soil samples with untreated wool, when inoculated with the pathogen also present more *Cellvibrio* sps. and *Paenarthrobacter* sps., but also an increased abundance of *Acidovorax* sps. and a reduction of *Mycobacterium* sps. *Acidovorax* sps. can cause disease in crops (Burdman & Walcott, 2012) and *Mycobacterium* sps. are causal agents of human diseases (Cook *et al.*, 2009).

Annex 2: Posters for the community meetings

Poster 1: Community Day April 2023

WAGENINGEN
UNIVERSITY & RESEARCH

Wool for crop resilience

Beatriz Andreo Jimenez[1], Tess van der Voorde[1], Alexander van Tuyll[2], Francisco Mondaca Duarte[2], Irene Sanchez Andrea[3], Evelien Maaskant[3], Paulien Harmsen[3]

[1]Biointeractions & Plant Health (WPR), [2]Greenhouse Horticulture & Bulbs (GHB), [3]Microbiology Group (WU), [4]Biobased products (BBP)

To which domain did you submit your proposal?

Textiles, Topic 5: recycling of discarded carbon-based materials

What are you exploring? With what objective?

We want test the re-use of a currently discarded keratin-rich side stream from farming activities i.e. **wool** as an alternative **growing media** for crops in greenhouses, and its potential as source of nutrients for microorganisms and plant protection against pathogens.

We aim to answer:

1) Which wool **treatment** is needed to keep plant performance?
2) Does wool let plants **grow** and **protect** against a pathogens?
3) What **microorganisms** are dominating in the wool-based media?
4) Bring together **partners** to further develop the use of wool in horticulture

Why is this interesting scientifically?

Previous work showed the **positive** effect on **yield** in wool-grown plants, compared to other substrates. Furthermore other **keratin-rich** products are protecting plants against **pathogens**. Our study will bring:

1) Knowledge on how wool can provide plant **support** and **protection**
2) Data on **microorganisms** that can grow on this media and which ones could potencially protect plants

How is this relevant to the materials transition?

This project could increase the societal impact in the direction of a renewable carbon-based material i.e. wool transition as:

a) Reduce the environmental **footprint**, by avoiding the burning of the wool
b) Reducing the need for non-recyclable **rockwool** and natural **peat**
c) Creating a **value chain** for wool, sheep farmers could benefit from all the product (wool)

What are the key activities or steps?

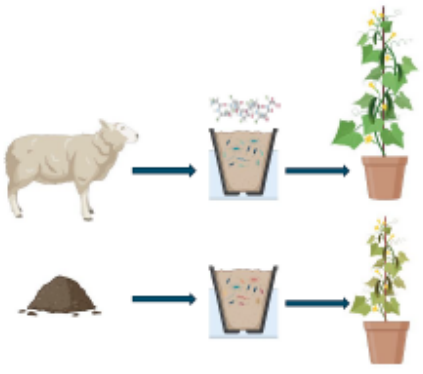
1. Preliminary test of wool physico-chemical properties and potential treatments to apply
2. Test of wool as growing media in the greenhouse
3. Pathogenicity bioassays of wool-media grown plants
4. Microbiome profiling of wool-based growing media

What are key deliverables?

- Data of the physico-chemical properties and treatment of wool based growing media
- Data on plant performance in wool based growing media
- Data on microorganisms that are enriched in wool-based media
- Collaboration with other projects working in a related topic
  - PPS Systematic Approach for Finding Alternatives to Peat Substrates
  - PPS Peat alternatives mushroom & horticulture sectors
  - KB-34 Microbiome connections in the circular production systems

One what issues would you like to get input from others?

Are there other materials that could we tested in a follow up project? Such as keratin/chitin/cellulose/lignocellulose rich side streams



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TRANSFORMATIVE Bioeconomies

BAPS number: KB-45-005-011-WPR

Poster 2: Community Day December 2023

Wool for crop resilience

Beatriz Andreo Jimenez[1], Tess van de Voorde[1], Carin Lombaers[1], Mirjam Schilder[1], Alexander van Tuyll[2], Evelien Maaskant[3], Willem Drost[3], Paulien Harmsen[3]

[1]Biointeractions & Plant Health (WPR), [2]Greenhouse Horticulture & Bulbs (GHB), [3]Biobased products (BBP)

WAGENINGEN UNIVERSITY & RESEARCH

Domain Flagship Textiles. Topic 5: recycling of discarded carbon-based materials.

Objectives

Which wool treatment is needed to keep plant performance? Does wool increase plant performance and reduce disease?
What microorganisms are dominating in the wool-based media? Bring together partners to further develop the use of wool in horticulture

Wool tests on chemical properties and plant performance

Wool fatty acid and nitrogen composition

We quantified the fatty acid and nitrogen content in wool. GC-MS analysis of the unwashed wool shows a broad signal and specific sharp signals that correspond to cholesterol and derivatives, present in wool grease. Washing the wool reduces the intensity of the broad signal, but cholesterol remains (**Figure 1**). The amount of nitrogen in the soil samples was comparable for the samples with 0% and 10% wool (v/v), whereas the sample with 25% wool had a much higher N-content.

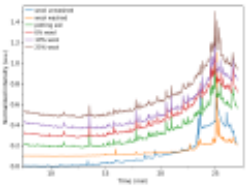

Figure 1. Normalized GC spectra for all samples measured at approximately 5 mg/mL.

Wool as growing media

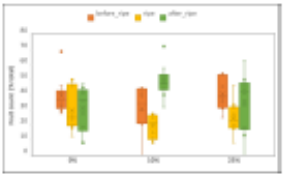

Figure 2. Greenhouse experiment with strawberry plants. We counted strawberry yield before, during and after ripening phase. Fruit count is given in proportion to the total.

We performed an experiment with strawberry plants in the greenhouse using washed wool mixed with coconut fibres and soil as growing media. Results showed that 10% v/v washed wool in the growing media allowed plants to grow, having a higher absolute plant weight, yield and less tip burn. Furthermore, strawberries ripened faster in 10% wool (**Figure 2**). 25% v/v wool substrate affected negatively to the plants.

Wool and disease suppression

We run a bioassay with garden cress and the pathogen *Pythium*, with washed and unwashed wool at two concentrations. Seven days after sowing (DAS) plants growing in wool (no pathogen) showed better photosynthesis efficiency (**Figure 3A**). Infected plants did not show differences in disease spread, all of them were sick. However, four DAS we could notice that seedlings with 0.1% w/w mixed into the soil delayed the disease spread (**Figure 3B**).

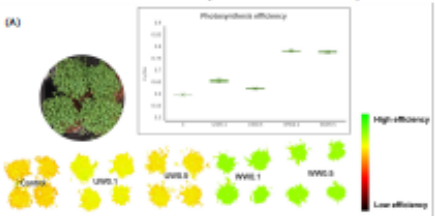

Figure 3. Pathogen bioassay with *Pythium*. A) Photosynthesis efficiency in plants without pathogen. B) Signs of disease in seedlings 5 DAS. WW: washed wool; UW: unwashed wool; 0.5: 0.5% wool w/w; 0.1: 0.1% wool w/w.

Lessons learned

Wool shows potential to be further applied in agriculture and horticulture

- ✓ washing the wool removed most of the fatty acids, and 25% w/w wool soil samples still contained detectable wool grease.
- ✓ wool contained high N from protein, especially the washed wool.
- ✓ Washed wool at 10% v/v helped fruits to ripe faster
- ✓ Washed wool improves photosynthesis efficiency in plants, and at 0.1% w/w seems to delay the disease spread.

Readiness

The project TRL is at around 6, where we are testing the capacity within a controlled environment that reflects the spatial-temporal context (greenhouse). However, some remain questions are unanswered. For example, we need to:

- dive into what microorganisms are associated to the wool, for safety and as biostimulants source (analysis ongoing)
- we need to test what type of wool treatment e.g. wash is the most appropriate to enhance plant productivity and/or reduce disease
- How is soil composition affecting these effects?

Next steps

What steps do we need to further develop wool research for a circular agriculture?

1. Finalize gathering all the data needed
2. Designing new experiments to test the effect of different treatments or wool composition
3. Discuss with stakeholders about our findings and new ideas

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