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# potatoMETAbiome

Harnessing the potato-microbiome interactions for development of sustainable breeding and production strategies

TU Graz

Institute of Environmental Biotechnology
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**Impressum**

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# Vorwort

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Nachhaltige Entwicklungen im Bereich der Landwirtschaft gewinnen zunehmend an Bedeutung. Sie werden in Zukunft viele konventionelle Prozesse, vor allem im Zusammenhang mit dem Anbau von Nutzpflanzen, ersetzen müssen, um die Weltbevölkerung ernähren zu können ohne weiter unsere Umwelt und das Klima negativ zu beeinflussen. Das natürliche Pflanzenmikrobiom bietet die bisher vielversprechendste Basis, um solche Entwicklungen voranzutreiben. Im internationalen sowie interdisziplinären Projekt potatoMETAbiome versuchen wir zum ersten Mal gezielt Wechselwirkungen zwischen unterschiedlichen Kartoffelkultivaren und ihrem spezifischen Mikrobiom zu entschlüsseln, um sie für zukünftige Anwendungen in der Landwirtschaft bereitzustellen.
Prof. Gabriele Berg hat Biologie, Ökologie und Biotechnologie in Rostock und Greifswald studiert und ihr Doktorat 1995 an der Universität Rostock abgeschlossen. Sie erhielt 2003 ein Heisenberg-Stipendium der DFG und 2005 eine Universitätsprofessur an der Technischen Universität Graz wo sie seither am Institut für Umweltbiotechnologie lehrt und forscht. Das unabhängige Analyseinstitut Clarivate Analytics hat sie in den Jahren 2018 sowie 2019 als eine der einflussreichsten Wissenschaftlerinnen im weltweiten Ranking gelistet.

# 1a. Description of the project

Results obtained within the PotatoMETAbiome project contribute to the development of sustainable forms of potato cultivation, currently largely based on high inputs of fertilizers, pesticides, and water, by exploiting the functions of the soil microbiome and enhanced interactions between plants and microbes. In order to achieve sustainable potato cultivation, it is necessary to consider plants as meta-organism: holobionts with its associated microbiome. In the project, important microbial strains were examined, which have a positive effect on the resilience of potato plants to biotic stress. Laboratory experiments were verified in field tests to verify their applicability under real conditions. The evaluation of socio-economic and environmental impacts of the developed strategies was conducted, including potato genotypes selected for their optimal interactions between plants and the microbiome, as well as involving stakeholders during the project further ensure the dissemination and implementation of the strategies. In particular, the development of plants with optimal soil utilization (improved root biomass) and microbiome acquisition will reduce the impact of abiotic stresses such as drought, as well as biotic stresses from pathogens. Additionally, these traits can improve the plant's resource use efficiency through interactions with beneficial microbes. This increase in potato resilience can lead to a reduction in fertilizer and pesticide use, thereby reducing the ecological footprint of potato farmers. For this purpose, selected bacteria were tested as part of this project for their antagonistic effects against the potato pathogen *Rhizoctonia solani* and, together with a booster substance, two consortia were established, which were then tested in the field. The treated plants had significantly fewer disease symptoms and a higher yield of marketable potato tubers. Using modern high-throughput sequencing techniques, changes in the microbiome were identified in microbial communities, including bacteria, fungi and oomycetes. Especially the treatment with the consortium, but also the presence of *R. solani*, can explain part of the microbiome composition. In a follow-up experiment, changes in the microbiome and plant parameters over the life cycle of potato plants stressed with *R. solani*, which were treated either with the consortium "Patricia" or with water, were investigated. The treated plants showed increased stress resilience, plant growth and had larger tubers with fewer disease symptoms. The microbial diversity increased over the course of the life cycle and tended to be higher in treated plants. Overall, we were able to show within the scope of this project, both under controlled conditions and in the open field, that the targeted

treatment of stressed potato plants with the constructed consortia can represent a sustainable and ecological alternative to the methods used up to now.

# 1b. Projektbeschreibung

Die Ergebnisse, die im Rahmen des PotatoMETAbiome-Projekts gewonnen wurden, tragen zur Entwicklung nachhaltiger Formen des Kartoffelanbaus bei, die derzeit weitgehend auf einem hohen Anteil an Düngemitteln, Pestiziden und Wasser beruhen, indem die Funktionen des Bodenmikrobioms und verbesserte Wechselwirkungen zwischen Pflanzen und Mikroben genutzt werden. Um einen nachhaltigen Kartoffelanbau zu ermöglichen, ist es notwendig Pflanzen als Meta-Organismen zu betrachten, die vielerlei Interaktionen mit ihrem Mikrobiom eingehen. Im Projekt wurden wichtige mikrobielle Stämme untersucht, welche sich positiv auf die Resilienz von den Kartoffelpflanzen, bei biotischem Stress, auswirken. Die aus Laborversuchen resultierenden Ergebnisse wurden in Feldversuchen bestätigt, um ihre Einsetzbarkeit unter realen Bedingungen zu verifizieren. Die Bewertung der sozioökonomischen und ökologischen Auswirkungen der entwickelten Strategien, einschließlich der Kartoffelgenotypen, die aufgrund ihrer optimalen Wechselwirkungen zwischen Pflanzen und des Mikrobioms ausgewählt wurden, und die Beteiligung der Interessengruppen während des Projekts konnten die Verbreitung und Umsetzung der Strategien weiters sicherstellen. Insbesondere die Entwicklung von Pflanzen mit optimaler Bodennutzung (verbesserte Wurzelbiomasse) und Mikrobiom-Akquirierung wird die Auswirkungen abiotischer Belastungen wie Trockenheit sowie biotischer Belastungen durch Krankheitserreger verringern. Zusätzlich können diese Eigenschaften die Ressourcennutzungseffizienz der Pflanze durch Wechselwirkungen mit nützlichen Mikroben verbessern. Diese Erhöhung der Widerstandsfähigkeit der Kartoffeln kann zu einem Rückgang des Einsatzes von Düngemitteln und Pestiziden führen, wodurch der ökologische Fußabdruck der Kartoffelproduktion verringert wird. Dafür wurden im Rahmen dieses Projekts ausgewählte Bakterien auf ihre antagonistischen Effekte gegen den Kartoffelpathogen *Rhizoctonia solani* getestet und gemeinsam mit einer Booster-Substanz wurden daraus zwei Konsortien erstellt, die danach im Feld getestet wurden. Die behandelten Pflanzen hatten signifikant weniger Krankheitssymptome und einen höheren Ertrag an marktfähigen Kartoffeln. Durch Einsatz von modernen Hochdurchsatzsequenzierungen wurden Veränderungen im Mikrobiom, sowohl bei Bakterien, Pilzen als auch Oomyceten, festgestellt. Vor allem die Behandlung mit dem Konsortium, aber auch das Vorkommen von *R. solani,* erklären einen Teil der Mikrobiom-Zusammensetzung. In einem Folgeexperiment wurden Veränderungen im Mikrobiom und Pflanzenparameter über den Lebenszyklus von, mit *R. solani* behandelten, Kartoffelpflanzen untersucht, die entweder mit dem Konsortium „Patricia“ oder mit

Wasser behandelt wurden. Die behandelten Pflanzen zeigten eine erhöhte Stressresilienz, verbessertes Pflanzenwachstum und hatten größere Knollen mit weniger Krankheitssymptomen. Die Diversität des Mikrobioms nahm im Laufe des Lebenszyklus zu, und war tendenziell höher bei den behandelten Pflanzen. Insgesamt konnten wir im Rahmen dieses Projekts, sowohl unter kontrollierten Bedingungen, als auch im freien Feld zeigen, dass die gezielte Behandlung von gestressten Kartoffelpflanzen, mit dem konstruierten Konsortien eine nachhaltige und ökologische Alternative zu den bisher angewandten Methoden darstellen kann.

# 2. Introduction

## 2.1 Scientific overview of the project

The European Union spends about 38% of its annual budget on agriculture, supporting an agricultural output worth over €400 Billion per year (Sgueo et al. 2016). Concerning potato production, 53 billion tons of raw potatoes were harvested in the EU in 2015 (Eurostat 2017). Additional to food production, a large number of potatoes harvested in Europe are used in industry for starch production. The majority (98.5%) of these potatoes stem from conventional potato growing.

Current agricultural practices are yet non-sustainable. Potato breeding strategies focus on high productivity, thus modern cultivars may have lost some of the traits needed to recruit host-specific beneficial root microbiota (Sawers et al., 2008) The majority of cultivars has been developed to sustain high yields in production systems with an increased need for external inputs such as chemical pesticides and fertilizers. This circumstance has negative impacts on soil biodiversity and contributes to environmental and food contamination (McCullough and Matson 2012).

Plants harbour a wide diversity of microorganisms that colonize the surface of the roots and the adhering soil that is enriched with specific root exudates (rhizosphere) or inner plant tissues (endophytes). Plants and their associated microbiomes form complex and dynamic mutualistic interactions (Mendes et al. 2011), and can be considered as a meta-organism the so called 'holobiont' (Vandenkoornhuyse et al. 2015). Native as well as crop plants provide ecological niches and easily utilizable carbon to their microbiome. The microbiome is seen as the plant's second genome (Turner et al. 2013), which provides complimentary plant-beneficial functions. It fulfils several substantial functions, particularly the rhizosphere and endophytes compartment, are known to suppress diseases (Mendes et al. 2011), to improve drought resistance, to alter aboveground herbivory (Hol et al. 2010) or even to modify flowering time, highlighting the extent to which the plant microbiome influences the overall fitness of the plant, which can go beyond the traits provided by plant genomes. Plants are able to specifically select their microbiome from the environment. The composition of the plant microbiome is dependent on the soil type, plant species (Berg and Smalla 2009).

The aim of the project potatoMETAbiome is to provide a new strategy for sustainable plant breeding. The ground-breaking concept of this project is based on using a large potato genotype bank (BRC) as basis for the selection of potato varieties that most effectively interact with soil microbes. The project is separated in five work packages. The first work package (WP1) focuses on the project management, dissemination and communication. In WP2 an initial pool of 1400 genotypes available at BRC will be screened in silico for relevant traits. The most promising 200 genotypes will be further tested using in vitro plants in climate chambers experiments for microbial interaction traits (MITs) (WP2). Subsequently, the 50 genotypes with higher MITs will be evaluated in greenhouse experiments in three different locations, where both plant (genome, transcriptome and metabolome) and microbiome aspects will be assessed, thus providing an overview of the functional genomic variations, microbiome and MITs (WP2-4), their higher resource use efficiency (WP3) and resistance to (a)biotic stress (WP4).

Finally, a selection of 10 genotypes will be evaluated further for their performance for the already mentioned functions in field trials in three countries. The data generated in field trails will be used to provide a social, economic and environmental perspective on how the new cultivars with higher MITs and associated biologicals can impact potato cropping, thus supporting sustainable production (WP5). A schematic overview of all project tasks is provided in Figure 1.

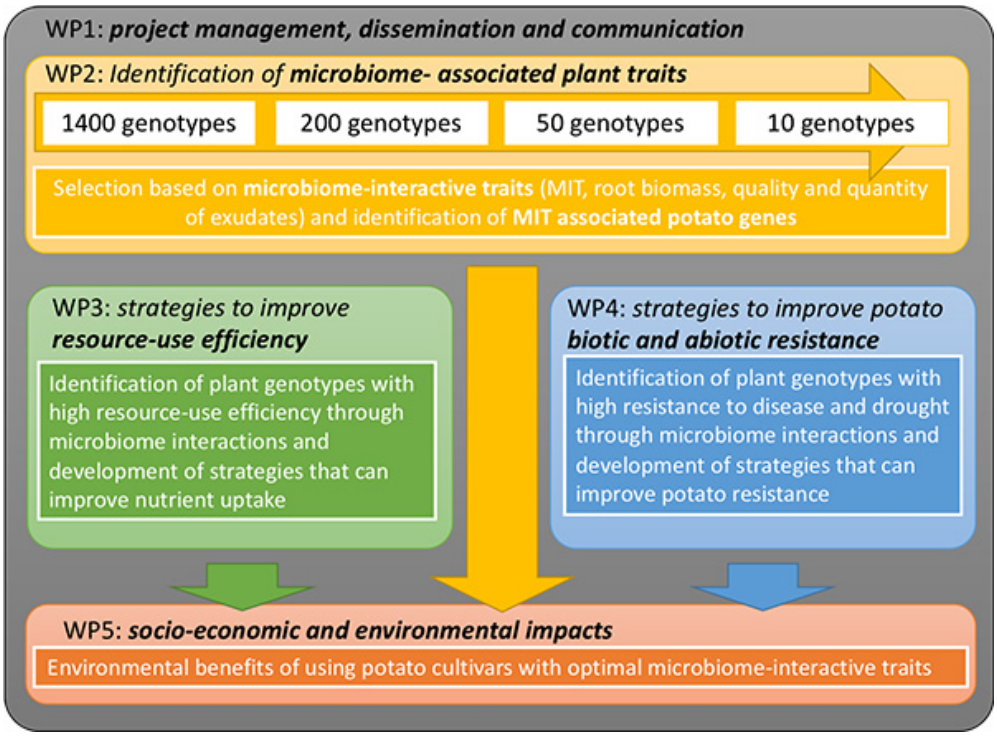



Figure 1: **Project structure outlining different Work Packages (WPs), their operational links and proposed approaches**.

## 2.2 Work Package 4 (biotic stress) – Strategies to improve potato resilience to biotic stress – Lead: Institute of Environmental Biotechnology (TU Graz)

The aim of WP4 is to promote the sustainable use of European arable soils through the development of potato cultivars that are resistant to biotic stress. The implementation of potato cultivars that are resistant to biotic stress will have great economic, environmental, and human health impact through the reduction in the input of pesticides. In this context, the use of microbial biological control agents represents a viable alternative, by promoting the successful suppression of the phytopathogens (*Rhizoctonia solani* and *Verticillium dahliae*) in the rhizosphere or phyllosphere. The efficiency of the biocontrol method depends on the survival of the biocontrol agent in the soil and efficient rhizosphere colonization, the latter being mediated by signal molecules, which are not well understood. Furthermore, the success of the applied bioinoculants also depends on how the introduced organisms interact with the complex biota living in the soil. Thus, regarding biotic stress, WP4 aims at improving promising biologicals with so far unexploited strategies. Specifically, we will com-

bine the use of beneficial consortia with novel formulation technologies to increase the applicability (shelf life) and efficiency (exploitation of microbial Volatile Organic Compounds [mVOC] activation in the strains).

For this approach, 10+1 genotypes will be combined by TU Graz with four-strain microbial consortia in combination with two formulations (consortia and/or formulation + pathogen). Microbial community profiling by amplicon sequencing will enable to assess responses of the indigenous soil microbiota to the introduced inoculate and the fate of the introduced microbial consortia throughout the plant's life cycle. Plant-microbiome interactions will be determined by plant transcriptomic approaches and metabolite analyses of the potato plants.

Subsequently, one of the project partners, the Technical University of Munich, will identify the mechanisms conferring higher biotic and abiotic resistance by performing meta-transcriptomics on the selected 10+1 genotypes, grown under greenhouse conditions, in the presence and absence of pathogens, under no abiotic stress, and in the absence of pathogen but under drought conditions.

By understanding the impact and fate of the introduced biocontrol in the soils and by developing a new method of application that improves efficiency, WP4 will generate knowledge that can be implemented in other biocontrol programs.

It will produce key information (genes, metabolites) on the resistance of the selected genotypes to both biotic and abiotic stress, thus contributing to predictive breeding technologies and development of new potato genotypes selected for improvement in plant health, protection, and resistance.

In summary, WP4 will contribute to a reduction in the environmental footprint of pesticide use in potato cropping by selecting efficient biocontrol microbial consortia that improve the resistance of the selected genotypes to biotic stress (the fungal soil-borne pathogens *V. dahliae* [Verticillium wilt] and *R. solani* [black scurf]), thus supporting the development and exploitation of novel integrated disease methods and practices.

# 3 Results

The main aim of WP 4 is to investigate novel, bio-based strategies to improve potato resilience to biotic stress.

## 3.1 Deliverables

Deliverable 1: Definition of four bacterial consortia with enhanced activity against potato pathogens and two prototype formulations.

Deliverable 2: Impact of inoculation on plant omics and indigenous microbial communities.

Deliverable 3: Report on mechanisms leading to higher resistance to stress.

## 3.2 Procedure for constructing the 4 four-strain bacterial consortia

For constructing the microbial consortia, we selected promising microbial isolates from the strain collection of antagonist microorganisms (SCAM), Institute of Environmental Biotechnology, TU Graz, Austria and screened them in vitro against *Rhizoctonia solani* and *Verticillium dahliae*. Then we selected the 25 most promising isolates, respectively, combined them to consortia and screened them again against the two model pathogens. After that, various natural extracts were added that served as so called 'boosters' for the consortia. Boosters act as positive supporters during establishment of the microorganisms in the new micro-habitat and generally improve the effect of the microbial consortia by provision of specific nutrients or enhancement of their antagonistic activity.

### 3.2.1 Microorganisms selected for the project

In the first step, we selected antagonistic isolates with antagonistic effects against the two model pathogens (*R. solani* and *V. dahliae*) from the 'in house' SCAM library (Fig. 2).

| | | | | | |
|---|---|---|---|---|---|
| *Bacillus amyloliquefaciens 4P2-4* | 1 | *P. putida L22-6-9* | 40 | Promising isolates of tomato A3 | 78 |
| *Bacillus amyloliquefaciens Sc-K143* | 2 | *P. putida L32-6-8* | 41 | Promising isolates of tomato A4 | 79 |
| *Bacillus subtilis Sc-S7* | 3 | *P. putida L11-1-3* | 42 | Promising isolates of tomato A5 | 80 |
| *Bacillus subtilis 1Pe4-13* | 4 | *P. putida L8-6-2* | 43 | Promising isolates of tomato A6 | 81 |
| *Enterobacter intermedius 4Rz1* | 5 | *P. putida L8-6-9* | 44 | Promising isolates of tomato A9 | 82 |
| *Erwinia crysanthemi LC21-3-3* | 6 | *P.putida B 6Kp8* | 45 | Promising isolates of tomato A10 | 83 |
| *Erwinia rhapontici 1Pe1-14* | 7 | *Pseudomonas trivialis 3Re2-7 *23* | 46 | Promising isolates of tomato A11 | 84 |
| *Paenibacillus peoriae 1P1-2* | 8 | *Pseudomonas reactans 3Re2-7* | 47 | Promising isolates of tomato A12 | 85 |
| *Pseudomonas chlororaphis L9-2-5* | 9 | *Serratia fonticola 3Rc3* | 48 | Promising isolates of tomato B7 | 86 |
| *Pseudomonas chlororaphis L30-3-6* | 10 | *Serratia fonticola R6* | 49 | Promising isolates of tomato B8 | 87 |
| *Pseudomonas chlororaphis L30-3-8* | 11 | *Serratia grimesii 4Rz6* | 50 | Promising isolates of tomato B9 | 88 |
| *Pseudomonas chlororaphis L30-3-9* | 12 | *Serratia grimesii 3Rp8* | 51 | Promising isolates of tomato B11 | 89 |
| *Pseudomonas chlororaphis 6R4-28* | 13 | *Serratia grimesii 9Ez29* | 52 | Promising isolates of tomato B12 | 90 |
| *Pseudomonas chlororaphis 6Kp10* | 14 | *Serratia grimesii 9Rz10* | 53 | Promising isolates of tomato C12 | 91 |
| *Pseudomonas corrugata 5Re4-12* | 15 | *Serratia grimesii 9Rz4* | 54 | Promising isolates of tomato D2 | 92 |
| *Pseudomonas corrugata 5Re4-6* | 16 | *Serratia grimesii R15* | 55 | Promising isolates of tomato D3 | 93 |
| *Pseudomonas corrugata 5Re4-21* | 17 | *Serratia grimesii R11* | 56 | Promising isolates of tomato D8 | 94 |
| *Pseudomonas fluorescens 2R1-7* | 18 | *Serratia grimesii L16-3-3* | 57 | Promising isolates of tomato D9 | 95 |
| *Pseudomonas fluorescens 2Re2-6** | 19 | *Serratia grimesii L21-3-10* | 58 | Promising isolates of tomato D10 | 96 |
| *Pseudomonas fluorescens L13-6-12* | 20 | *Serratia odorifera R1* | 59 | Promising isolates of tomato D11 | 97 |
| *P. putida 8Kz4* | 21 | *Serratia odorifera 4Rx13* | 60 | Promising isolates of tomato D12 | 98 |
| *P. putida 8Kz8* | 22 | *Serratia plymuthica 3Re4-18* | 61 | Promising isolates of tomato E1 | 99 |
| *P. putida 4Kc15* | 23 | *Serratia plymuthica HRO-C48 (oilseed rape)* | 62 | Promising isolates of tomato E2 | 100 |
| *P. putida 8Rr24* | 24 | *Serratia proteamaculans 3Rc15* | 63 | Promising isolates of tomato E4 | 101 |
| *P. putida 4Kc13* | 25 | *Streptomyces rochei 5R3-11* | 64 | Promising isolates of tomato E5 | 102 |
| *P. putida 10Kx10/2* | 26 | *Streptomyces setonii RP87* | 65 | Promising isolates of tomato E8 | 103 |
| *P. putida 9Bc8* | 27 | *Trichoderma gamsii AT1-2-4 (sugar beet)* | 66 | Promising isolates of tomato E10 | 104 |
| *P. putida 9Bc3* | 28 | *Trichoderma velutinum G1/8; FN675871* | 67 | Promising isolates of tomato E11 | 105 |
| *P. putida 3R1-19* | 29 | *Xenorhabdus nematophilus R19* | 68 | Promising isolates of tomato E12 | 106 |
| *P. putida 6R5-24* | 30 | Promising isolates of tomato Ale 1 | 69 | Promising isolates of tomato F7 | 107 |
| *P. putida 5Rel-25* | 31 | Promising isolates of tomato Ale 2 | 70 | Promising isolates of tomato F8 | 108 |
| *P. putida 5R2-27* | 32 | Promising isolates of tomato Ale 3 | 71 | *Ralstonia pickettii CE1* | 109 |
| *P. putida 3Re4-21* | 33 | Promising isolates of tomato Ale 4 | 72 | *Ralstonia pickettii CE5* | 110 |
| *P. putida 5Re2-6* | 34 | Promising isolates of tomato Ale 5 | 73 | *Ralstonia pickettii CE11* | 111 |
| *P. putida 5Re2-10* | 35 | Promising isolates of tomato Ale 6 | 74 | Bacillus subtilis Sc-S7 | 112 |
| *P. putida 5Re2-17* | 36 | Promising isolates of tomato Ale 7 | 75 | Bacillus amyloliquefaciens Rs-Ms-87 | 113 |
| *P. putida 5Re2-28* | 37 | Promising isolates of tomato A1 | 76 | Bacillus amyloliquefaciens Sc-K143 | 114 |
| *P. putida L7-2-3* | 38 | Promising isolates of tomato A2 | 77 | Bacillus aerius Rs-Ts-276 | 115 |
| *P. putida L12-5-4* | 39 | | | | |

Figure 2: **List of the selected strains that were obtained from SCAM (Strain collection of antagonist microorganisms, Institute of Environmental Biotechnology, TU Graz, Austria).**

## 3.2.2 Screening of isolates

In total, 115 isolates were screened in triplicates in each assay. The antagonistic effect of soluble metabolites (Dual culture assay 'DCA') (Fig. 3a, b) and volatiles (6-well Two Clamp VOCs Assay 'TCVA' and Petri Dish VOCs Assay 'PDVA') against the pathogens *R. solani* and *V. dahliae* were investigated. During the first assessments, the two pathogen models showed different growth characteristics. Therefore, we had to adjust the set-up of the DCA and VOCs assays, we tested the antagonistic effect of VOCs against the mycelia of *R. solani* and the spores of *V. dahliae* by using the PDVA. For investigating the antagonistic effect against the mycelia of *V. dahliae* we chose the TCVA (Fig 3c,d; Fig 4a,b). For performing the DCA's we tested the antagonistic effect against the mycelia of R. solani and the spores of *V. dahliae*.

Evaluation of the antagonistic effect of the isolates' metabolites via DCA showed inhibition zones between 6 and 10 mm against the mycelia of *R. solani* (Fig. 3a) and between 4 and 13 mm against the spores of *V. dahliae* (Fig. 3b). The antagonistic inhibition rates of VOCs

of the best performing 25 isolates were between 17% and 73% against mycelium formation of *R. solani* (Fig. 6c) and between 7% and 56% (Fig. 6d) against *V. dahliae*.

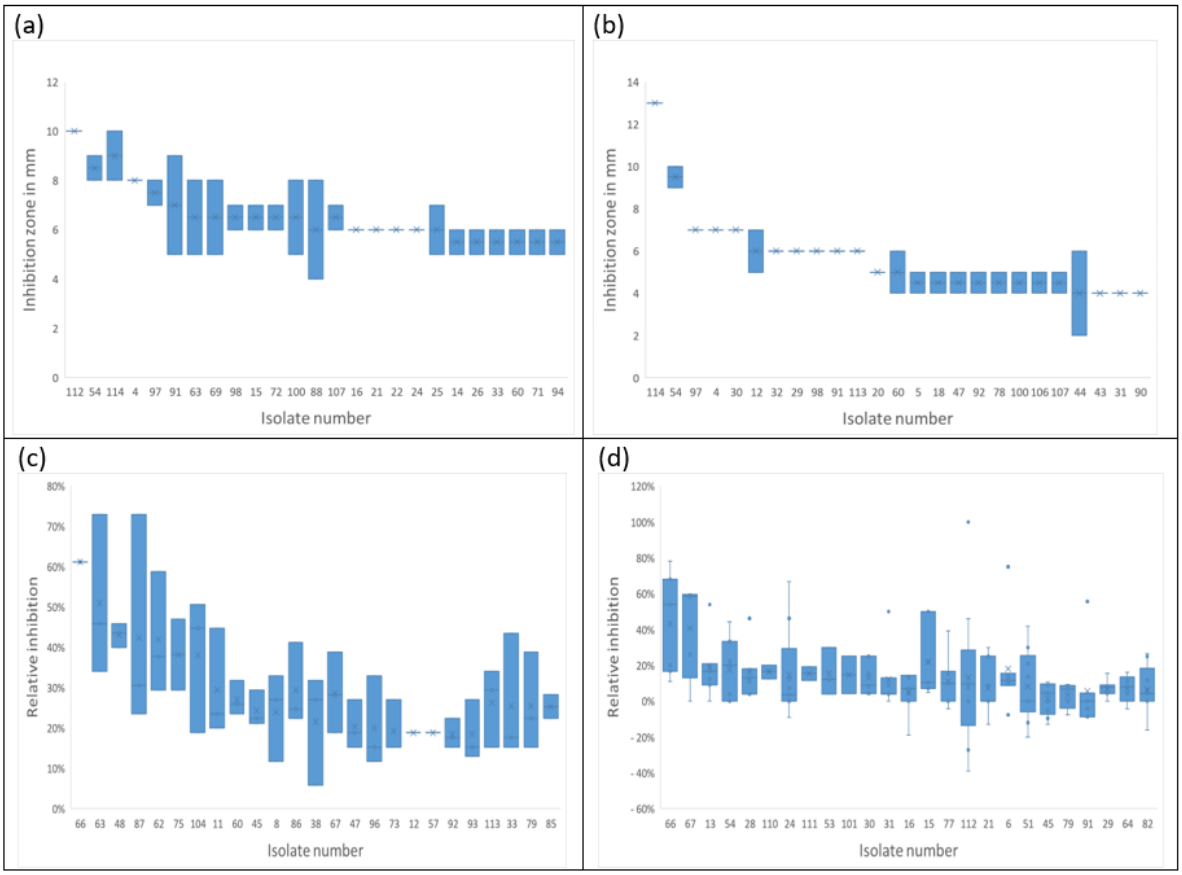



Figure 3: **Antagonistic effect of the metabolites and volatiles of the 25 best performing isolates against fungal pathogens**. (a) Dual culture assay against *R. solani* (mycelia) on Waksman agar. The inhibition zone in mm was measured after the control was overgrown with fungal mycelia. (b) Dual culture assay against *V. dahliae* (mycelia) on Waksman agar. The inhibition zone in mm was measured after the control was overgrown with fungal mycelia. (c) Relative inhibition of volatiles against *R. solani*. The assay was stopped after the fungal mycelia of the control reached the rim of the Petri dish. (d) Relative inhibition of volatiles against *V. dahliae*. (mycelia) The TCVA was stopped after 11 days. All assays were performed in triplicates.

The PDVA assays with the selected isolates against the spores of *V. dahliae* were conducted in triplicates. The inhibition grade per isolate was summarized to a total inhibition grade for the evaluation. The 25 best performing isolates showed inhibition grades between one and five (Fig. 4).

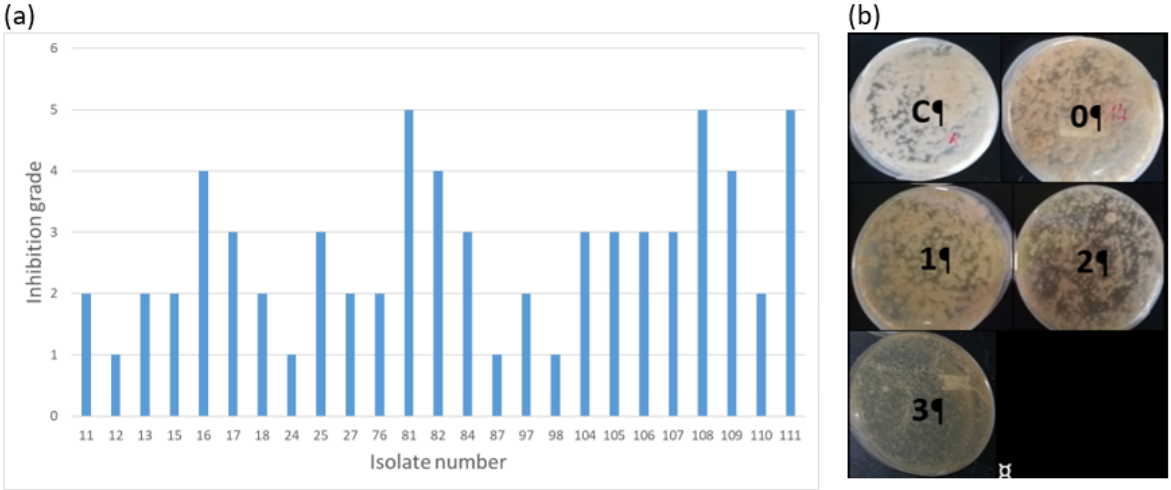



Figure 4: **Antagonistic effect of the volatiles of the 25 best performing isolates against fungal pathogens**. (a) PDVA against *V. dahliae* (spores). The inhibition grade was visually measured after six days of incubation. 5,000 fungal spores per assay were applied. The assay was performed in triplicates. (b) Visuable inhibition grade from 'C' control, '0' no inhibition to '3' very high inhibition. The Inhibition grade 4 for one isolate shows no fungal growth. The sum of the three results per isolate reveals the inhibition grade (maximum inhibition grade is 12).

### 3.2.3 Screening of consortia

The 25 best performing isolates per assay were selected and combined to assemble four strain consortia. The aim of the construction of consortia was to cover the best performing isolates per assay in one consortium (Tab. 1). Therefore, isolates were ranked in each assay and combined (e.g. Isolate 1 was the best performing in DCA *V. dahliae* spores, isolate 2 was the best performing in DCA *R. solani* mycelia and so on). For the other consortia we changed the isolates per assay. The best performing isolate in PDVA was replaced with the second-best performing isolate. With this procedure we covered all antagonistic effects against the two pathogens. Additionally, we reduced the risk of antagonistic effect within the consortia. It is possible, that the best performing isolates show antagonistic effect against them, but the second-, third- or fourth-best isolates are supporting each other.

Table 1: **Example of the procedure that was implemented for consortia combinations.** The aim was to construct the consortia based on the best performing isolates in the five implemented assays.

| **Consortia** | **PDVA** ***R. solani*** **mycelia** | **DCA** ***R. solani*** **mycelia** | **TCVA** ***V. dahliae*** **mycelia** | **PDVA** ***V. dahliae*** **spores** | **DCA** ***V. dahliae*** **spores** |
|---|---|---|---|---|---|
| **Isolate 1** | - | 3 | - | - | 1 |

| | | | | | |
|---|---|---|---|---|---|
| **Isolate 2** | - | 1 | 11 | - | - |
| **Isolate 3** | - | - | 1 | 5 | - |
| **Isolate 4** | 1 | - | - | - | - |

On some occasions it was observed that single microorganisms themselves do not show antagonistic effects, but in combination with other strains they do. Therefore, we decided to also include the five worst isolates in the combination during testing to avoid omitting potential antagonists.

In total 180 different consortia were tested for antagonistic effects against the pathogens (Fig. 5).

| Consortia | Isolates | | | |
|---|---|---|---|---|
| 1 | 114 | 112 | 13 | 66 |
| 2 | 111 | 112 | 13 | 66 |
| 3 | 16 | 112 | 13 | 66 |
| 4 | 30 | 112 | 13 | 66 |
| 5 | 12 | 112 | 13 | 66 |
| 6 | 114 | 4 | 13 | 66 |
| 7 | 114 | 97 | 13 | 66 |
| 8 | 114 | 15 | 13 | 66 |
| 9 | 114 | 16 | 13 | 66 |
| 10 | 114 | 112 | 28 | 66 |
| 11 | 114 | 112 | 24 | 66 |
| 12 | 114 | 112 | 110 | 66 |
| 13 | 114 | 112 | 109 | 66 |
| 14 | 114 | 112 | 84 | 66 |
| 15 | 114 | 112 | 16 | 66 |
| 16 | 114 | 112 | 15 | 66 |
| 17 | 114 | 112 | 13 | 63 |
| 18 | 114 | 112 | 13 | 48 |
| 19 | 114 | 112 | 13 | 87 |
| 20 | 114 | 112 | 13 | 62 |
| 21 | 114 | 112 | 13 | 60 |
| 22 | 114 | 112 | 13 | 47 |
| 23 | 114 | 112 | 13 | 104 |
| 24 | 114 | 112 | 13 | 45 |
| 25 | 114 | 112 | 13 | 86 |
| 26 | 114 | 112 | 13 | 67 |
| 27 | 111 | 63 | 97 | 28 |
| 28 | 114 | 63 | 97 | 28 |
| 29 | 16 | 63 | 97 | 28 |
| 30 | 30 | 63 | 97 | 28 |
| 31 | 109 | 63 | 97 | 28 |
| 32 | 15 | 63 | 97 | 28 |
| 33 | 81 | 63 | 97 | 28 |
| 34 | 108 | 63 | 97 | 28 |
| 35 | 104 | 63 | 97 | 28 |
| 36 | 110 | 63 | 97 | 28 |
| 37 | 111 | 66 | 97 | 28 |
| 38 | 111 | 48 | 97 | 28 |
| 39 | 111 | 87 | 97 | 28 |
| 40 | 111 | 62 | 97 | 28 |
| 41 | 111 | 60 | 97 | 28 |
| 42 | 111 | 47 | 97 | 28 |
| 43 | 111 | 104 | 97 | 28 |
| 44 | 111 | 86 | 97 | 28 |
| 45 | 111 | 67 | 97 | 28 |
| 46 | 111 | 63 | 4 | 28 |
| 47 | 111 | 63 | 112 | 28 |
| 48 | 111 | 63 | 12 | 28 |
| 49 | 111 | 63 | 31 | 28 |
| 50 | 111 | 63 | 30 | 28 |
| 51 | 111 | 63 | 97 | 13 |
| 52 | 111 | 63 | 97 | 24 |
| 53 | 111 | 63 | 97 | 67 |
| 54 | 111 | 63 | 97 | 15 |
| 55 | 111 | 63 | 97 | 45 |
| 56 | 111 | 63 | 97 | 110 |
| 57 | 4 | 16 | 24 | 48 |
| 58 | 97 | 16 | 24 | 48 |
| 59 | 112 | 16 | 24 | 48 |
| 60 | 12 | 16 | 24 | 48 |
| 61 | 4 | 111 | 24 | 48 |
| 62 | 4 | 114 | 24 | 48 |
| 63 | 4 | 30 | 24 | 48 |
| 64 | 4 | 109 | 24 | 48 |
| 65 | 4 | 15 | 24 | 48 |
| 66 | 4 | 81 | 24 | 48 |
| 67 | 4 | 108 | 24 | 48 |
| 68 | 4 | 104 | 24 | 48 |
| 69 | 4 | 110 | 24 | 48 |
| 70 | 4 | 16 | 13 | 48 |
| 71 | 4 | 16 | 28 | 48 |
| 72 | 4 | 16 | 67 | 48 |
| 73 | 4 | 16 | 109 | 48 |
| 74 | 4 | 16 | 31 | 48 |
| 75 | 4 | 16 | 30 | 48 |
| 76 | 4 | 16 | 15 | 48 |
| 77 | 4 | 16 | 112 | 48 |
| 78 | 4 | 16 | 110 | 48 |
| 79 | 4 | 16 | 24 | 66 |
| 80 | 4 | 16 | 24 | 63 |
| 81 | 4 | 16 | 24 | 87 |
| 82 | 4 | 16 | 24 | 62 |
| 83 | 4 | 16 | 24 | 60 |
| 84 | 4 | 16 | 24 | 47 |
| 85 | 4 | 16 | 24 | 104 |
| 86 | 4 | 16 | 24 | 45 |
| 87 | 4 | 16 | 24 | 86 |
| 88 | 4 | 16 | 24 | 67 |
| 89 | 4 | 16 | 24 | 45 |
| 90 | 4 | 16 | 24 | 109 |
| 91 | 30 | 87 | 15 | 67 |
| 92 | 16 | 87 | 15 | 67 |
| 93 | 111 | 87 | 15 | 67 |
| 94 | 114 | 87 | 15 | 67 |
| 95 | 12 | 87 | 15 | 67 |
| 96 | 30 | 66 | 15 | 67 |
| 97 | 30 | 63 | 15 | 67 |
| 98 | 30 | 48 | 15 | 67 |
| 99 | 30 | 62 | 15 | 67 |
| 100 | 30 | 60 | 15 | 67 |
| 101 | 30 | 47 | 15 | 67 |
| 102 | 30 | 104 | 15 | 67 |
| 103 | 30 | 86 | 15 | 67 |
| 104 | 30 | 45 | 15 | 67 |
| 105 | 30 | 109 | 15 | 67 |
| 106 | 30 | 87 | 4 | 67 |
| 107 | 30 | 87 | 112 | 67 |
| 108 | 30 | 87 | 109 | 67 |
| 109 | 30 | 87 | 16 | 67 |
| 110 | 30 | 87 | 15 | 13 |
| 111 | 30 | 87 | 15 | 24 |
| 112 | 30 | 87 | 15 | 28 |
| 113 | 30 | 87 | 15 | 45 |
| 114 | 30 | 87 | 15 | 110 |
| 115 | 6 | 109 | 101 | 71 |
| 116 | 15 | 109 | 101 | 71 |
| 117 | 94 | 109 | 101 | 71 |
| 118 | 98 | 109 | 101 | 71 |
| 119 | 6 | 39 | 101 | 71 |
| 120 | 6 | 13 | 101 | 71 |
| 121 | 6 | 109 | 101 | 71 |
| 122 | 6 | 109 | 115 | 71 |
| 123 | 6 | 109 | 111 | 71 |
| 124 | 6 | 109 | 101 | 36 |
| 125 | 6 | 109 | 101 | 86 |
| 126 | 6 | 109 | 101 | 12 |
| 127 | 15 | 7 | 102 | 36 |
| 128 | 6 | 7 | 102 | 36 |
| 129 | 94 | 7 | 102 | 36 |
| 130 | 98 | 7 | 102 | 36 |
| 131 | 15 | 109 | 102 | 36 |
| 132 | 15 | 39 | 102 | 36 |
| 133 | 15 | 13 | 102 | 36 |
| 134 | 15 | 7 | 115 | 36 |
| 135 | 15 | 7 | 111 | 36 |
| 136 | 15 | 7 | 102 | 71 |
| 137 | 15 | 7 | 102 | 86 |
| 138 | 15 | 7 | 102 | 12 |
| 139 | 94 | 39 | 115 | 86 |
| 140 | 15 | 39 | 115 | 86 |
| 141 | 6 | 39 | 115 | 86 |
| 142 | 98 | 39 | 115 | 86 |
| 143 | 94 | 101 | 115 | 86 |
| 144 | 94 | 109 | 115 | 86 |
| 145 | 94 | 13 | 115 | 86 |
| 146 | 94 | 39 | 102 | 86 |
| 147 | 94 | 39 | 101 | 86 |
| 148 | 94 | 39 | 111 | 86 |
| 149 | 94 | 39 | 115 | 36 |
| 150 | 94 | 39 | 115 | 71 |
| 151 | 94 | 39 | 115 | 12 |
| 152 | 98 | 13 | 111 | 12 |
| 153 | 15 | 13 | 111 | 12 |
| 154 | 94 | 13 | 111 | 12 |
| 155 | 6 | 13 | 111 | 12 |
| 156 | 98 | 101 | 111 | 12 |
| 157 | 98 | 39 | 111 | 12 |
| 158 | 98 | 109 | 111 | 12 |
| 159 | 98 | 13 | 102 | 12 |
| 160 | 98 | 13 | 115 | 12 |
| 161 | 98 | 13 | 101 | 12 |
| 162 | 98 | 13 | 111 | 36 |
| 163 | 98 | 13 | 111 | 86 |
| 164 | 98 | 13 | 111 | 71 |
| 165 | 6 | 7 | 101 | 71 |
| 166 | 94 | 7 | 115 | 86 |
| 167 | 98 | 7 | 111 | 12 |
| 168 | 15 | 7 | 101 | 71 |
| 169 | 111 | 62 | 31 | 28 |
| 170 | 111 | 62 | 97 | 67 |
| 171 | 111 | 62 | 97 | 110 |
| 172 | 66 | 63 | 67 | 46 |
| 173 | 48 | 63 | 46 | 13 |
| 174 | 48 | 46 | 13 | 28 |
| 175 | 63 | 62 | 46 | 13 |
| 176 | 63 | 46 | 13 | 28 |
| 177 | 114 | 16 | 13 | 8 |
| 178 | 114 | 16 | 13 | 11 |
| 179 | 4 | 16 | 30 | 8 |
| 180 | 4 | 16 | 30 | 11 |

Figure 5: **List of consortia including the isolate numbers that were included in the four-membered strain assemblies.**

For investigation of these consortia, the same five assays were used (Fig. 6a-d). The TCVA consists of two plates, one containing the consortia, the other the pathogen, and they are fixed with two clamps. A perforated silicon foil with holes separates the two plates. Thus, chambers are formed, where the volatiles produced by the consortia can interact with the fungus (Fig. 6a). Same interaction is shown through the PDVA. Herein, two Petri dishes are faced to each other. One is holding the fungal mycelia and the other contains the consortia. The PDVA is sealed with a Parafilm (Fig. 6b). In the DCA the four strains of the consortia are streaked out and tested against fungal mycelia of *R. solani* (Fig. 6c) or spores of *V. dahliae* (Fig. 6d).

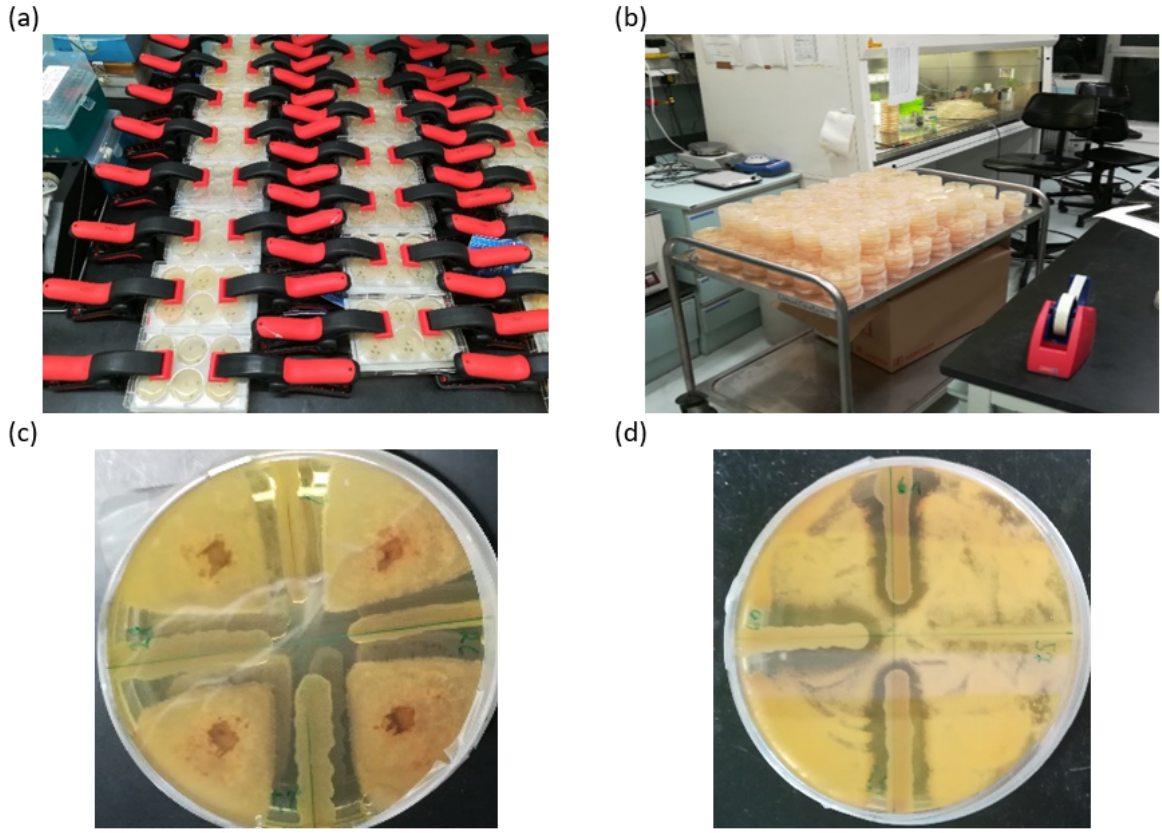



Figure 6: **The assays used for investigating antagonistic effects of consortia against fungal pathogens.** (a) TCVA against *V. dahliae* (mycelia). (b) PDVA against fungal mycelia of *R. solani* and spores of *V. dahliae*. (c) DCA against fungal mycelia of *R. solani*. (d) DCA against fungal spores of *V. dahliae*.

In addition, it was assessed if there is a substantial difference between streaking the consortia and homogenizing them with defined amounts of liquid buffer per isolate against *R. solani*. For the design of the consortia, 250 µl per isolate with an $OD_{600}$ of 0.8 were mixed to a total amount of 1,000 µl. Differences between these two assays were seen. The first divergence was the different antagonistic effect against *R. solani*. The PDVAs with the mixed consortia showed lower inhibition rates (Fig. 7b, Fig. 8), than the PDVAs with the streaked isolates. Additionally, some of the PDVAs with the streaked isolates illustrated an uneven inhibition (Fig. 7a). Based on the uneven growth we assumed, that some microorganisms of the consortia metabolize one or more volatiles of their consortium-partners. This would explain the lower antagonistic effect of the mixed consortia.

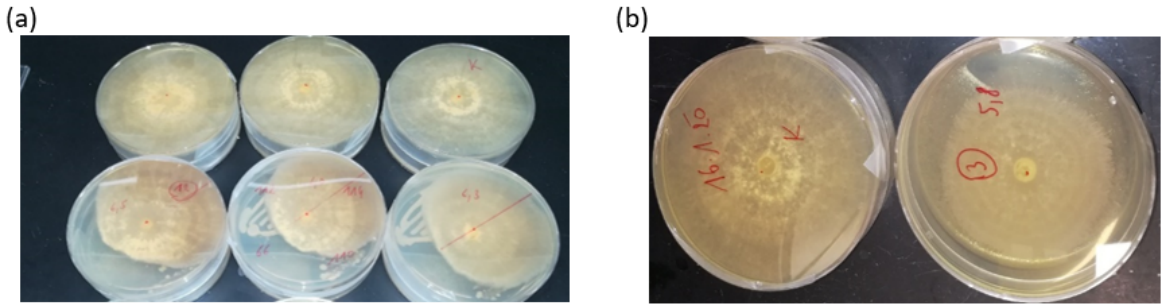



Figure 7: **Antagonistic effects of consortia against fungal pathogens.** (a) PDVA against *R. solani* (mycelia) whereby the isolates of the consortia are streaked out separately. (b) PDVA against fungal mycelia of *R. solani* by using consortia with mixed isolates. The same amount of liquid per isolate is used for combining the consortia.

As mentioned before, the consortia were assessed with PDVA and TCVA experiments to check the influence of volatiles on the growth of the pathogens. The 15 best performing consortia were selected for further analysis. The growth rates of *R. solani* were between 38% and 79% of the PDVA with the streaked consortia and between 74% and 97% of the PDVA with the mixed consortia. For the PDVA with the streaked consortia the best performing consortia was number 96 with an inhibition rate of 62% and for the second PDVA the consortia number 3 with an inhibition rate of 26%. The growth rates of the best performing consortia against the mycelia of *V. dahliae* were between 78% and 103%. Herein, even the consortium number 3 was the best performing with an inhibition rate of 22%. To determine the growth rate against the spores of *V. dahliae*, we prepared for this visual evaluation a scale from 12 (no inhibition; 100% growth) to 0 (maximum inhibition; 0% growth). Growth rates between 25% and 84% were observed. The best performing consortia were 110 and 163, the latter with an inhibition rate of 75% (Fig 8).

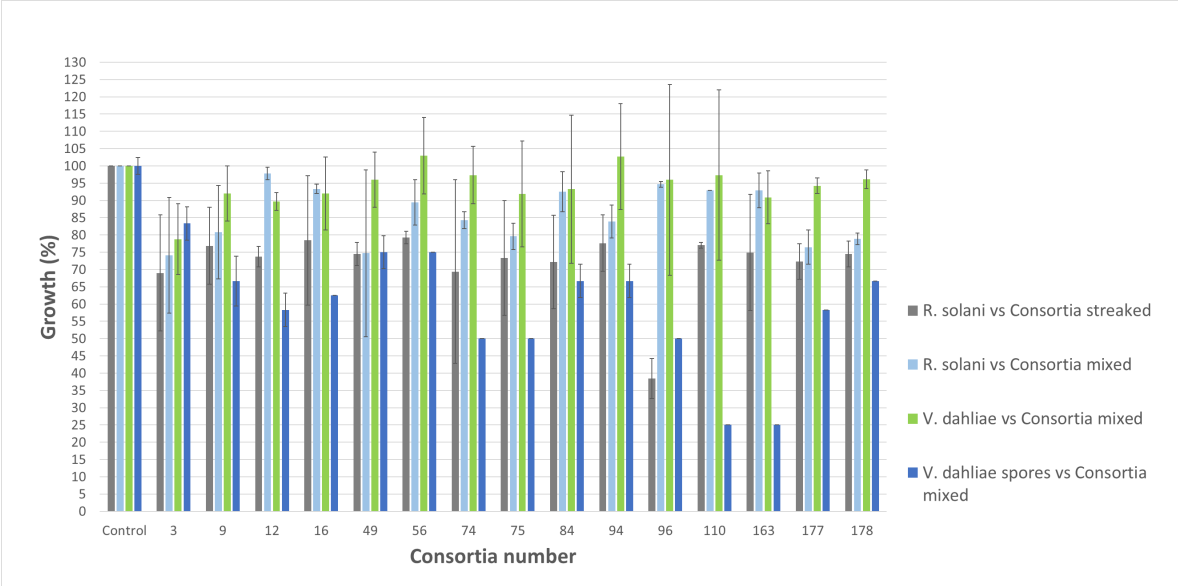



Figure 8: **Antagonistic effects of consortia against fungal pathogens.** (grey) PDVA against *R. solani* (mycelia) whereby the isolates of the consortia are streaked out separately. (azure) PDVA against fungal mycelia of *R. solani* by using consortia with mixed isolates. The same amount of liquid per isolate is used for combining the consortia. (green) TCVA against fungal mycelia of *V. dahliae* by using consortia with mixed isolates. (blue) PDVA against fungal spores of *V. dahliae* by using consortia with mixed isolates. The growth rate of the fungal mycelia or spores is shown. The sample size was n=3.

Following the evaluation of the growth rate of the fungi through volatile interaction, a DCA was performed to check if the isolates fit together *in vitro*. Therefore, the isolates were streaked onto a Waksman agar plate including a fungal plaque of *R. solani* or a spore suspension of *V. dahliae*. If the bacteria were overgrown with the pathogenic fungus (#) the microorganisms of the consortia were classified as not compatible in vitro. If the *Trichoderma* isolate overgrew the whole plate (*), the bacteria of the consortia supported this beneficial fungus against the pathogen. Some isolate came into contact with the pathogen (0), but were not killed through the pathogen or inhibited the pathogen. Other isolates of the consortia showed clear inhibition zones up to 10 mm (3) (Tab. 2). Through combining the results of the VOCs assays and the Dual culture assays, we selected consortia number 3, 9, 75, 96, 177 and 178 for further experiments. For the selection of the consortia we first focussed on the result of the VOCs assays. Furthermore, we wanted to increase the chance of getting positive results in further experiments. Therefore, we did not implement consortia 74 and 75 together, because they were of a too similar composition and displayed similar effects. Consortium 163 is a consortium composed of four of the worst isolates. It displayed positive antagonistic effects of volatiles, but the DCA approaches of this consortium showed negative influences between the isolates. Thus, we did not use this consortium for the next

experiments. Considering that isolates which show negative influences to each other *in vitro*, could affect together positive *in vivo,* we keep the non-selected consortia as backup.

Table 2: **Dual culture assay of the consortia**. Example of the procedure of consortia combination. The isolates were streaked onto a Waksman agar plate including a fungal plaque of *R. solani* or a spore suspension of *V. dahliae*. Subsequent criteria were selected: # = bacteria are overgrown with the fungus; x = contaminated; 0 = come in contact with bacteria; 1 = 0-3 mm; 2 = 4-6mm; 3= >6 mm; *=Trichoderma overgrew the plate.

| **Consortia** | ***R. solani* (mycelia)** | | ***V. dahliae* (spores)** | |
|---|---|---|---|---|
| | **a** | **b** | **a** | **b** |
| **3** | 2/*/*/* | 2/*/*/* | 0/1/0/* | 0/1/0/* |
| **9** | 3/*/*/* | 3/*/*/* | 2/0/0/* | 2/0/0/* |
| **12** | x | 3/1/#/3 | 3/2/#/* | 2/3/#* |
| **16** | 3/1/*/* | */1/*/* | 2/2/2/* | 3/2/2/* |
| **49** | #/2/0/2 | #/2/0/1 | #/2/2/0 | #/2/2/0 |
| **56** | #/2/2/# | #/2/2/# | #/2/2/# | #/2/3/# |
| **74** | 2/2/#/1 | 1/1/#/2 | 3/0/2/2 | 3/0/2/2 |
| **75** | 2/1/#/1 | 2/1/#/1 | 3/0/0/2 | 3/0/0/2 |
| **84** | 2/2/2/0 | 2/2/1/0 | 3/0/0/2 | 3/0/0/2 |
| **94** | 3/*/*/* | 3/3/2/3 | 3/1/0/3 | 3/1/0/3 |
| **96** | */*/*/* | */*/*/* | 2/*/0/3 | 2/*/0/3 |
| **110** | 0/2/2/# | 0/1/2/# | 2/2/0/0 | 2/1/0/0 |
| **163** | 2/0/#/1 | 2/0/#/2 | 3/0/#/1 | 3/0/#/1 |
| **177** | 3/0/0/1 | 3/0/0/1 | 3/0/0/1 | 2/0/0/1 |
| **178** | 3/0/0/1 | 3/0/0/1 | 3/0/0/1 | 3/0/0/1 |

### 3.2.4 Screening of consortia including a booster substance

In the first step, the boosters were prepared. Seven extracts were prepared from house compost, commercial bio compost, coffee ground, fresh horse dung, one-year-old horse dung, two-year-old horse dung and from potato shoots. For this approach, 1 ml $dH_2O$ per

100 mg sample were filled in 50 ml falcon tubes. The extracts were sterilized in the autoclave at 121°C for 20 minutes, then centrifuged for 20 minutes at 5,000 g and sterilized through a filter 0.20 µm. For the screening additional a commercial fertilizer and a bioactive alkyl-pyrazine (2-ethyl-pyrazine: 5-isobutyl-2,3-dimethylpyrazine; 1:1) were used. Subsequently, PDVA-based assessments against the mycelia of *R. solani* were conducted. Here, 1% of the extracts or commercial fertilizer were added to the consortia 3, 9, 75, 96, 177 and 178. For the alkyl-pyrazine, 0.3% was added to the consortium mix. After mixing the consortia with the boosters, the PDVA was prepared. For the consortia 3 and 9 no booster displayed a better effect *in vitro*. In contrast, the consortia 75, 96 and 178 increased their inhibition rate by the addition of pyrazine. Additionally, consortium 96 displayed better effects with bio-compost and commercial fertilizer as booster, and consortium 177 showed better inhibition by addition of bio-compost. The highest antagonistic effect was caused by adding pyrazine to consortium 75. The inhibition rate was increased for 15 percentage points (Fig 9).

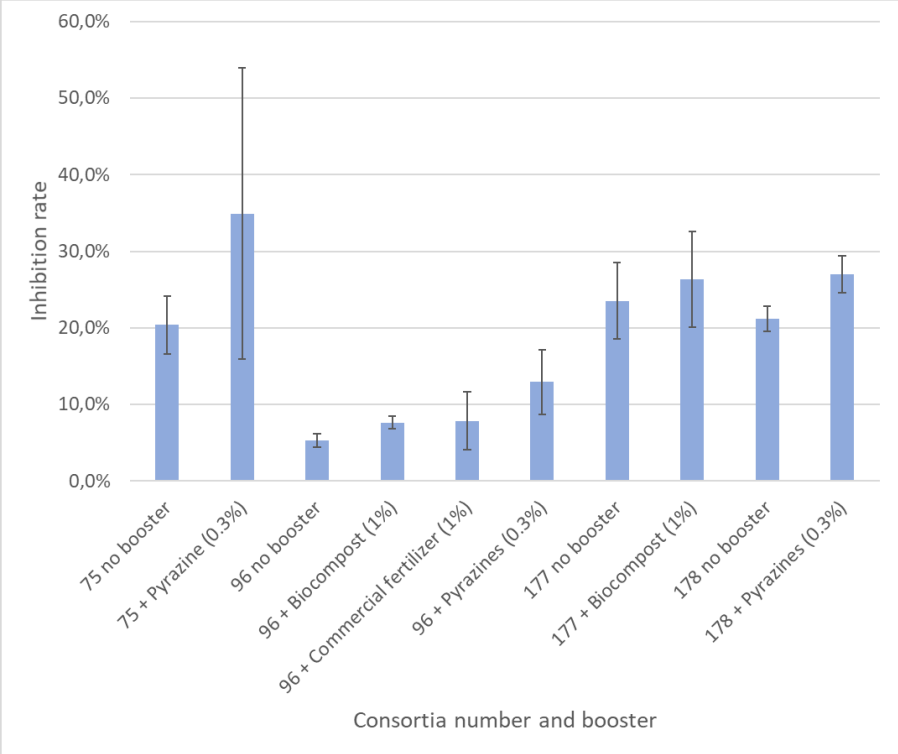



Figure 9: **Antagonistic effects of microbial consortia and consortia supplemented with different boosters against** *R. solani* **(mycelia).** Consortia number 75, 96, 177 and 178 displayed higher inhibition rates by adding booster. The sample size was n=3

### 3.2.5 Narrowing the formulation by performing greenhouse trials

For the pre-greenhouse-trial the potato breed 'SOLO' was used and challenged with *V. dahliae*. Different formulations were used for the first assessments. We tested the consortia that included boosters and the consortia without boosters but with different compositions. For the approach of the four bacterial strains in phosphate buffer 1 ml in total, 250 µl per isolate was used. In contrast, for the approach of the same number of cells per isolate were used, we counted the cells and adjusted them to the isolate with the lowest amounts of cells. As additional booster of *Trichoderma*, the fungus was incubated in coffee ground or millet for at least 22 days (Fig 10). These *Trichoderma* boosters were added in the same way as the Verticillium pathogen system. Five beads of millet overgrown with fungi were placed approximately 2 cm above the potato tuber. In the case of the coffee ground booster the size of the coffee ground added were the same as the millet beads. In addition to the synthetic consortia, we tested a natural consortium consisting of earthworm dung (Tab. 3).

Figure 10: **Preparation of a** *Trichoderma* **booster.** *T. gamsii* AT-1-2-4 was incubated on sterile millet for at least 22 days.

Table 3: P**re-greenhouse-trial – List of consortia including their formulation.** Potato breed 'SOLO' was investigated against *V. dahliae.*

| Consortia | Formulation |
|---|---|
| **A** | Control |
| **B** | Control + *V. dahliae* |
| **C** | Consortia 3 Four bacterial strains in phosphate buffer + *V. dahliae* |
| **D** | Consortia 3 Same number of cells per isolate + *V. dahliae* |
| **E** | Consortia 3 Four bacterial strains in phosphate buffer + coffee booster + *V. dahliae* |
| **F** | Consortia 3 Four bacterial strains in phosphate buffer + millet booster + *V. dahliae* |

| | |
|---|---|
| **G** | Consortia 75 Four bacterial strains in phosphate buffer + *V. dahliae* |
| **H** | Consortia 75 Same number of cells per isolate + *V. dahliae* |
| **I** | Consortia 75 Four bacterial strains in phosphate buffer + pyrazine (0.3%) booster + *V. dahliae* |
| **J** | Consortia 96 Four bacterial strains in phosphate buffer + *V. dahliae* |
| **K** | Consortia 96 Same number of cells per isolate + *V. dahliae* |
| **L** | Consortia 96 Four bacterial strains in phosphate buffer + coffee booster + *V. dahliae* |
| **M** | Consortia 96 Four bacterial strains in phosphate buffer + millet booster + *V. dahliae* |
| **N** | Consortia 177 Four bacterial strains in phosphate buffer + *V. dahliae* |
| **O** | Consortia 177 Same number of cells per isolate + *V. dahliae* |
| **P** | Consortia 177 Four bacterial strains in phosphate buffer + bio-compost extract (1.0%) booster + *V. dahliae* |
| **Q** | Consortia 178 Four bacterial strains in phosphate buffer + *V. dahliae* |
| **R** | Consortia 178 Same number of cells per isolate + *V. dahliae* |
| **S** | Consortia 178 Four bacterial strains in phosphate buffer + pyrazine (0.3%) booster + *V. dahliae* |
| **T** | Vermi-compost (natural consortium) + *V. dahliae* |

For the analysis of the pre-greenhouse-trials, the number of shoots was assessed and their length measured. Five pots for each consortium were planted. Interestingly, the potato plants that were not challenged with the pathogens showed lower amounts of shoots and a lower shoot length when compared to the potato plants with the pathogen. Thus, the total biomass of the infected potato plant was higher, than the biomass of the uninfected potato plant. The potato plants inoculated with consortia displayed different effects on the biomass. The highest amount of biomass was investigated following inoculation with a natural consortium. Only consortium 178, where the four bacterial strains in phosphate buffer were used for inoculation, had more shoots than the control infected with *Verticillium*. Consortium 3 including the coffee ground booster and consortium 177, when using the same number of cells per isolate, resulted in the same number of shoots. All other consortia formulations resulted in comparatively lower numbers (Fig. 11a). Consortia 3, 96 including the millet booster, consortia 75, 178 with the four bacterial strains in phosphate buffer used, consortium 96 with the same number of cells per isolate used and consortia 177, 178 including the boosters had longer shoots in average (Fig. 11b). In summary, for potatoes inoculated with the natural consortia the highest biomass was observed. In the case of the

synthetic consortia, even positive effects on biomass were investigated. Particularly, consortia including boosters and consortia combined with the four bacterial strains in phosphate buffer, displayed positive effects on biomass.

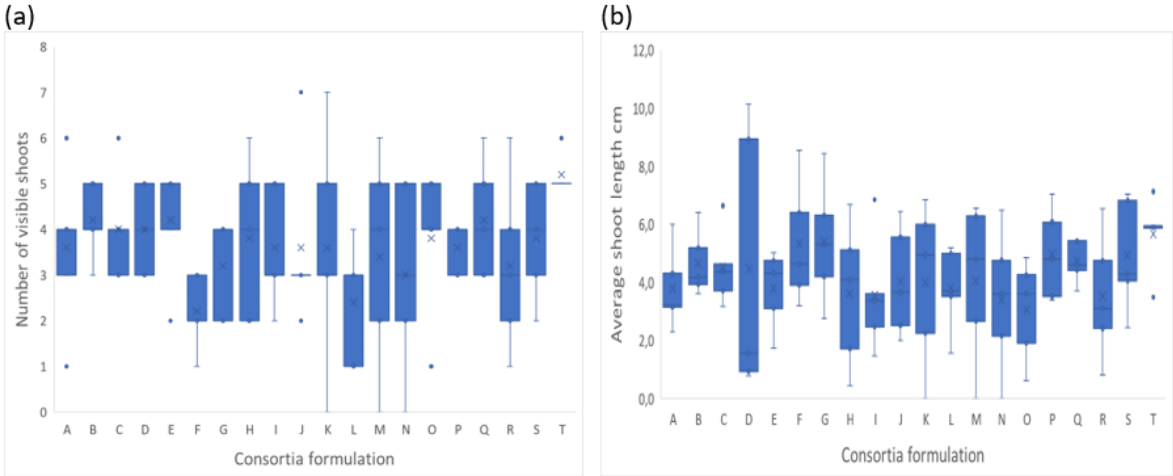



Figure 11: **Evaluation of antagonistic effects of consortia formulations against** *V. dahliae* **in the greenhouse.** *V. dahliae* was grown on millet beads for at least 22 days. The potato cultivar 'SOLO' was investigated for the interaction approach with the consortia for mulations. The sample size was n=5.

After harvesting the potato plantlets no direct correlation between positive effects on biomass (number of shoots, shoot length) and wilt disease or loss of shoots was observed, whereby a direct correlation of robustness of shoots and the wilt disease was found (Tab. 4). If the shoots were generally robust, no wilt disease was monitored. This effect was observed for consortium 75 with the four bacterial strains in phosphate buffer and consortium 96 including the coffee booster. For consortium 3 including the millet booster two plants out of five were robust, one plant displayed normal growth and two plants revealed wilt disease. It can be assumed that this consortium is unstable under the tested conditions. Thus consortium 3 was implemented in greenhouse trials against *R. solani*. Additionally, consortia 96 and 178 with four bacterial strains in phosphate buffer, consortia 75 and 178 including the pyrazine buffer and the natural consortia, no plant or only one plant revealed visual symptoms of wilt disease (Tab. 4). These consortia were additionally used for the subsequent greenhouse trial. Formulation K containing consortium 96 using the same number of cells per isolate was removed from further experiments, because treated potato shoots changed their colour to wine-red (Fig. 12).

Table 4: **Pre-greenhouse-trial – Consortia applied to potato tubers for protecting against** *V. dahliae.* Potato breed 'SOLO' was infected with *V. dahliae.* The number of plants displaying wilt disease or lost shoots, and robust plants were counted. The sample size was n=5.

| Consortium | Wilt/Shoot Loss | Robustness |
|---|---|---|
| **A** | 0 | 0 |
| **B** | 3 | 0 |
| **C** | 2 | 0 |
| **D** | 3 | 0 |
| **E** | 4 | 1 |
| **F** | 2 | 2 |
| **G** | 0 | 3 |
| **H** | 4 | 0 |
| **I** | 0 | 0 |
| **J** | 1 | 0 |
| **K** | 1 | 0 |
| **L** | 0 | 3 |
| **M** | 3 | 0 |
| **N** | 3 | 0 |
| **O** | 2 | 1 |
| **P** | 3 | 0 |
| **Q** | 1 | 1 |
| **R** | 1 | 0 |
| **S** | 0 | 0 |
| **T** | 0 | 0 |

Figure 12**: Evaluation of antagonistic effects of consortium formulation K against** *V. dahliae* **in the greenhouse**. The potato cultivar 'SOLO' was investigated for the interaction approach with the consortium formulation K. Potatoes inoculated with formulation K, containing consortium 96 using the same number of cells per isolate, displayed a wine-red colour.

### 3.2.6 Assessment of the formulations in greenhouse trials

For the pre-greenhouse-trial the potato breed 'Arkula' was used and challenged with *R. solani*. Arkula is known for being susceptible to *R. solani* infections. The best performing formulations of the first assessment were implemented in the second assessment. For the four bacterial strains in phosphate buffer 250 µl inoculum per isolate was used (1 ml in total). As additional booster of *Trichoderma*, the fungus was incubated in coffee ground or millet for at least 22 days. Five beads of millet overgrown with fungi were placed approximately 2 cm above the potato tuber. In the case of the coffee ground booster, the size of the coffee ground added were the same as the millet beads. The pyrazine booster was added as a liquid to the consortia with the concentration of 0.3% v/v. In addition to the synthetic consortia, we tested a natural consortium consisting of earthworm dung (Tab. 5). The pathogen system of *R. solani* was prepared in analogy to the *Trichoderma* booster; however, barley grains were used instead of millet.

Table 5: **Pre-greenhouse-trial – List of consortia including their formulation**. Potato breed 'Arkula' was investigated against *R. solani.*

| Consortia | Formulation |
|---|---|

| | |
|---|---|
| **A** | Control |
| **B** | Control + *R. solani* |
| **F** | Consortia 3 Four bacterial strains in phosphate buffer + millet booster + *R. solani* |
| **G** | Consortia 75 Four bacterial strains in phosphate buffer + *R. solani* |
| **I** | Consortia 75 Four bacterial strains in phosphate buffer + pyrazine (0.3%) booster + *R. solani* |
| **J** | Consortia 96 Four bacterial strains in phosphate buffer + *R. solani* |
| **L** | Consortia 96 Four bacterial strains in phosphate buffer + coffee booster + *R. solani* |
| **Q** | Consortia 178 Four bacterial strains in phosphate buffer + *R. solani* |
| **S** | Consortia 178 Four bacterial strains in phosphate buffer + pyrazine (0.3%) booster + *R. solani* |
| **T** | Vermi-compost (natural consortium) + *R. solani* |

For the analysis of the pre-greenhouse-trials, the number of shoots was assessed and their length measured. Additionally, an infection factor was used for the evaluation, it ranged from the lowest (1) to the highest infection score (4) (Fig. 13).

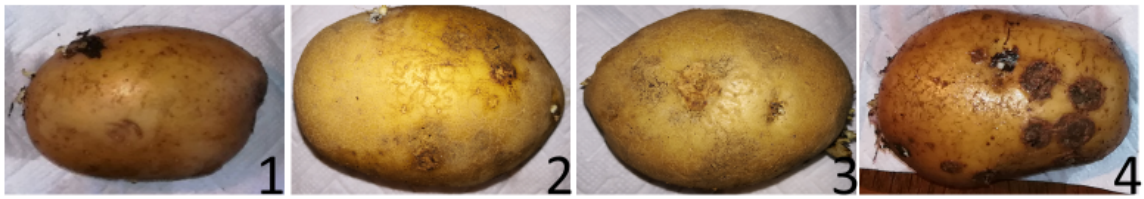



Figure 13: **Evaluation of antagonistic effects of consortia formulations against R. solani on the potato tubers**. The potato cultivar 'Arkula' was evaluated in terms of infection symptoms of the pathogen *R. solani* by using the lowest infection (1) to the highest infection score (4).

Six pots per consortium formulation were planted. Generally, the average number of shoots of the potato plants were in the range between four and six shoots per pot. Herein, no significant differences were observed (Fig. 14a). For the average length of the shoots, in the control containing the pathogen, the potatoes had the shortest shoots followed by the control without the pathogen. The potatoes inoculated with consortia had longer shoots than the controls, whereby the natural consortium resulted in the highest amount of biomass (Fig. 14b). In addition to the biomass the infection factor was investigated. Herein, no direct correlation between biomass and degree of infection was observed. The control without

the pathogen and consortium 178 with the four bacterial strains in phosphate buffer revealed the lowest number of symptoms of pathogen infection, followed by the natural consortium and consortium 75 containing the pyrazine booster. For all other consortia the same or a higher number of visible traits of the pathogen was observed (Fig. 14c).

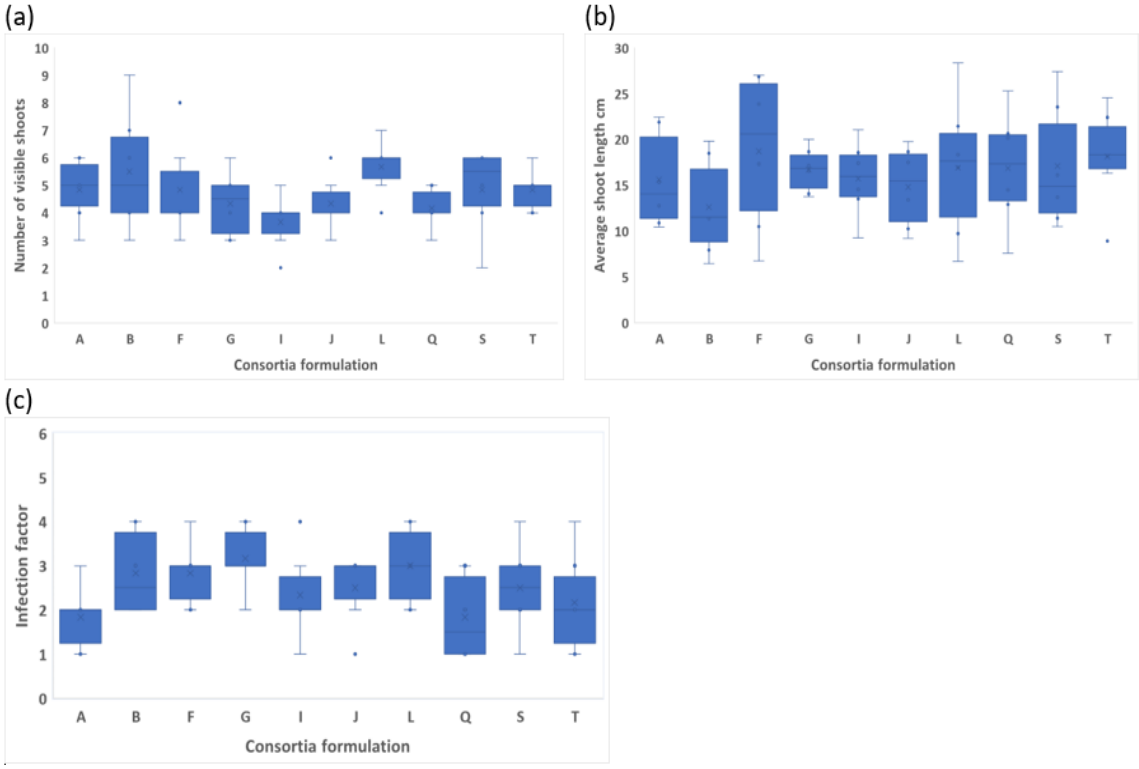



Figure 14: **Evaluation of antagonistic effects of consortia formulation against R. solani in the greenhouse**. *R. solani* was grown on barley beads for at least 22 days. The potato cultivar 'Arkula' was used to evaluate the consortia formulations. The sample size was n=6.

The evaluation of the infection factor provided indications that the pyrazine booster improved the effect of consortium 75 but impaired the effect of consortium 178 against *R. solani.* Therefore, consortia 75 and 178 were later evaluated on the field with and without the pyrazine booster respectively.

By obtaining these results, the first milestone to define four bacterial consortia with enhanced activity against potato pathogens and two prototype formulations was reached. Based on these results, we selected four formulations which we labelled as 'Patricia' (consortium 75), 'Lilly' (consortium 178) and Timi (consortium 96) (Tab. 6). In addition, we have identified four additional bacterial consortia and formulations with enhanced activity, which will serve as a backup.

Table 6: **Selected consortia including their prototype formulation**. Four consortia were assembled and include four microbial strains in each consortium.

| Consortium | Microorganisms | Formulation |
|---|---|---|
| **Patricia** | *Bacillus subtilis 1Pe4-13,*<br>*Pseudomonas corrugata 5Re4-6,*<br>*P. putida 6R5-24,*<br>*Serratia fonticola 3Rc3* | Consortium 75 with four bacterial strains in phosphate buffer + pyrazine (0.3%) booster |
| **Timi** | *Pseudomonas corrugata 5Re4-12,*<br>*P. putida 6R5-24,*<br>*Trichoderma gamsii AT 1-2-4,*<br>*Trichoderma velutinum G1/8* | Consortium 96 with four strains in phosphate buffer |
| **Lilly** | *Pseudomonas chlororaphis L30-3-8,*<br>*Pseudomonas chlororaphis 6R4-28,*<br>*Pseudomonas corrugata 5Re4-6,*<br>*Bacillus amyloliquefaciens Sc-K143* | Consortium 178 with four bacterial strains in phosphate buffer |
| **Lilly and Booster** | *Pseudomonas chlororaphis L30-3-8,*<br>*Pseudomonas chlororaphis 6R4-28,*<br>*Pseudomonas corrugata 5Re4-6,*<br>*Bacillus amyloliquefaciens Sc-K143* | Consortium 178 with four bacterial strains in phosphate buffer + pyrazine (0.3%) booster |

### 3.2.7 Selection of the formulation with the most suppressive effect against *R. solani* in the field.

The field experiment was performed at ´Leibniz-Institut für Gemüse- und Zierpflanzenbau (IGZEV)´ in Großbeeren (Germany). The aim of this experiment was to identify the most promising formulation of microbial consortia that suppress the pathogen *R. solani* in combination with the potato cultivar ´Arkula´. Two microbial consortia were selected and applied either with or without the pyrazine-based booster on the field (Tab. 7).

Table 7: **Microbial consortia including their prototype formulation for suppression of** *R. solani* **Ben3 on the field.** Four formulations of microbial consortia were assembled. Each consortium includes four microbial strains.

| Consortia | Microorganisms | Formulation |
|---|---|---|
| **Patricia** | *Bacillus subtilis 1Pe4-13,*<br>*Pseudomonas corrugata 5Re4-6,*<br>*P. putida 6R5-24,*<br>*Serratia fonticola 3Rc3* | Consortium 75 - Four bacterial strains in phosphate buffer |

| | | |
|---|---|---|
| **Patricia and booster** | *Bacillus subtilis 1Pe4-13,*<br>*Pseudomonas corrugata 5Re4-6,*<br>*P. putida 6R5-24,*<br>*Serratia fonticola 3Rc3* | Consortium 75 - Four bacterial strains in phosphate buffer + pyrazine (0.3%) booster |
| **Lilly** | *Pseudomonas chlororaphis L30-3-8,*<br>*Pseudomonas chlororaphis 6R4-28,*<br>*Pseudomonas corrugata 5Re4-6,*<br>*Bacillus amyloliquefaciens Sc-K143* | Consortium 178 - Four bacterial strains in phosphate buffer |
| **Lilly and booster** | *Pseudomonas chlororaphis L30-3-8,*<br>*Pseudomonas chlororaphis 6R4-28,*<br>*Pseudomonas corrugata 5Re4-6,*<br>*Bacillus amyloliquefaciens Sc-K143* | Consortium 178 - Four bacterial strains in phosphate buffer + pyrazine (0.3%) booster |

A further aim of this experiment was to evaluate if the microbial consortia establish in the rhizosphere of the potato plants and if the pyrazine booster has an impact on the establishment of the bacteria. Therefore, rifampicin mutants (75 µg/ml rifampicin) were constructed for the conducted field trial. Another important question addressed the impact of the microbial formulation on the plant associated microbiome. By using amplicon sequencing, shifts within the microbiome and potential recruitment of beneficial microbes via the microbial consortia can be investigated. Therefore, samples for a detailed microbiome analysis were obtained.

The field experiment was conducted in four stages: (i) planting and first treatment, (ii) second treatment, (iii) sampling for amplicon sequencing at flowering time and (iv) assessment at harvesting.

For the first treatment, potato tubers of the cultivar ´Arkula´ were subjected to 11 different treatments (Tab. 8) and planted in a box parcel containing diluvial sand. The surface of potato tubers was spray-treated with the microbial consortia (108 CFU ml-1 per microorganism diluted in tap water) for all samples with microbial consortia containing 0.3% pyrazine booster or the pyrazine booster (0.3 % diluted in tap water) itself. After planting, the plants were exposed to 10 barley grains overgrown with *R. solani* per potato tuber (Tab. 8).

Table 8**: Treatment of the potato cultivar ´Arkula´.** The microbial consortia (108 CFU ml-1 per strain; $^{+}/_{-}$ pyrazine booster 0.3%) were sprayed onto the surface of the potato tubers. Subsequently, 10 barley grains infected with *R. solani* Ben3 were added to the tubers. Subsequently, 10 barley grains infected with *R. solani* Ben3 were added to the tubers for targeted pathogen treatment.

| Variant | Treatment |
|---|---|
| **1** | Arkula + *R. solani* Ben3 |
| **2** | Arkula (non-treated) |
| **3** | Arkula + pyrazine booster |
| **4** | Arkula + consortium Patricia |
| **5** | Arkula + consortium Lilly |
| **6** | Arkula + consortium Patricia + pyrazine booster |
| **7** | Arkula + consortium Lilly + pyrazine booster |
| **8** | Arkula + consortium Patricia + *R. solani* Ben3 |
| **9** | Arkula + consortium Lilly + *R. solani* Ben3 |
| **10** | Arkula + consortium Patricia + pyrazine booster + *R. solani* Ben3 |
| **11** | Arkula + consortium Lilly + pyrazine booster + *R. solani* Ben3 |

Before planting, the soil (diluvial sand) of the field was fertilized in order to reach 150 kg $ha^{-1}$ nitrogen and 300 kg $ha^{-1}$ potassium concentrations. The experiment was implemented in a randomized plot design. The size of one parcel was 2m x 2m, wherein 14 potato tubers were planted. The treatments were repeated four times (Tab. 9).

Table 9: **Randomized plot design of the field experiment.** In total, 11 potato tuber treatments (indicated by first number) were repeated randomly four times (indicated by second number) and planted on two fields (groups 1 and 2).

| **Group 1:** | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| 1/1 | 3/1 | 5/1 | 7/1 | 9/1 | 11/1 | 2/2 | 5/2 | 4/2 | 10/2 | 11/2 |
| 2/1 | 4/1 | 6/1 | 8/1 | 10/1 | 1/2 | 3/2 | 6/2 | 8/2 | 7/2 | 9/2 |
| **Group 2:** | | | | | | | | | | |
| 4/3 | 1/3 | 3/3 | 10/3 | 11/3 | 2/3 | 1/4 | 8/4 | 6/4 | 10/4 | 2/4 |
| 7/3 | 5/3 | 6/3 | 8/3 | 9/3 | 4/4 | 5/4 | 11/4 | ¾ | 9/4 | 7/4 |

The second treatment was performed approximately 10 days after all potato shoots emerged. For this treatment, 100 ml of the formulations were applied per plant. Additionally, the shoot number was evaluated at this time. This assessment did not reveal significant differences in the number of shoots between treatments.

During the flowering stage, two potato plants per parcel were harvested for sampling of the DNA of the rhizosphere and for assessment of the aboveground biomass. Therefore, five grams of root biomass per plant were washed in a stomacher. One ml of the resulting suspension was plated on NA agar plates containing rifampicin (75 µg $ml^{-1}$) and cycloheximide (100 µg $ml^{-1}$) for evaluation of the establishment of the microbial consortia in the rhizosphere (Tab. 10). The residual suspension was centrifuged and the pellet was used for DNA extraction.

The strains of the applied microbial consortia were shown to be established in the rhizosphere. The number of the remaining, non-inoculum bacteria in the rhizosphere was calculated to be approximately $10^4$ CFU $ml^{-1}$. Even in the fields, containing the controls and the pyrazine booster itself, rifampicin mutants were observed. The detection of these mutants can be explained by the application of rifampicin mutants in former experiments (Tab. 10). The assessment of the aboveground biomass was included for the evaluation of the number of shoots, shoot length and the wet/dry weight of the aboveground biomass. Within this assessment, no significant differences between the treatments were observed.

Table 10: **Evaluation of the establishment of the microbial consortium strains in the rhizosphere.** In order to obtain insights in the establishment of the microbial consortia in the rhizosphere, rifampicin mutants (75 µg $ml^{-1}$) were constructed. Five grams of root biomass per plant were homogenized in a stomacher. One ml of the obtained suspension was plated on NA agar plates containing rifampicin (75 µg $ml^{-1}$) and cycloheximide (100 µg $ml^{-1}$). For assessment of the CFU $ml^{-1}$, the colonies on the plates were counted.

| Treatment number | Treatment | CFU $ml^{-1}$ |
|---|---|---|
| **1** | Control (+ *R. solani* AG-3) | 4.50E + 01 |
| **2** | Control (untreated) | 1.10E + 02 |
| **3** | Pyrazine booster | 1.17E + 03 |
| **4** | Patricia | 9.30E + 04 |
| **5** | Lilly | 9.78E + 04 |
| **6** | Patricia and Booster | 4.93E + 04 |
| **7** | Lilly and Booster | 7.28E + 04 |

| | | |
|---|---|---|
| **8** | Patricia (+ *R. solani* AG-3) | 7.49E + 04 |
| **9** | Lilly (+ *R. solani* AG-3) | 3.03E + 04 |
| **10** | Patricia and Booster (+ *R. solani* AG-3) | 6.56E + 04 |
| **11** | Lilly and Booster (+ *R. solani* AG-3) | 7.18E + 04 |

For the evaluation of the impact of the microbial formulation on the plant microbiome, the DNA of the potato rhizosphere was extracted. The DNA was used for detailed analyses of the microbial community (bacteria, fungi, oomycetes) via amplicon sequencing.

DNA was extracted from 270 mg of rhizosphere soil with the Qiagen PowerSoil kit according to the manufacturers‘ protocol. The extracted rhiozospheric DNA was used for amplicon library preparation of the V4 region from the 16S rRNA gene fragment and ITS1 region with a one-step PCR. For 16S rRNA amplification the universal primers 515F (5‘-GTGCCAGCMGCCGCGGTAA-3‘) and 806R (5‘-GGACTACHVGGGTWTCTAAT-3‘), with sample-specific barcodes attached, were used. The PCR mix contained 1x Taq&Go, 0.2 mM of each primer and 1 µl of the 1:10 diluted template and was performed in triplicates to each 30 µl. PCR conditions were 96°C for 5 min, followed by 30 cycles of 96°C for 1 min, 54°C for 1 min and 74°C for 1 min, and a final elongation at 74°C for 10 min. Successful amplification was confirmed by gel electrophoresis on a 1% TAE gel. For amplification of the fungal ITS1 region the primer ITS1f (5‘-CTTGGTCATTTAGAGGAAGTAA-3‘) and ITS2r (5‘-GCTGCGTTCTTCATCGATGC-3‘) were used. The PCR mix and program was the same as for 16S rRNA amplification, except for the annealing temperature of 58°C. For analysis of the oomycetal community a two-step PCR protocol was applied. For the first step the primer ITSF_Oo (5‘-AGTATGYYTGTATCAGTG-3‘) and ITS4r (5‘-TCCTCCGCTTATTGATATGC-3‘), both attached with a primer pad, were used. The PCR mix contained 2 µl of Taq&Go, 1.2 µl of 25mM $MgCl_2$, 0.1 µl of each primer (10 µM), 1 µl of the template and 5.6 µl water to reach a total volume of 10 µl. The PCR was performed in triplicates and PCR conditions were as followed: 95°C for 5 min, followed by 30 cycles of 95°C for 30s, 58°C for 35s and 72°C for 40s, and a final elongation at 72°C for 10 min. For the second step PCR, 1 µl ot the previous PCR was used as template and the PCR mix was the same as in the above described one step PCR protocol. The PCR conditions varied slightly, with an initial denaturation at 95°C for 5 min, followed by 15 cycles of 95°C for 30s, 53°C for 30s and 72°C for 30s, and a final elongation at 72°C for 5 min. All PCR products were purified, and equimolar amounts of the samples were pooled. The sequencing was performed by Eurofin on an Illumina MiSeq platform with 250 bp paired-end sequencing. The data was

analysed with Qiime2, R (version 4.2.2.) using Posit (Version 2022.12.0.353) with the packages phyloseq and vegan.

The amplicon sequencing resulted in 4,650,602, 1,272,380 and 319,180 reads, which were assigned to 9,829 bacterial, 5,537 fungal and 353 oomycetal ASVs, respectively.

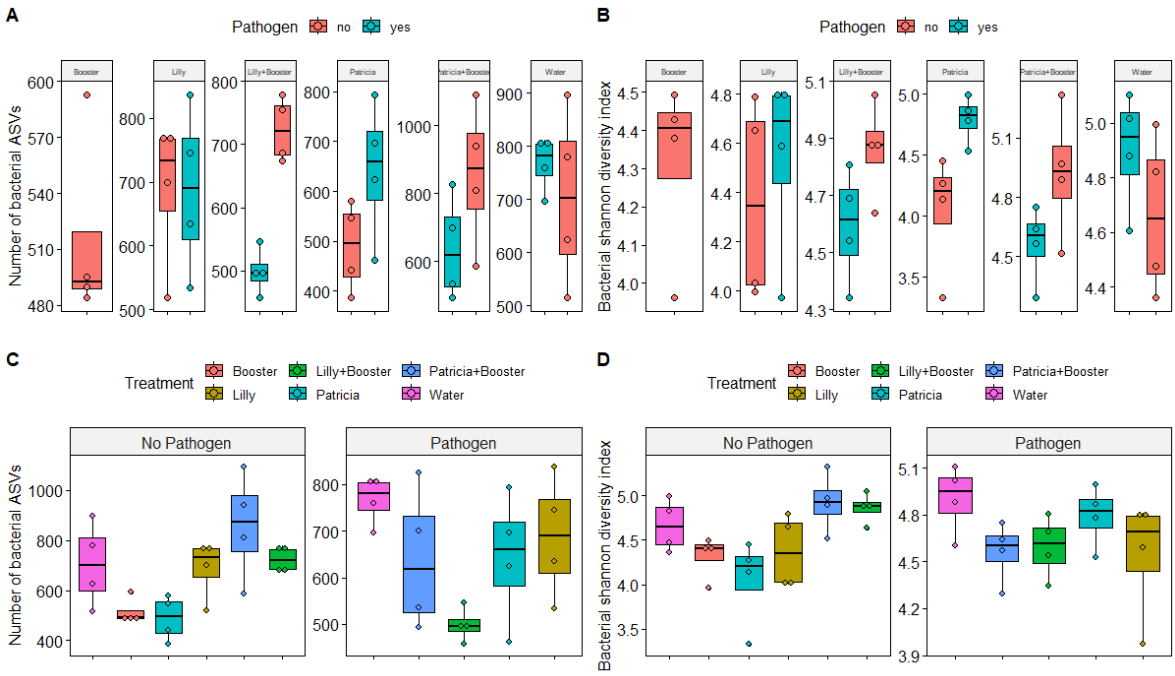



Figure 15: **Bacterial observed ASVs (A,C) and Shannon diversity (B,D).**

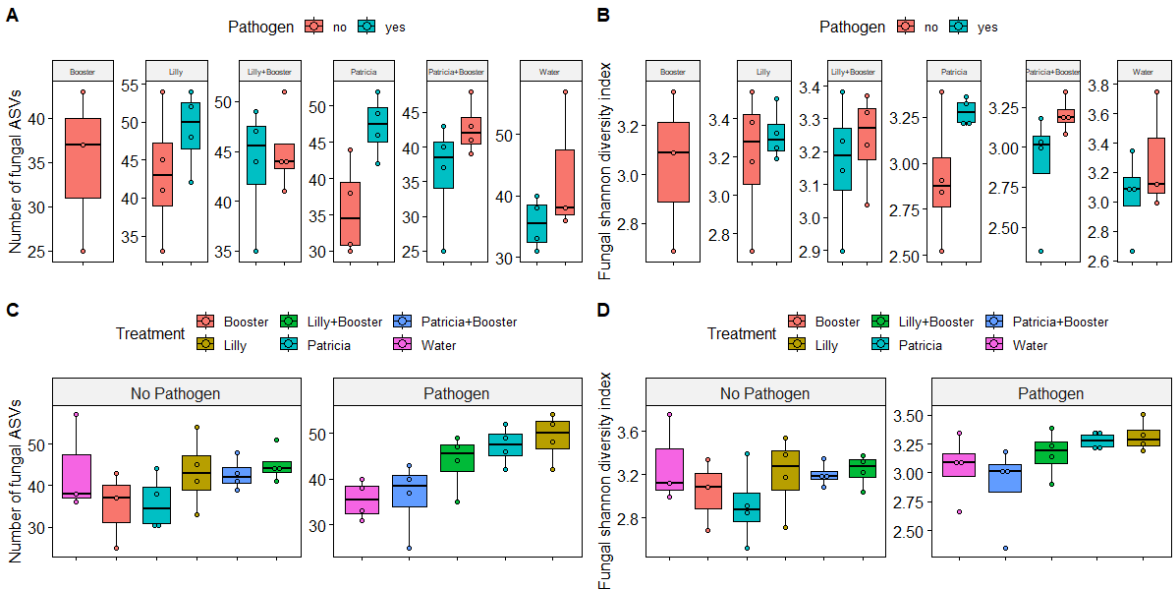



**Figure 16: Fungal observed ASVs (A,C) and Shannon diversity (B,D).**

We observed a trend that the bacterial diversity in the treatments was lower compared to the water control, especially when stressed with *R. solani* (Fig. 15). This could result from the strong presence of the bacterial strains from the consortium, which we were able to proof with the rifamipicin mutants (Table 10). We performed Kruskal-Wallis statistics on the number of observed ASVs and Shannon diversity against the factor Treatment*Pathogen (p=0.0076 and p=0.0006, respectively), followed by pairwise Wilcoxon test with p-value adjustment (fdr), which resulted in no significant differences. The fungal diversity in the treated samples showed a trend to increase when stressed with the pathogen compared to the water control.

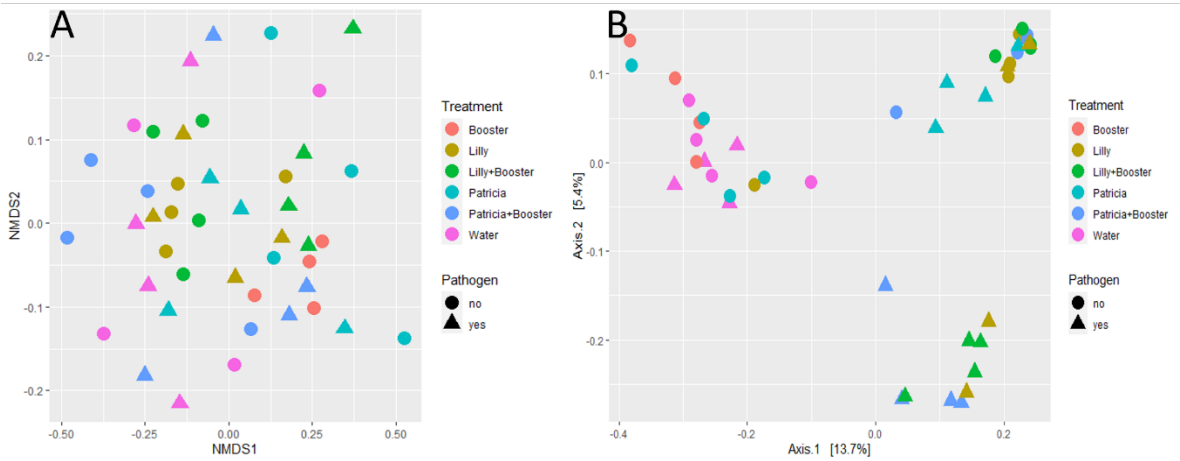



Figure 17: **Bacterial (A) and fungal (B) beta diversity obtained with a Bray-Curtis distance matrix.**

Concerning the microbial community composition, we observed significant differences in the composition mainly between the treatments and to a lesser extent between the presence of a pathogen and/or the booster substance. PERMANOVA analysis showed that the treatment explained 12.7% (p=0.001) and 16.1% (p=0.001) of the bacterial and fungal community variation, respectively. The infection with the pathogen described only 3.2% (p=0.015) and 3.3% (p=0.013) of the bacterial and fungal community change, respectively. Addition of the booster substance had no significant influence on the bacterial community composition variation and with 3.2% (p=0.014) accounted only for minor part of the fungal community variation. In a NMDS and PCoA plot for the bacterial and fungal communities, respectively, these discrepancies could also be seen (Fig. 17).

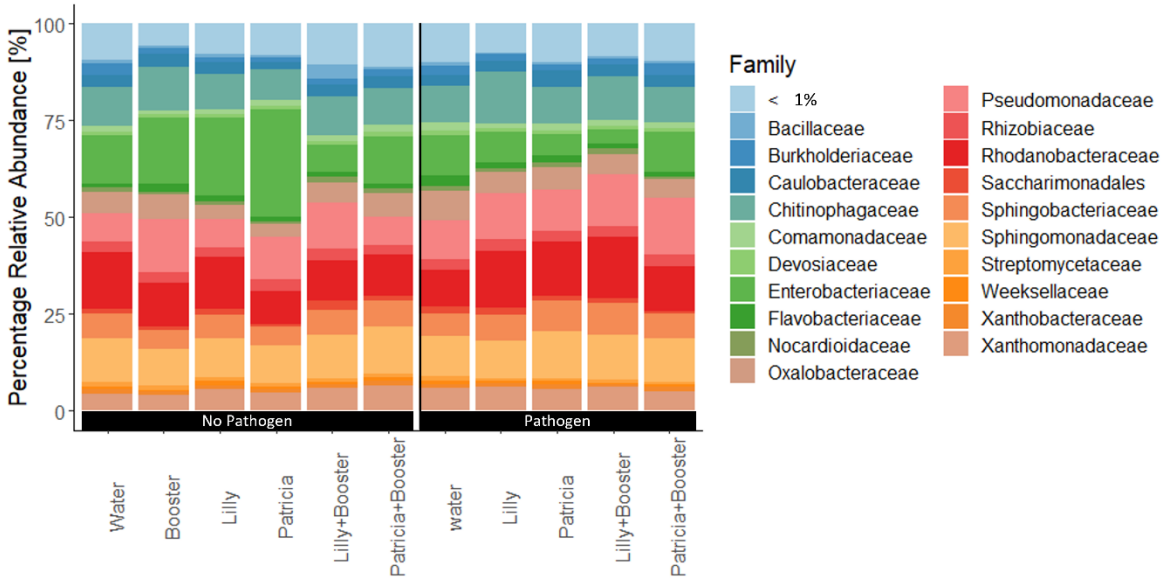



Figure 18: **Bacterial taxonomic composition on family level.**

In the bacterial dataset, *Proteobacteria* (72%) was the most abundant bacterial phylum, followed by *Bacteriodota* (19.7%) and *Actinobacteriota* (4.2%). On family level, differences between samples were observed: samples not stressed with a pathogen had higher levels of *Enterobacteriaceae* (16.1% compared to 7.5%), while the presence of the pathogen led to slightly increased abundance in *Pseudomonaceae* (12% compared to 9.7%) and *Rhodanobacteriaceae* (11.5% compared to 13%) (Fig. 18). *Pseudomonaceae* were also increased in the samples treated with the consortium and/or booster (11.2%) compared to the water control samples (8.7%), which could be explained by the presence of *Pseudomonas* sp. strains in the consortium.

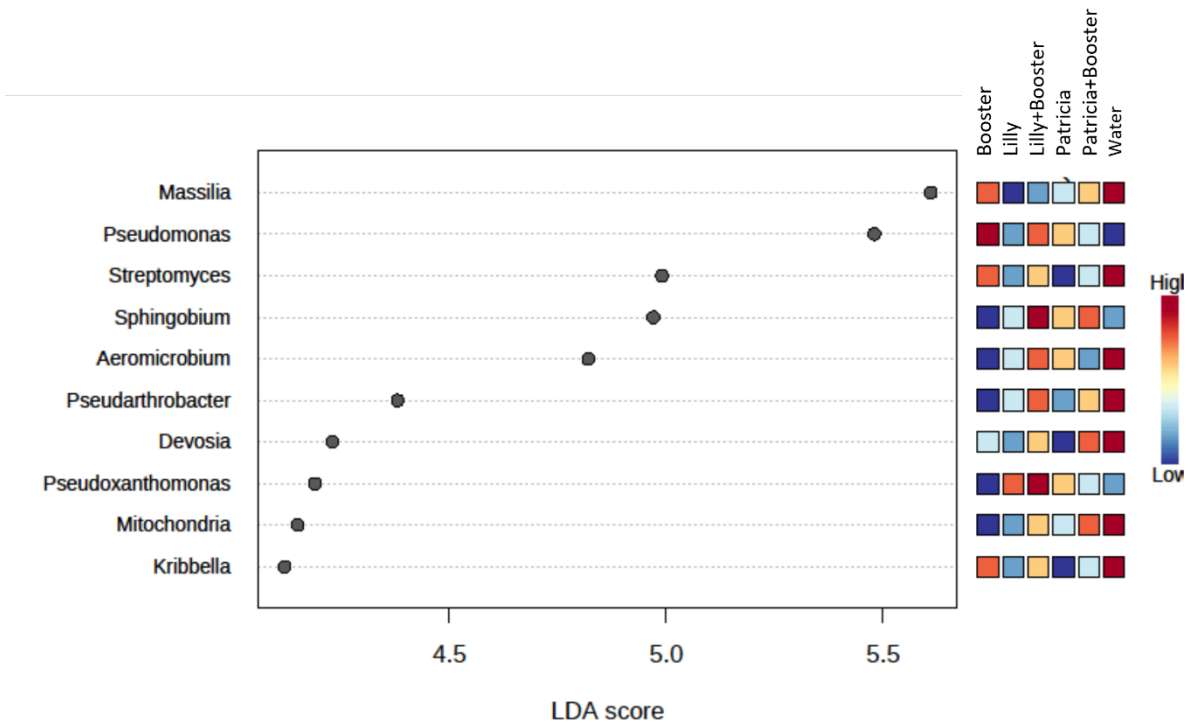



Figure 19: **Differentially abundant bacterial genera that were identified between the treatments, regardless of the presence of the pathogen.**

*Streptomyces*, *Massilia* and *Kribbella* were enriched in the water and booster controls, while *Sphingobium* was enriched in the treatments (Fig. 19). Lilly with and without booster resulted in an enrichment of *Pseudoxanthomonas*, while this genus generally was more enriched in the treatments than in the control. *Devosia* and *Kribbella* were low abundant when only the bacterial consortium was applied.

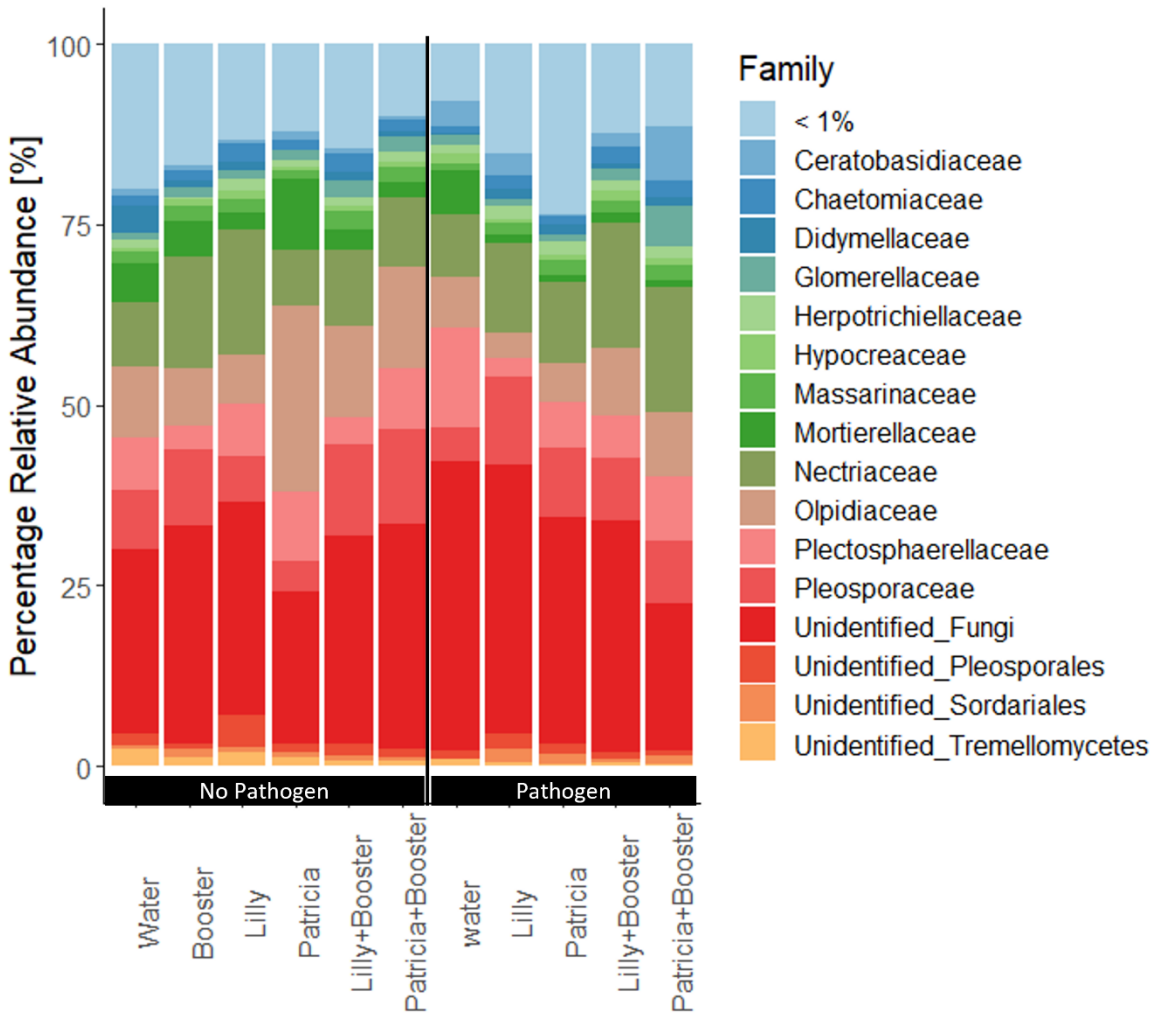



Figure 20: **Fungal community composition on family level.**

For the fungal community, we identified *Ascomycota* (49.3%) as dominant phylum, while a substantial number of ASVs (30%) could not be assigned to a specific fungal phylum. In the control samples without pathogen, *Olpidiomycota* was more dominant (12.8%) than in the samples with pathogen (6.9%). Therefore, *Ascomycota* were slightly more abundant in the samples with pathogen (52.2% compared to 46.9%). On family level, the most prevalent hits were assigned to unidentified fungi, followed by the rare ones which were merged to „<1%“ (14.3%) and *Nectriceae* (12.5%), which were more abundant in the samples treated with consortium and/or booster (13.2%) than in the untreated water controls (8.8%) (Fig. 20). The pathogen *R. solani* i spart of the family *Ceratobasidiaceae*, which did not belong to one of the more dominant fungal families, but they were a lot more abundant in the samples with pathogen (3.2%) than in the untreated control samples (0.7%). All samples, except for Paricia+Booster, showed lower abundance of the *Ceratobasidiaceae* than the water control.

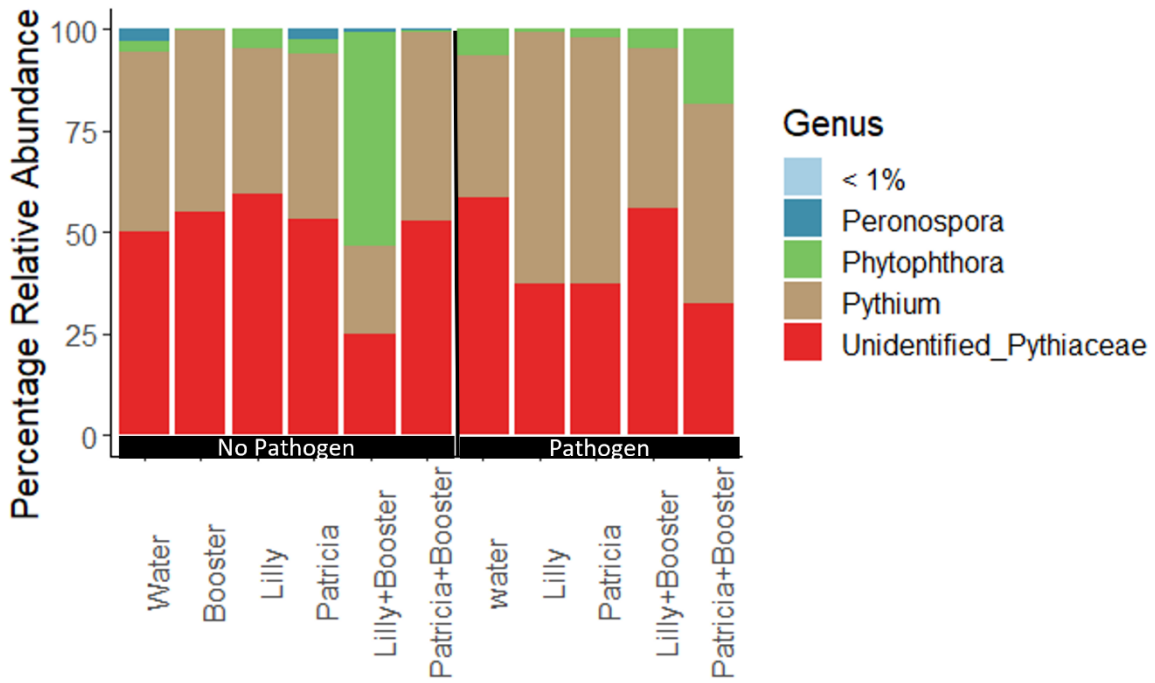



Figure 21: **Oomycetal taxonomic composition on genus level.**

For the oomycetal community, the most abundant family in most samples were *Pythiaceae* (95%), followed by *Peronosporales* (4.4%). Only in the samples treated with Lilly+Booster and without the pathogen the *Peronosporales* were more dominant (52.3%) than *Pythiaceae* (46.6%). On genus level the most dominant groups were unidentified *Phyticaceae* (47%) and *Pythium* (43.6%) (Fig. 21).

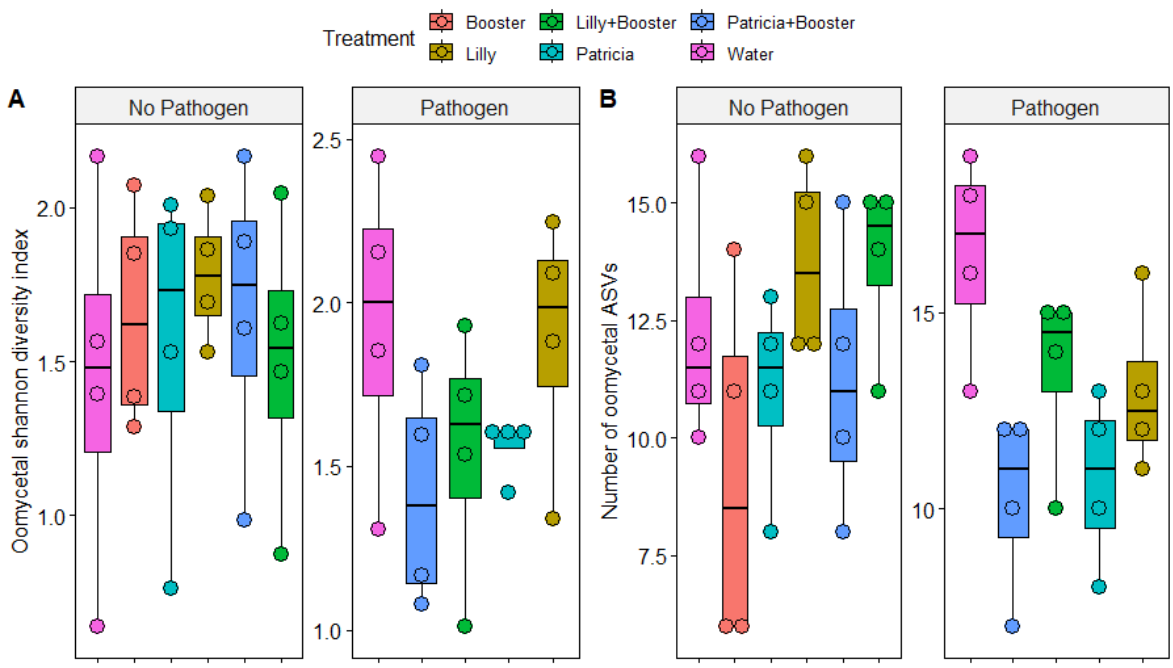



Figure 22: **Oomycetal shannon diversity (A) and observed ASVs (B).**

The Shannon diversity and number of observed ASVs for the oomycetes community were both slightly higher in the water control samples, especially when artificially stressed with the pathogen (Fig. 22). We observed a moderate, positive correlation between the increase of the observed ASVs with the increase in bacterial diversity (r=0.49), when stressed with the pathogen. On the other hand, when no pathogen was present, the fungal observed ASVs (r=0.39) and Shannon diversity (r=0.49) positively correlated with an increase in bacterial diversity. Only minor positive correlation (r=0.28) was observed between ooymcetal Shannon diversity and bacterial diversity, when the pathogen is present. Interestingly, when not stressed with the pathogen, we detected a minor negative correlation between oomycetal and bacterial Shannon diversity (r=-0.29), which indicates that the oomycetal diversity increases when the bacterial diversity decreases.

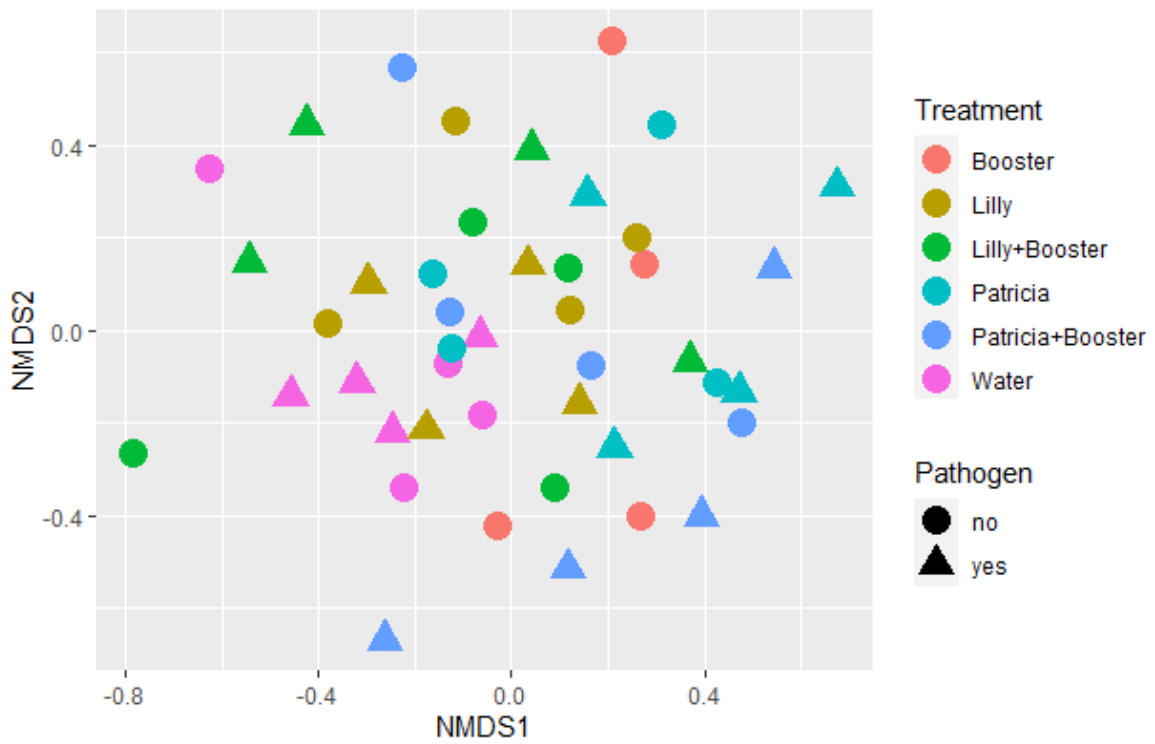



Figure 23: **Oomycetal beta diversity retrieved by Bray-Curtis distance matrix.**

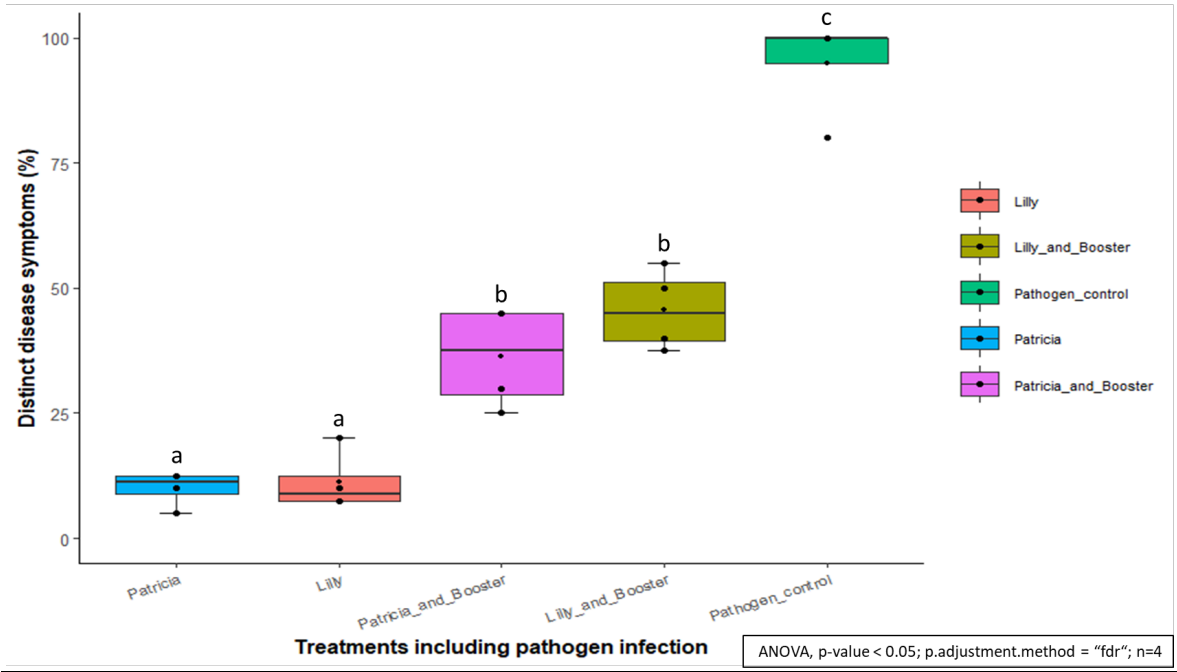
We observed a significant variation in the oomycetal community composition mainly between the treatments (12.2%, p=0.024) and no significant differences between the presence of the pathogen and/or the booster substance. Especially the clustering of the water control samples was evident when visualized in a NMDS plot (Fig. 23).

At harvesting time, the potato tubers were evaluated in terms of marketable and non-marketable tubers. Furthermore, the potato tubers were assessed concerning visible infections with *R. solani*, wherein the tubers were visually inspected for occurrence of sclerotia and the distinct symptoms of potato tubers, respectively. Additionally, the occurrence of scab disease on the tuber surface was assessed.

Concerning disease symptoms, the bacterial consortia were shown to significantly decrease the symptoms which are visible as dry cores and tuber deformation. The control group (untreated potato cultivar 'Arkula') infected with *R. solani* AG-3 was holistically infected with the pathogen. In contrast, the percentage of disease symptoms on the potato tubers treated with the consortium/booster combination ranged between 30% - 55%. The best results were observed after the inoculation of the potato tubers via the pure microbial consortia which substantially decreased the percentage of distinct disease symptoms in a range

between 5% and 20% (Fig. 24a). Even within the treatments without artificial pathogen infection, distinct disease symptoms decreased to a percentage of 5% to 15%. This is a reduction of 10% of the distinct symptoms compared to the respective control group (Fig. 24b).

(a)




(b)

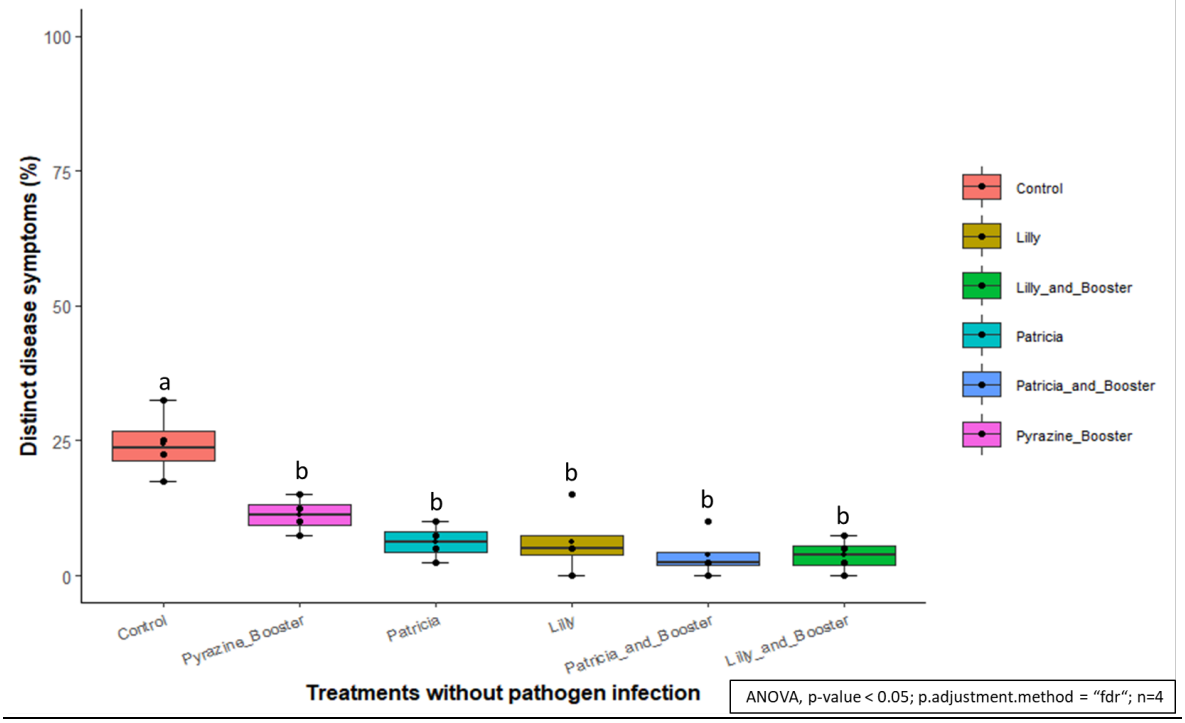



Figure 24: **Assessment of the impact of consortia formulations on the distinct symptoms caused by** *R. solani* **in the field.** *R. solani* was grown on barley beads for at least 22 days. The potato cultivar 'Arkula' was used to evaluate the efficiency of the consortia formulations. The sample size was n=4.

The assessment of sclerotia formation and scab disease occurrence on potato tubers revealed the same positive effects of the bacterial formulations as the assessment of the distinct disease symptoms displayed. For the assessment, tubers were grouped in terms of very low infection (0.0% - 0.9%), low infection (1.0% - 4.9%), high infection (5.0 – 9.9%) and very high infection (more than 10%); the percentage describes the proportion of the tuber surface that was affected. Concerning the sclerotia infestation, the percentage of potato tubers revealing very low infestation of sclerotia substantially increased. Compared to the control group exposed to the pathogen, treatment with the consortium 'Patricia' resulted in approximately 60% more potato tubers featuring very low infection rates, followed by consortium 'Lilly' (45%), 'Patricia + Booster' (40%) and 'Lilly + Booster' (38%) (Fig. 25a). A reduced incidence of sclerotia was even observed after the application of the bacterial formulation in the treatments without artificial infection with *R. solani* AG-3. Herein, the percentage of very low infection compared to the control increased in a range from 10% ('Pyrazine Booster', 'Patricia', 'Lilly and Booster') to 20% ('Lilly', 'Patricia and Booster') (Fig. 25b).

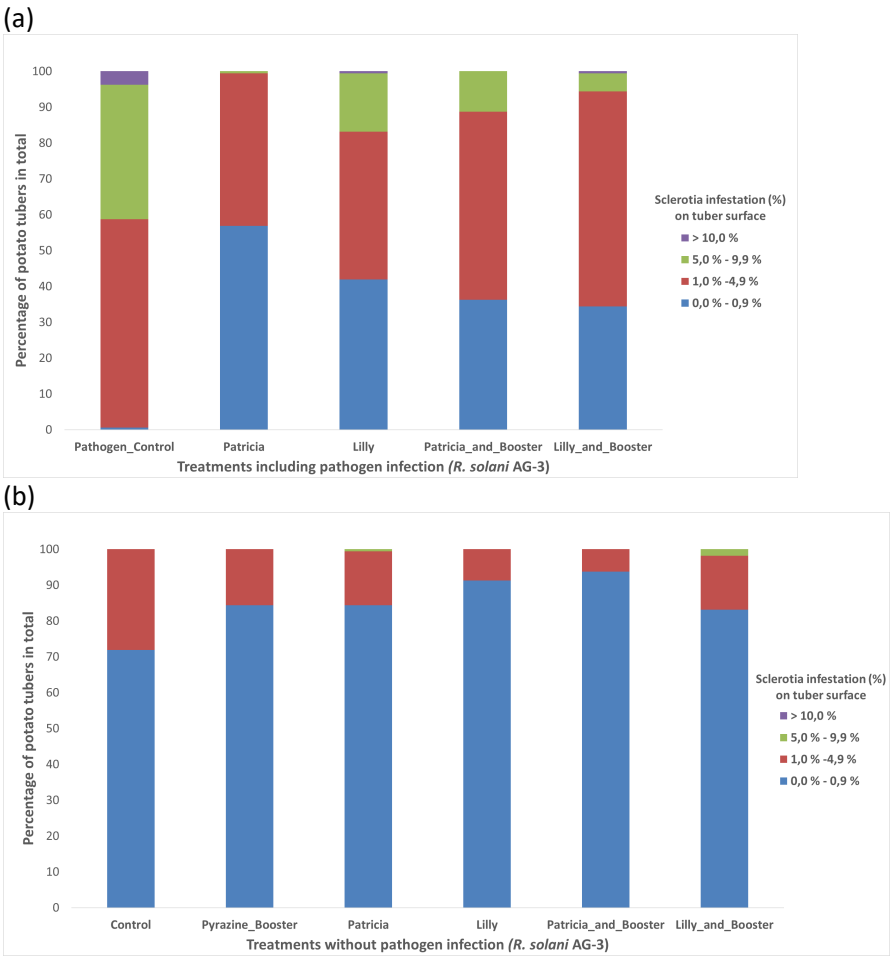
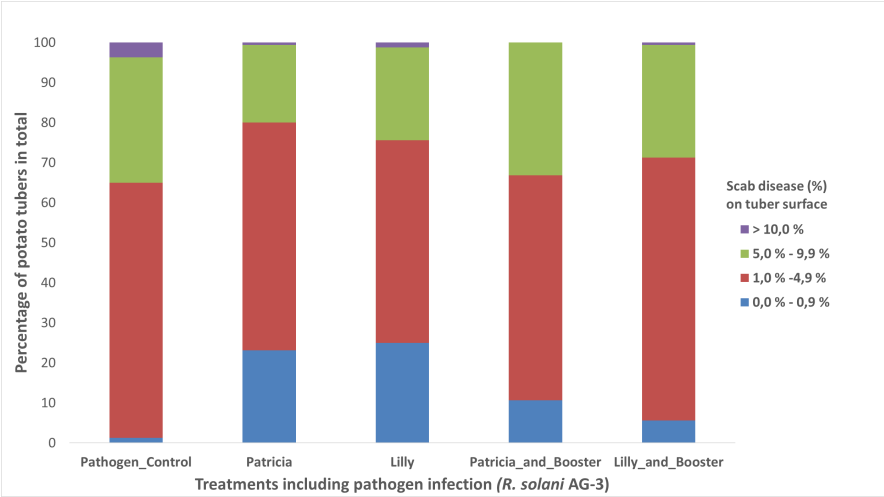



Figure 25: **Assessment of the impact of consortia formulations on the sclerotia infestation caused by** *R. solani* **in the field**. *R. solani* was grown on barley beads for at least 22 days. The potato cultivar 'Arkula' was used to evaluate the consortia formulations. The sample size was n=4. ANOVA analysis: p-value (<0.05; p.adjustment.method = "fdr").

For the assessment of scab disease on the tuber surface of potatoes the same categories were used as for the assessment of sclerotia infestation. In the treatments containing the pathogen *R. solani* AG-3 a decrease of scab disease was observed. Compared to the control containing only the pathogen, the consortia 'Patricia' and 'Lilly' increased the "very low infection" group to 20%. The treatments containing the pyrazine booster improved the percentage of very low infection to 5% - 10% (Fig. 26a). The positive effects of the bacterial formulations in terms of scab disease incidence were higher in the treatments without artificial infection with *R. solani* AG-3. Compared to the control, the percentage of "very low" infection was improved from 40% ('Patricia', 'Lilly', 'Patricia and Booster') to 15% ('Pyrazine Booster') and 5% ('Lilly and Booster') (Fig. 26b).

(a)




(b)

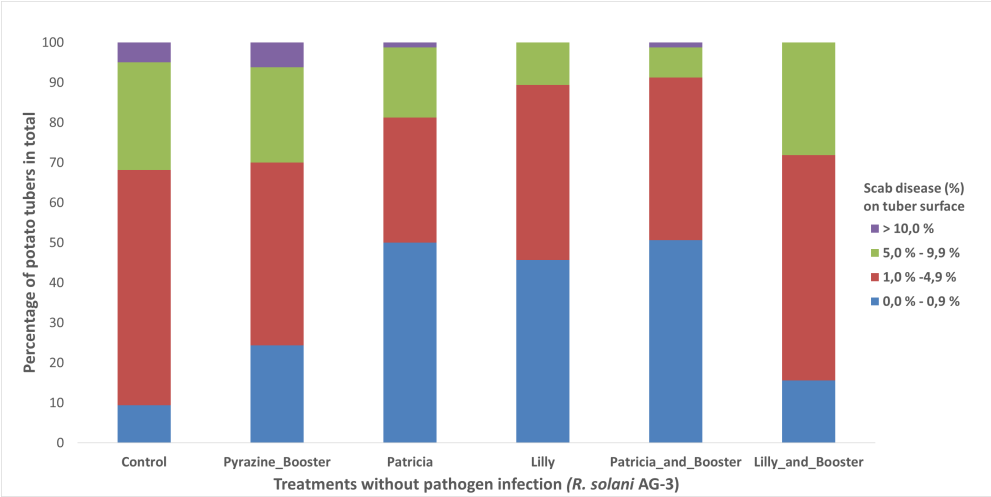



Figure 26: **Assessment of the impact of consortia formulations on scab disease in the field.** *R. solani* was grown on barley beads for at least 22 days. The potato cultivar 'Arkula' was used to evaluate the consortia formulations. The sample size was n=4. ANOVA analysis: p-value (<0.05; p.adjustment.method = "fdr").

Furthermore, the weight of the marketable tubers was assessed. In total, 12 plants per block were harvested and their weight of potato tubers evaluated. The weight of the tubers of all treatments ranged between 9,000 g and 10,000 g. The marketable tubers were assessed by categorizing them in terms of size (30 mm – 60 mm), distinct disease symptoms (no visible symptoms required), sclerotia infestation and scab disease (max. 1% infection of the tuber surface). Compared to the control group that was exposed to the pathogen, the bacterial consortium 'Patricia' revealed the highest yield of marketable tubers with approximately

6,200 grams, followed by 'Lilly' (5,000 g) and the formulations containing the pyrazine booster (3,000 g) (Fig. 27).

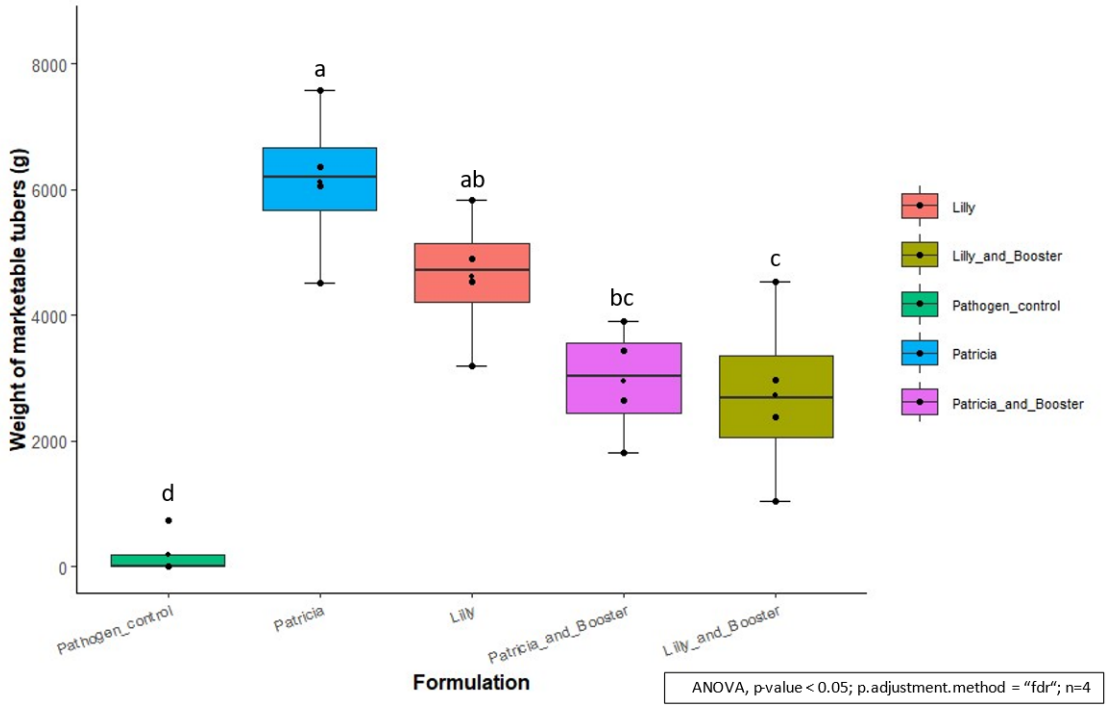



Figure 27: **Assessment of the impact of consortia formulations on the weight of marketable tubers in the field**. *R. solani* was grown on barley beads for at least 22 days. The potato cultivar 'Arkula' was used to evaluate the consortia formulations. The sample size was n=4.

Additionally, the weight of marketable tubers was separated in two different categories. The tubers of category one did not show any disease symptoms. In contrast, the tubers of category two were affected by sclerotia infestation and scab disease of max. 1% of the tuber surface. The control exposed to the pathogen did not yield any potato tubers of category one. Contrary, the bacterial consortia 'Patricia' (1,600 g) and 'Lilly' (1,100 g) yielded more marketable tuber of category one than the control without artificial infection with the pathogen (700 g). The formulations containing the pyrazine booster resulted in lower yields ('Patricia and Booster', 700 g; 'Lilly and Booster', 300 g) than the bacterial consortia (Fig. 28).

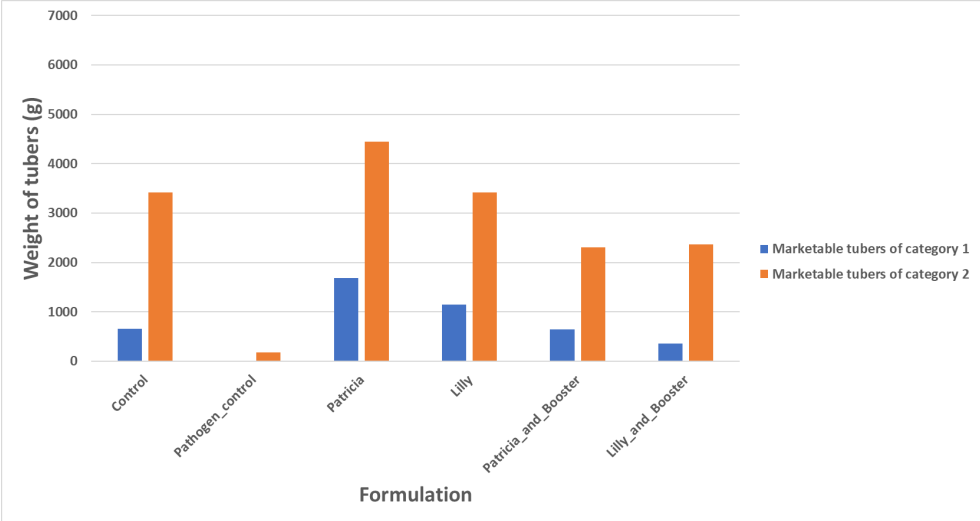



Figure 28: **Assessment of the impact of consortia formulations on the weight of marketable tubers of different tuber categories in the field**. *R. solani* was grown on barley beads for at least 22 days. The potato tubers of all treatments, except the control, were infected with *R. solani.* The potato cultivar 'Arkula' was used to evaluate the consortia formulations. The sample size was n=4. ANOVA analsysis: p-value (<0.05; p.adjustment.method = "fdr").

Overall, significant positive effects were observed when the designed bacterial consortia were applied. However, the pyrazine booster did not improve the effect of the bacterial consortia. Therefore, new booster formulations must be assessed during the next months.

### 3.2.8 Evaluation of formulation efficiency with different potato genotypes

The aim of this experiment was to identify the most fitting potato genotypes, which showed the best interaction effects with the beneficial microbial consortia against *R. solani*, out of the eleven that were preselected by the international project partners. The experiments were conducted with the same soil as used by the PotatoMetaBiome project partner in Munich in order to increase the comparability. Furthermore, the interactions of the plant-associated microbiome will be evaluated in the frame of this experiment. Therefore, the required pots were filled with soil (delivered from Bavaria) and watered for one week. After one week the plantlets were planted into the pots. The plantlets were acclimatized for two weeks via addition of more water than the water holding capacity (WHC) of 60%. After acclimatization, the soil is adjusted to the WHC 60% and the plantlets were grown for three additional weeks. In the fifth week, the stress induction of the plantlets via infection with the pathogen was conducted. In the treatment groups, the plantlets were inoculated with the beneficial consortia. In the seventh week, leaves were harvested for the detection of plant volatiles and sent to the partner MPI in Potsdam. The final harvesting was conducted

in week eight; here, the DNA sampling of the rhizosphere and further amplicon sequencing will be conducted. Additionally, the biomass was evaluated, and the roots were sampled and sent to the partner UPPA in Pau for detection of endophytes (Fig. 29).

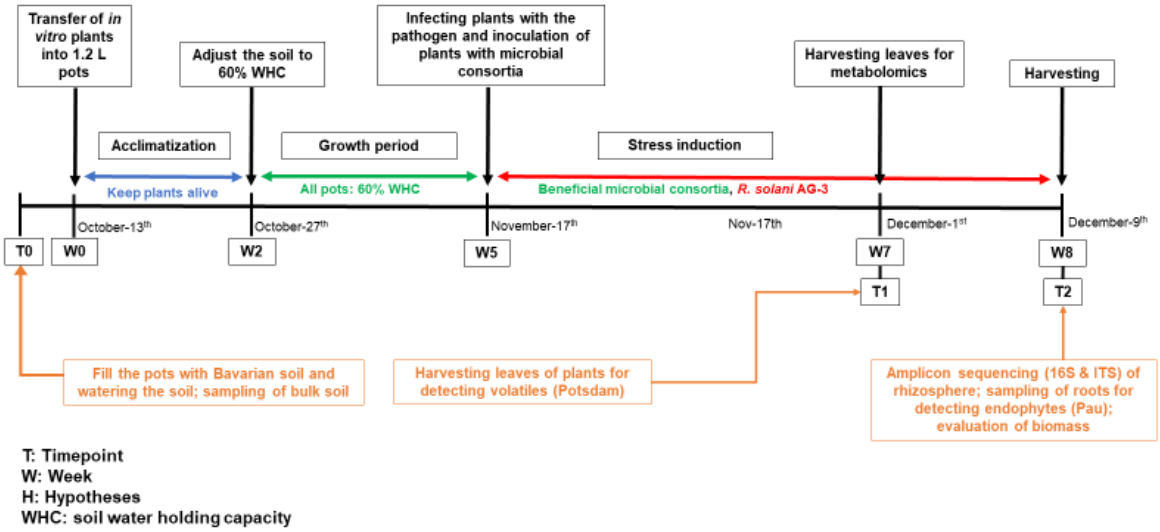



Figure 29: **Experimental design for the selection of 11 potato genotypes and the evaluation of antagonistic effects of consortia formulations against** *R. solani* **in the greenhouse.** *R. solani* was grown on barley grains for at least 22 days. In total, 11 different genotypes of potato plantlets were evaluated in the frame of the interaction approach with the consortia formulations in soil (WHC 60%).

The designed microbial consortia, containing the pyrazine booster, revealed negative interactions with the potato plantlets (Fig. 30). Therefore, the greenhouse experiment was not successful and will be repeated with the already tested microbial consortia in combination with new boosters.

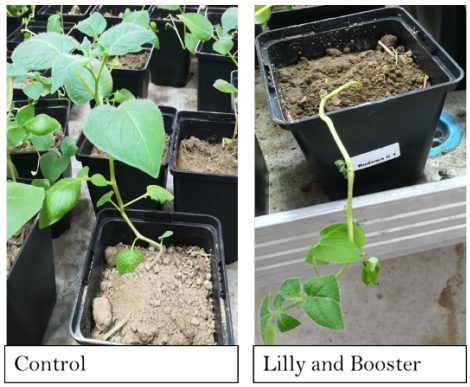



Figure 30: **Evaluation of formulation efficiency with different potato genotypes against** *R. solani* **in the greenhouse**. *R. solani* was grown on barley grains for at least 22 days. In total, 11 different genotypes of potato plantlets were evaluated in the frame of the interaction approach with the consortia formulations in soil (WHC 60%).

### 3.2.9 Targeted screening for boosters for the beneficial consortium 'Patricia'

The microbial consortium 'Patricia' was identified as the best performing one, therefore booster substances that improve the beneficial effect of the consortium against the pathogens *R. solani* AG-3 and *V. dahliae* ELV-16 were specifically searched for this consortium. In total, 109 substances were screened in terms of their improvement of antifungal traits of the bacterial consortium 'Patricia' (Fig. 31).

| Number | Booster | Number | Booster | Number | Booster |
|---|---|---|---|---|---|
| 1 | Dodecane | 41 | xanthine | 81 | 3,5 Dinitrosalicylic acid |
| 2 | Pentamethylheptane | 42 | L-valine | 82 | L-Tartaric acid |
| 3 | Linalool | 43 | Horse dung (fresh) | 83 | Caprylic acid |
| 4 | Phenoxyethylacetate | 44 | Harnstoff | 84 | Pelargonic acid |
| 5 | Bisabolene | 45 | Horse dung (>2 years) | 85 | 2-Undecanone |
| 6 | BioTrissol | 46 | Hauskompost commercial | 86 | Lauric acid |
| 7 | Casamino acid | 47 | L-arabinose | 87 | Myristic acid |
| 8 | Fe3Fertilizer | 48 | D-Fucose | 88 | Palmitic acid |
| 9 | PDA | 49 | D-Fructose | 89 | Arachidic acid |
| 10 | Glc 40% | 50 | D-Galactomannan | 90 | Tridecanol |
| 11 | Gal 20% | 51 | Lactose monohydrate | 91 | 2,3 butanediol |
| 12 | Substral | 52 | Maltose | 92 | S-methyl-cysteine |
| 13 | Mix 6/8/12 | 53 | D-Mannose | 93 | Acetic acid |
| 14 | Coffee ground | 54 | D-Ribose | 94 | Methoxyacetate |
| 15 | House compost | 55 | D-Mannit | 95 | Acetone |
| 16 | T/Peptone | 56 | Melitose | 96 | 2-methyl-1-butanol |
| 17 | Shoot extract | 57 | Octadecane | 97 | 3-methyl-1-butanol |
| 18 | L-asparginic acid | 58 | L-Rhamnose monohydrate | 98 | 2-butanone |
| 19 | y-aminobutyric acid | 59 | D-Xylose | 99 | Artificial root exudate |
| 20 | L-cysteiniumchloride | 60 | Trehalose | 100 | Citrate |
| 21 | L-asparagine | 61 | D-Sorbit | 101 | 1-Decanol |
| 22 | L-aspartic acid | 62 | D-Saccharose | 102 | D-Pantothenic acid Calcium salt |
| 23 | L-cysteine | 63 | Caproic acid | 103 | 1-Dodecanol |
| 24 | L-citruline | 64 | Cholesterin | 104 | Valeric acid |
| 25 | 3,4-dihydroxy-L-phenylalanine | 65 | Capric acid | 105 | Fumaric acid |
| 26 | Dicarboxybenzoyl-L-arginine | 66 | p-coumaric acid | 106 | Lactic acid |
| 27 | L-glutamic acid | 67 | Chitin | 107 | L-alanine, (S)-2-Aminopropansäure |
| 28 | L-glutamine | 68 | Caffeine | 108 | L-argenine |
| 29 | L-histidine | 69 | Citric acid | 109 | 1-Butanol |
| 30 | L-isoleucine | 70 | Malonic acid | | |
| 31 | Inosine | 71 | Maleic acid | | |
| 32 | L-lysine monohydrate | 72 | Diethyl-oxalacetate | | |
| 33 | L-leucine | 73 | 4-Hydroxybenzoesäure | | |
| 34 | L-methionine | 74 | Eisen(III)sulfide | | |
| 35 | L-phenylalanine | 75 | 6-aminocaproic acid | | |
| 36 | L-tryptophane | 76 | Proprionsäureamid | | |
| 37 | L-proline | 77 | ß-alanine | | |
| 38 | L-threonine | 78 | L-malic acid | | |
| 39 | L-tyrosine | 79 | Benzoic acid | | |
| 40 | L-serine | 80 | Succinic anhydride | | |
| | | | | | |

Figure 31: **List of potential 'booster' substances that were screened for improvement of the traits of the bacterial consortium 'Patricia'.**

In the first step, the improvement of the production of antifungal volatiles was assessed with a PDVA against *R. solani* AG-3. For this purpose, the boosting substances were either diluted with water or DMSO, sterilized through a filter (0.20 µm) and added to the NA agar. Additionally, the best performing boosters were assessed via a contact-based dual-culture assay containing the boosting substances on WAKSMAN agar. The aim of the assessment with the Dual Culture Assay was the exclusion of potentially growth-enhancing effects of the substance on the fungal pathogens *R. solani* AG-3 and *V. dahliae* ELV-16. By integration of the results of both assays, the best performing substances were identified as Lauric acid (14 mM), 2-methyl-1-butanol (0.5% v/v), D-Pantothenic acid Calcium salt (25 mM), Octadecane (0.5% v/v) and Palmitic acid (25 mM) (Fig. 32).

PDVA assessments revealed additional inhibition rates of the fungal mycelia of *R. solani* AG-3 by Lauric acid (18%), 2-methyl-1-butanol (30%), D-Pantothenic acid Calcium salt (21%), Octadecane (28%) and Palmitic acid (32%). In addition, controls were included to assess potential antifungal effects by the substances itself (Fig. 32).

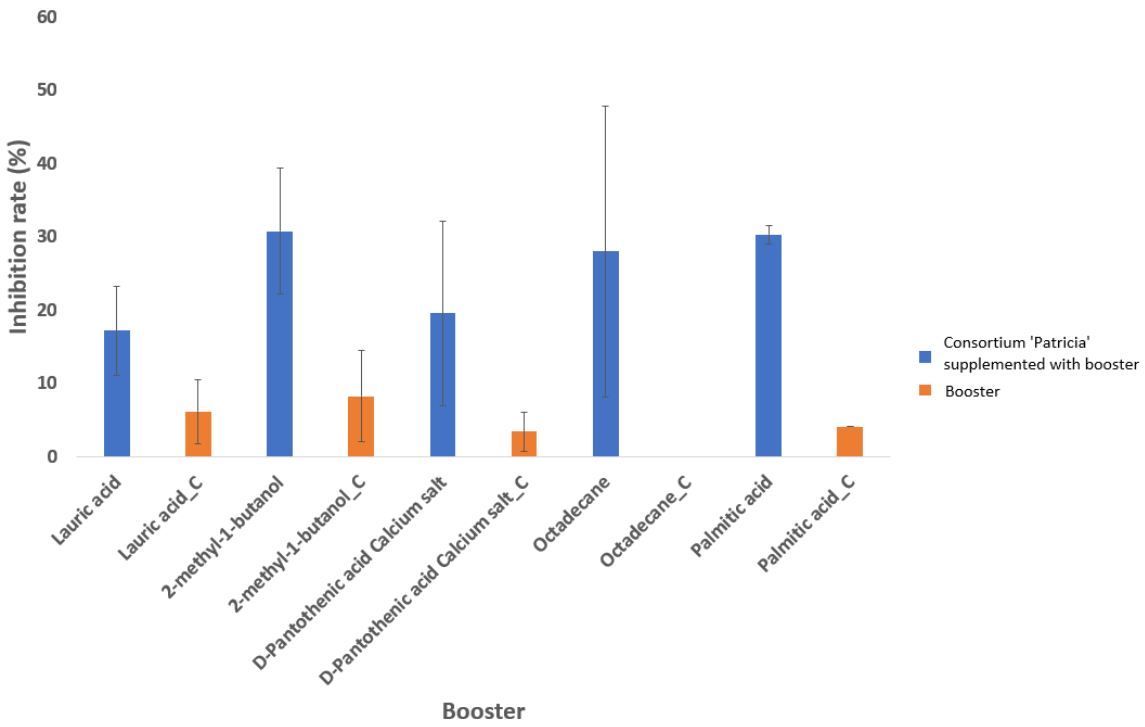



Figure 32: Antagonistic effects of the microbial consortium 'Patricia' supplemented with different boosters against R. solani (mycelia). The five best performing substances for boosting antifungal traits of the bacterial consortium are shown. The sample size was n=6.

Compared to the control group, the bacterial consortium 'Patricia' supplemented with the booster substances revealed an increased inhibition zone against both pathogens. The best performing substance in the Dual Culture Assay was Lauric acid followed by Octadecane, 2-methyl-1-butanol, Palmitic acid and D-Pantothenic acid Calcium salt (Fig. 32).

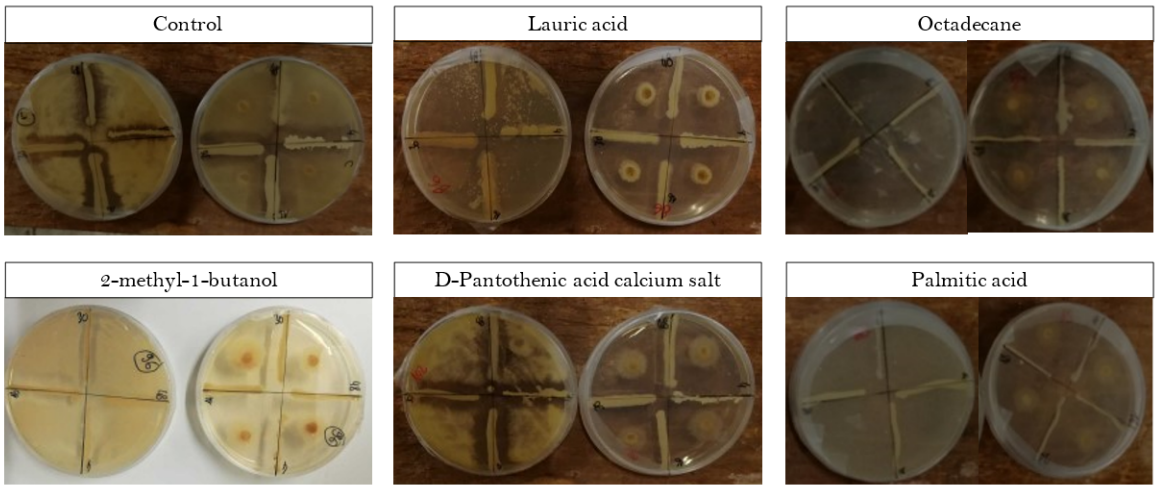



Figure 33: **Antagonistic effects of microbial consortium 'Patricia' supplemented with different boosters against** *R. solani* **and** *V. dahliae* **(mycelia).** The five best performing substances for boosting antifungal traits of the bacterial consortium are shown. Dual Culture Assay against *V. dahliae* (left) and *R. solani* (right). The sample size was n=3.

Combinations of these five substances were investigated, but indicated decreasing inhibition rates or even negative effects when compared to the application of pure substances.

In addition, a preliminary greenhouse experiment was performed to observe the effect of the formulation on plant fitness. The tomato breed 'Salattomate' (Hornbach Baumarkt AG, Germany) was inoculated with 20 ml of the formulation containing the consortium 'Patricia' (1*108 CFU $ml^{-1}$) and the boosting substances Lauric acid (14 mM), 2-methyl-1-butanol (0.5% v/v), D-Pantothenic acid Calcium salt (25 mM), Octadecane (0.5% v/v) and Palmitic acid (25 mM). The experiment was stopped after three weeks. Tomato plants were used instead of potato plants to obtained distinct results in a short time period with a plant from the same plant family. Only consortium 'Patricia' containing the volatile compound 2-methyl-1-butanol showed a good compatibility with the plant. Other tomato plants which were inoculated with the formulations containing Panthothenic acid calcium salt, Octadecane, Palmitic acid, or Lauric acid died within one week (Fig. 34).

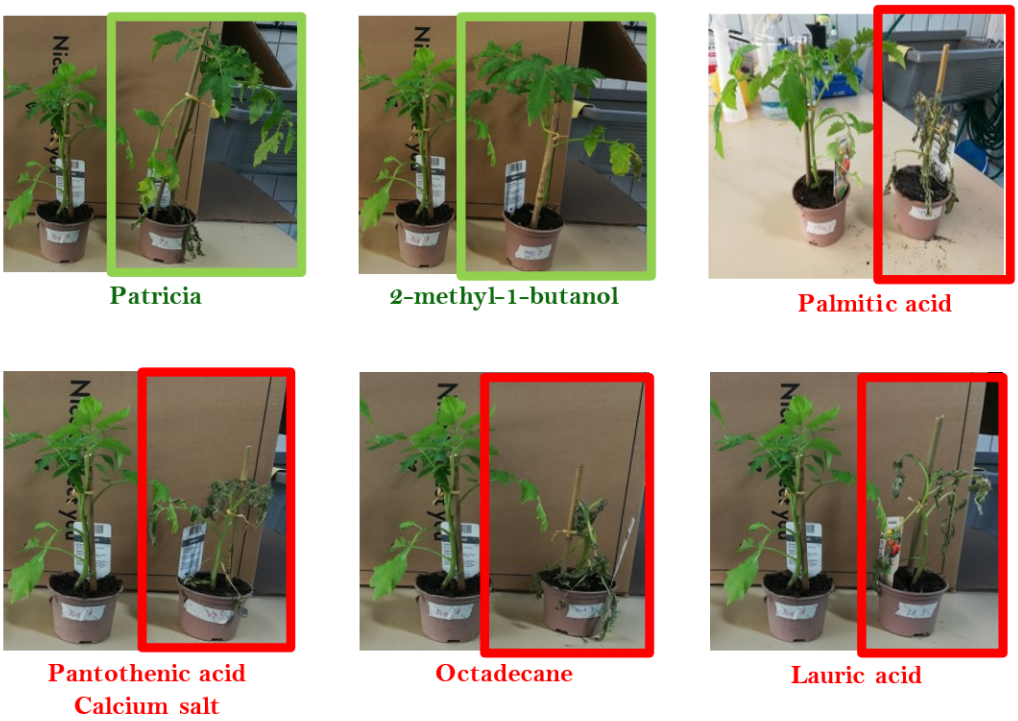



Figure 34: **Observable effects of the microbial consortium 'Patricia' supplemented with different boosters on the fitness of tomato plants**. Inocula of 20 ml with the five best performing formulations containing substances for boosting antifungal traits of the bacterial consortium were inoculated to the tomato breed 'Salattomate'. (Green boxes) Positive performing formulations. (Red boxes) Negative performing formulations. The sample size was n=6.

Due to these observations, the formulation containing consortium 'Patricia' and 2-methyl-1-butanol was selected for further greenhouse experiments.

### 3.2.10 Effect of the beneficial consortium 'Patricia' containing 2-methyl-1-butanol on the plant's life cycle

The potato breed 'Desiree' was inoculated with the newly constructed formulation containing consortium 'Patricia' mixed with the volatile 2-methyl-1-butanol (0.5 % v/v). The aim was to observe phenotypic differences of the plant as well as the rhizosphere microbiome during different life cycles of the potato plant. Pots with a volume of 10 L were filled with a composite of 80 % Einheitserde Profisubstrat Classic (Balster Einheitserdewerke GmbH, Germany) and 20 % Flairstone Estrichsand 0 – 4 mm (Hornbach Baumarkt AG, Germany). The potato tubers of the breed 'Desiree' were spray-treated with the formulation containing consortium 'Patricia' with a concentration of $10^8$ CFU $ml^{-1}$ per isolate and 2-methyl-1-butanol (0.5 % v/v) and planted into the pot with a depth of 10 cm. Further, ten barley grains infected with *R. solani* AG-3 were directly added to the potato tubers. A second treatment

was conducted when the shoots of all plants were visible. For the second treatment, 100 ml of the formulation per plant or water (negative control) were poured to the pots. The assessment of plant growth and rhizosphere sampling were performed at four different timepoints (T1: Sprouting; T2: Development of tubers; T3: Flowering; T4: Ripening) (Fig. 35).

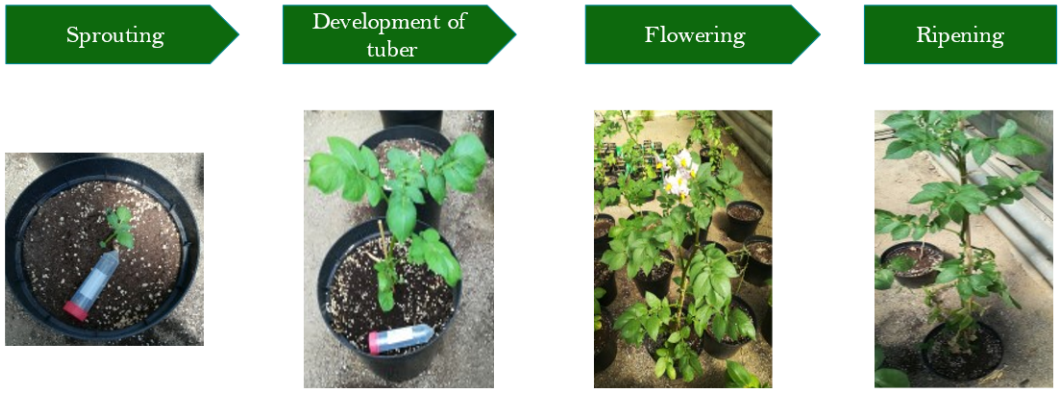



Figure 35: **Four timepoints that were analysed during the life cycle of the potato plant.** The potato breed 'Desiree' was inoculated with consortium 'Patricia' supplemented with 2-methyl-1-butanol. Sampling was performed at four different timepoints. Plants implemented as negative controls were supplemented with tap water. The sample size was n=6.

The potato plants treated with the formulation completed their life cycle stags faster when compared to the plants treated with water. At timepoint three 'Flowering' 59% of the plants treated with the formulation were flowering, for 33% of the plants no flower was observed for that timepoint and 8% of the plants were so heavily damaged or stressed that they have been harvested. For the plants treated with water only 33% were flowering, 42% were not flowering and the plants which were heavily damaged by the pathogen increased to 25% (Fig. 36).

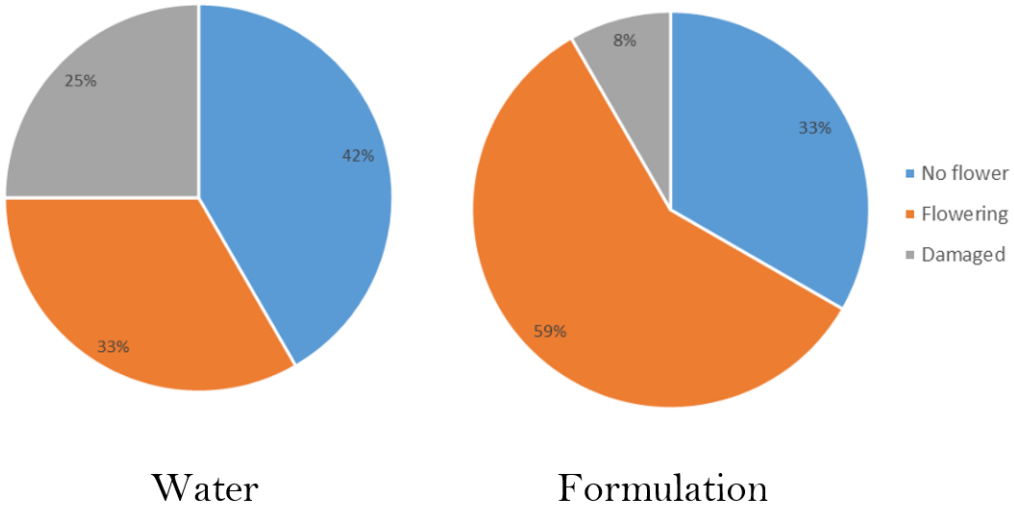



Figure 36: **Assessment of potato plants at the flowering development stage.** The plants of the potato breed 'Desiree' treated with consortium 'Patricia' supplemented with 2-methyl-1-butanol were earlier flowering and less stressed compared to the plants treated with water. The sample size was n=12.

In addition, the untreated plants showed more stress symptoms compared to the treated ones (Fig. 37). Between timepoint two 'Development of tuber' and timepoint three 'Flowering' the day temperature in the greenhouse reached up to 40°C in average. During this time, a positive influence against heat stress of the formulation on the plants was observed.

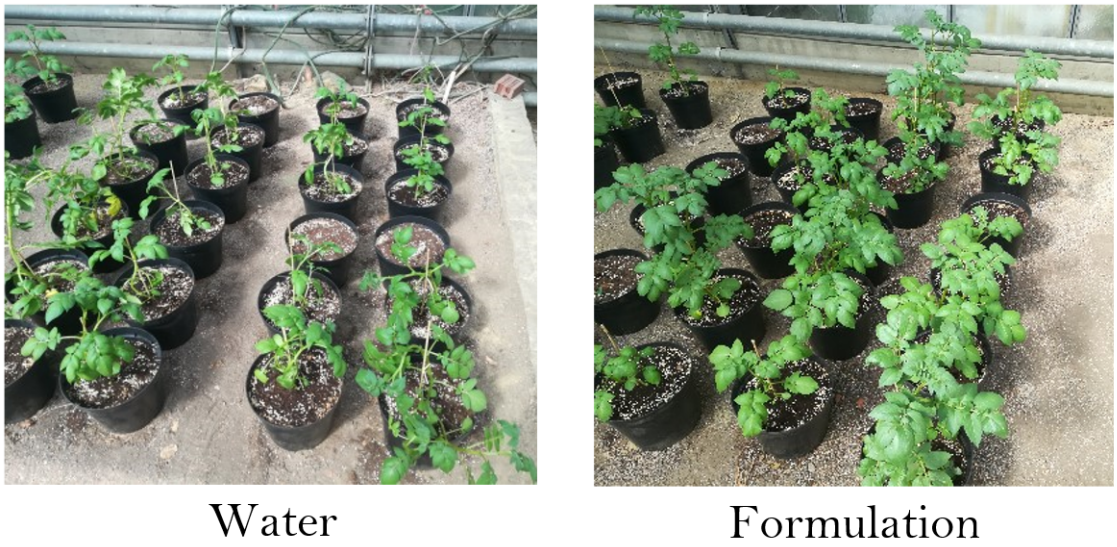



Figure 37: **Potato breed 'Desiree' between timepoint two 'Development of tuber' and timepoint three 'Flowering'.** The plants of the potato breed 'Desiree' treated with consortium 'Patricia' supplemented with 2-methyl-1-butanol showed higher resilience to heat stress compared to the plants treated with water.

The formulation increased the length of the shoots between the 'Development of tuber' (T2 = Timepoint) and 'Flowering' (T3) (Fig. 38). However, at 'Ripening' (T4) no difference in the length of the potato shoots was observed. Furthermore, there was no difference in the length of the roots between both treatments. At 'Ripening' (T4) the number of tubers, the weight of tubers and the disease symptoms of *R. solani* were assessed. The number of tubers were not differing between the treated and untreated tubers. Additionally, sclerotia infestation was not observed. The potato plants treated with the formulation produced higher tuber weights and the tubers had a lower percentage of the distinct disease symptoms (tuber deformation, dry core) (Fig. 39).

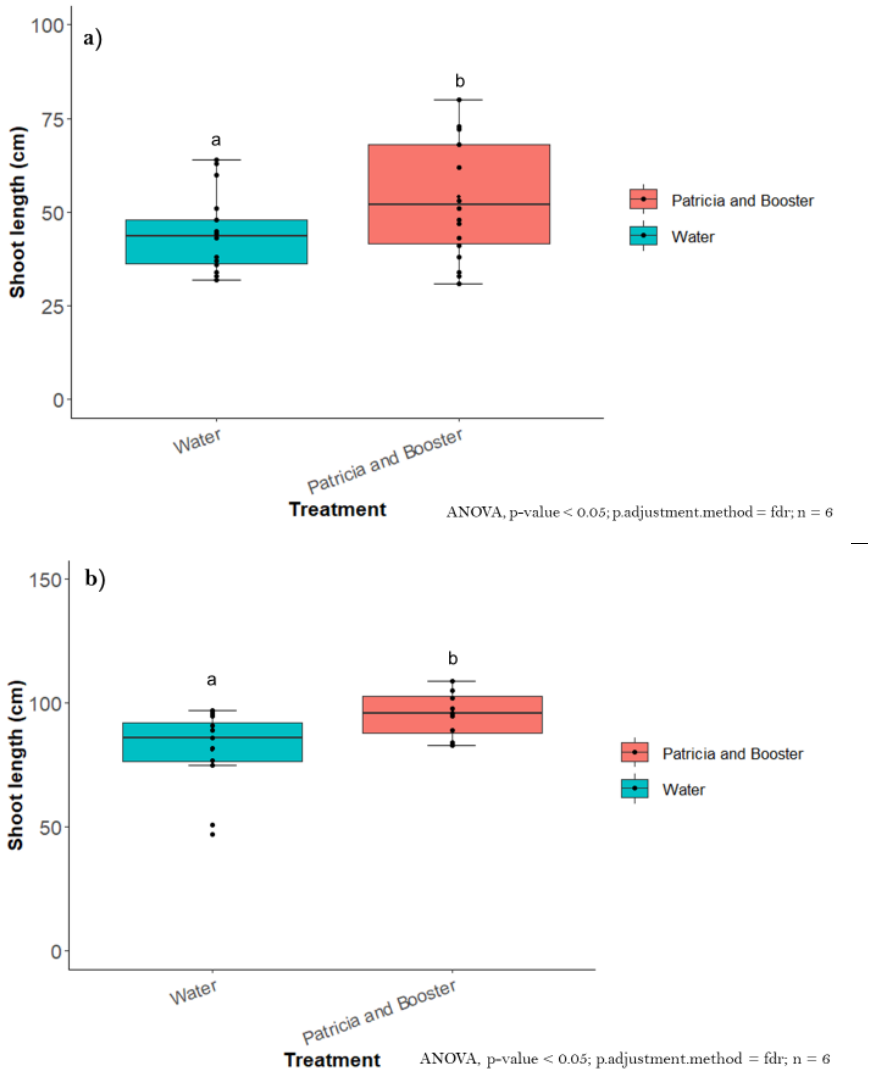



Figure 38: **Assessment of the length of shoots of potato breed 'Desiree' at different life cycle timepoints.** The plants of the potato breed 'Desiree' were treated with consortium 'Patricia' supplemented with 2-methyl-1-butanol and water. The plants were infected with *R. solani* AG-3. (a) Shoot length at 'Development of tuber'. (b) Shoot length at 'Flowering'. The sample size was n=6.

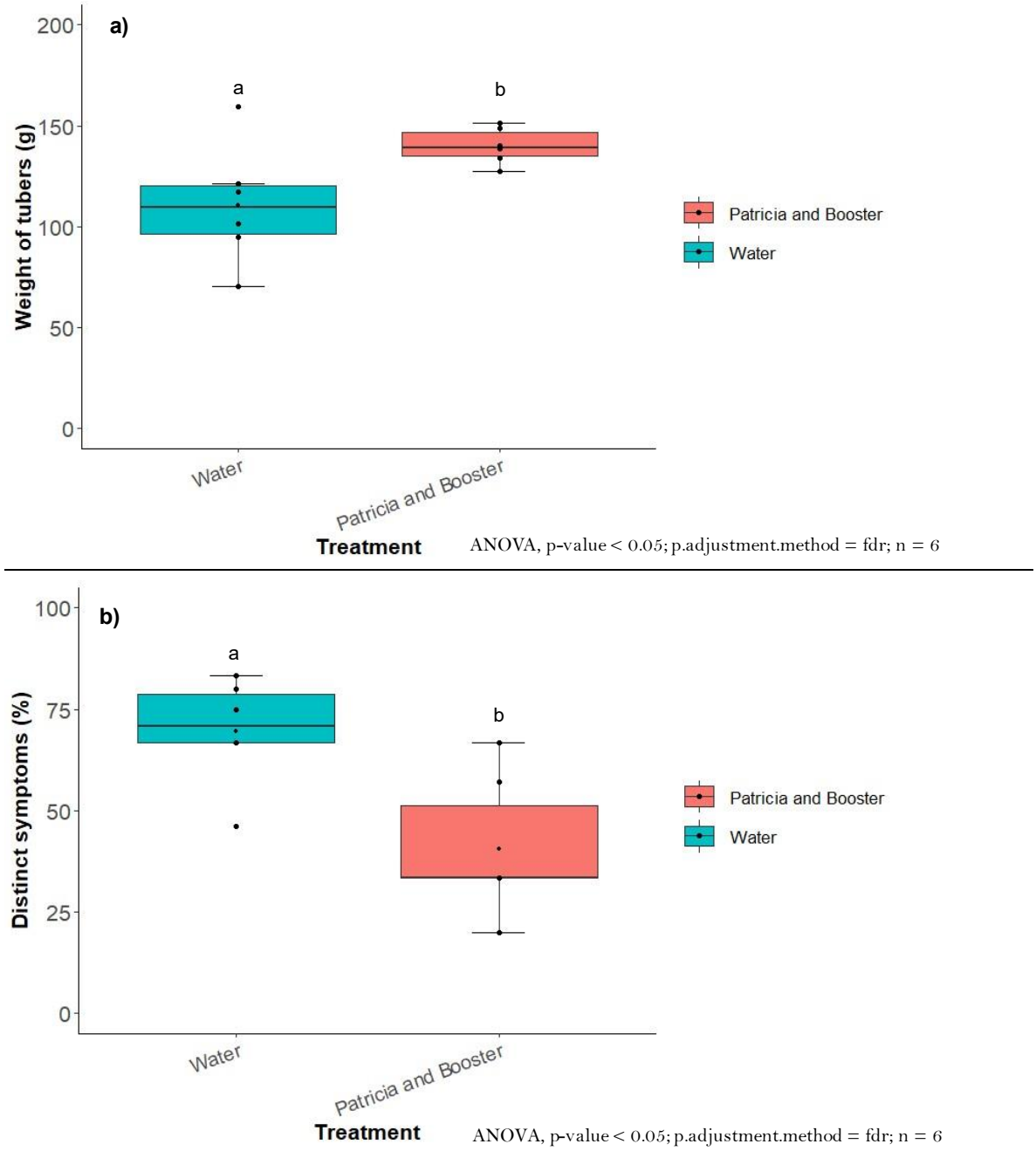



Figure 39: **Assessment of disease symptoms in potato breed 'Desiree' at 'Ripening'.** The plants of the potato breed 'Desiree' were treated with consortium 'Patricia' supplemented with 2-methyl-1-butanol and water. In parallel, the plants were exposed to the pathogen *R. solani* AG-3. (a) Weight of tubers in gram. (b) Percentage of the distinct disease symptoms. The sample size was n=6.

Additionally, to the assessment of plant parameters, the microbial community of the rhizosphere was determined at the four sampling time points. DNA extraction and 16S rRNA and ITS library preparation were performed as described in chapter 3.2.7.

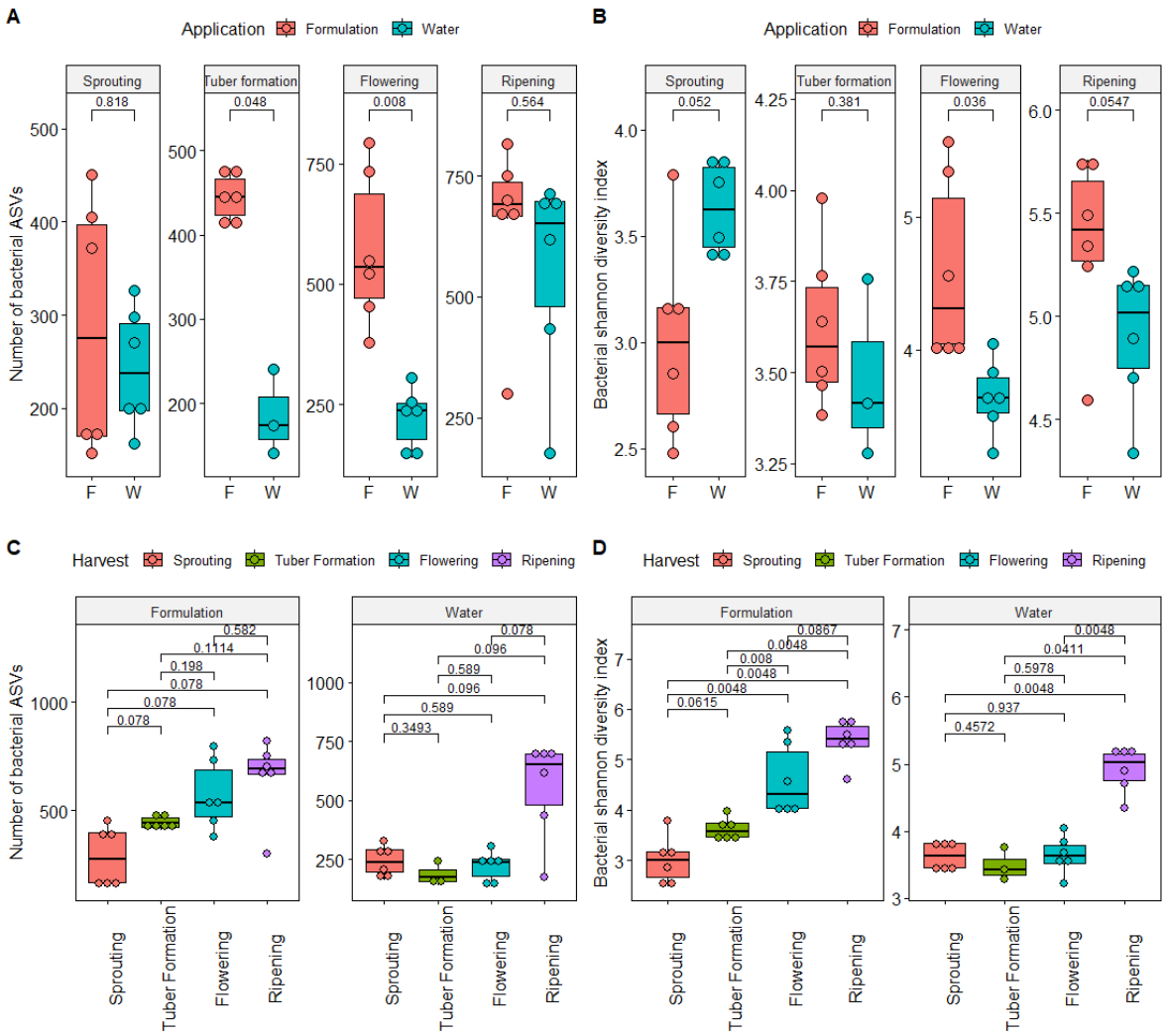



Figure 40: **Bacterial alpha diversity, depicted as observed (A, C) and Shannon (B, D) diversity**. Non-parametric Kruskal-Wallis test was performed, followed by pairwise Wilcoxon test with p-value adjustment-method „fdr“. Sample size was n=6.

The amplicon sequencing approach resulted in 3,168,555 and 1,290,149 high-quality reads, which were assigned to 9,722 bacterial and 2,014 fungal ASVs, respectively. Generally, there were several observable differences in the bacterial diversity between the plants treated with formulation and the untreated water control. While for the formulation both, Shannon diversity and observed ASVs, gradually increased, the diversity in the water control treatment stayed unchanged for the first three sampling timepoints (Fig. 41). Only for the last

sampling time point „Ripening“, a strong increase in diversity was observed (Fig. 41C). Except for the shannon diversity at time point „Sprouting“ the formulation showed similar or higher diversity compared to the water control. The sharp rise in diversity for the control between „Flowering“ and „Ripening“ could result from the heat stress in the glasshouse. It was previously described that abiotic stress can lead to a short-term increase of diversity, while in the long run the diversty decreases (Berg and Cernava 2022).

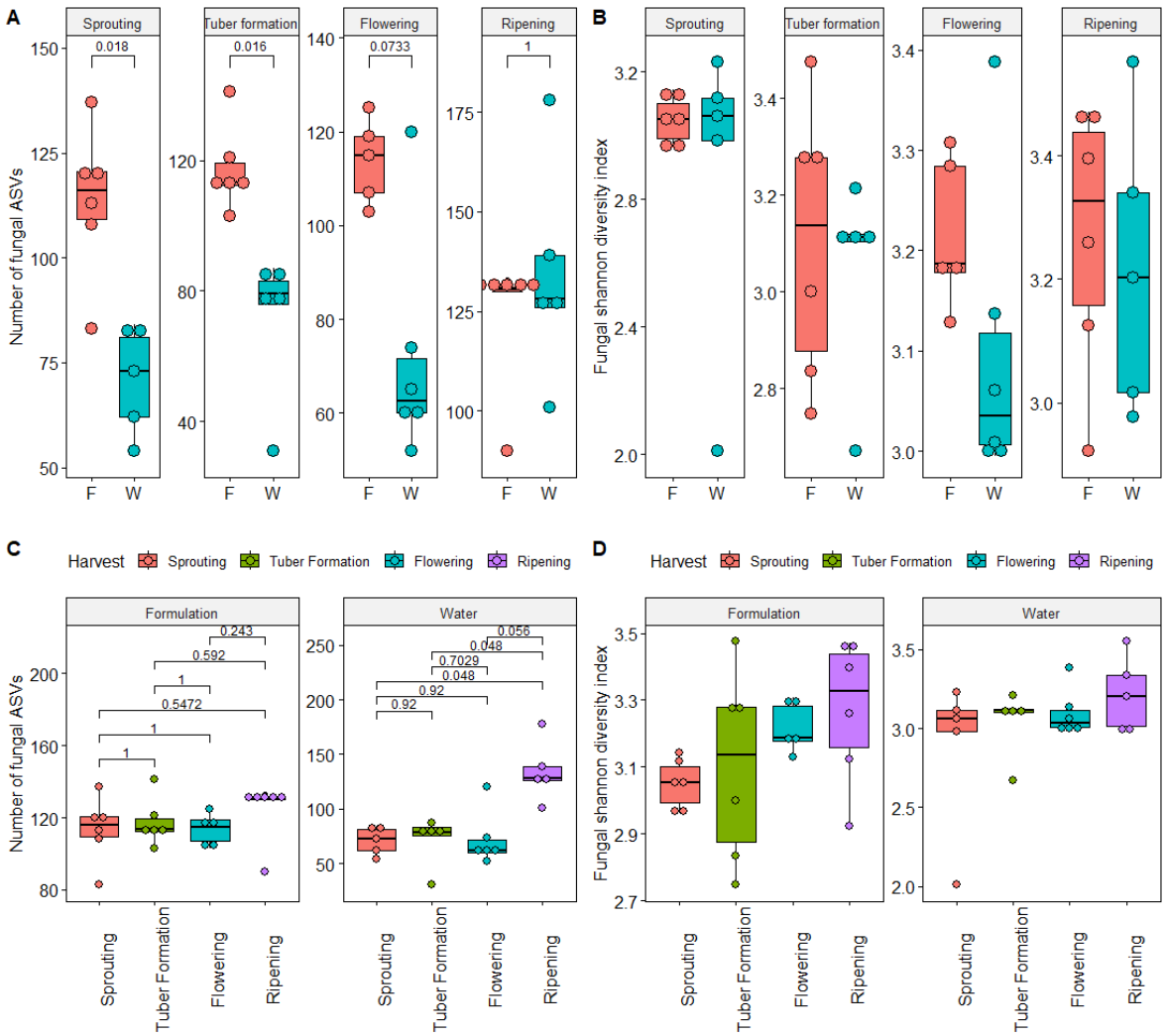



Figure 41: **Fungal alpha diversity depicted as observed (A, C) and shannon (B, D) diversity.** Kruskal-Wallis test was applied, followed by pairwise Wilcoxon test with „fdr“ as p-value adjustment. The sample size was n=6.

Similar results were detected for the fungal diversity. Plants treated with formulation yielded similar or higher number of ASVs compared to the control (Fig. 42A). Shannon diversity showed no significant result, yet the treated samples had always similar or higher diversity compared to the control (Fig. 42B).

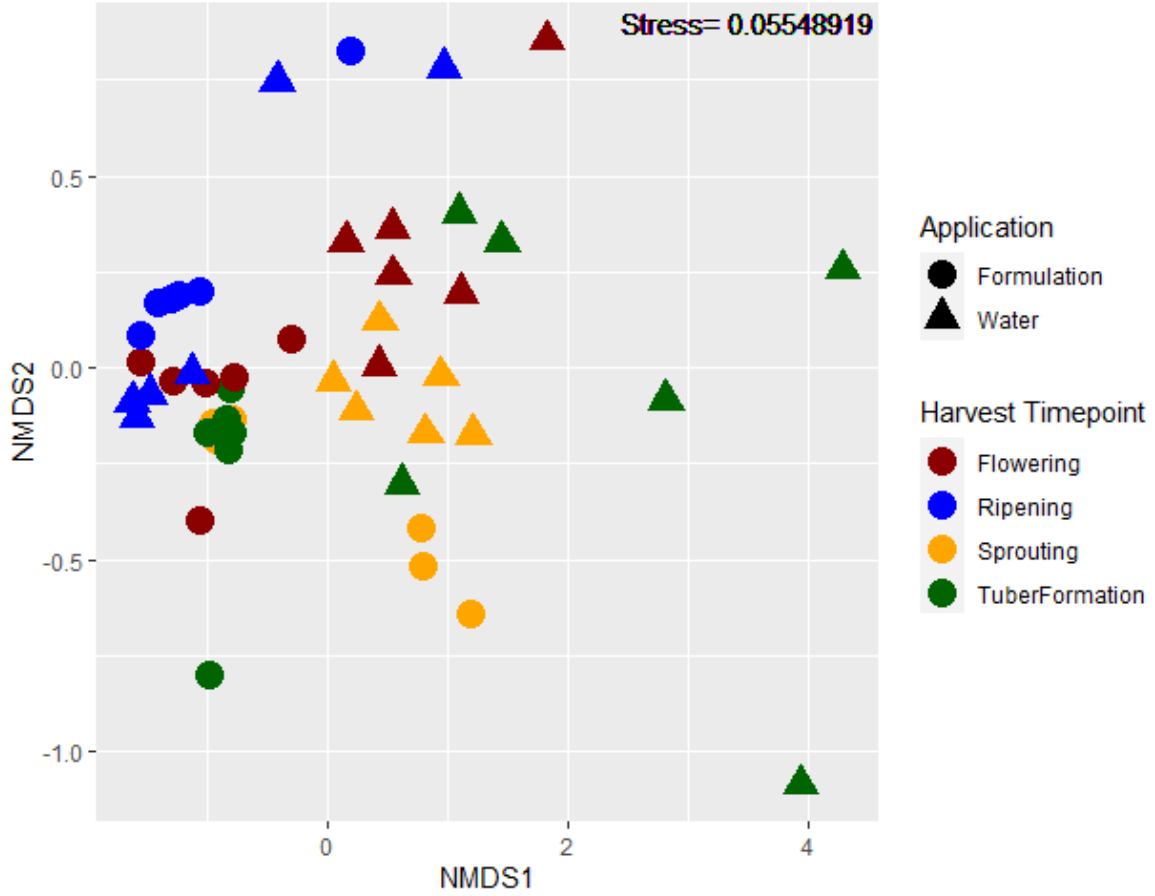



Figure 42: **Bacterial beta diversity obtained from a Bray-Curtis distance matrix displayed as NMDS plot.**

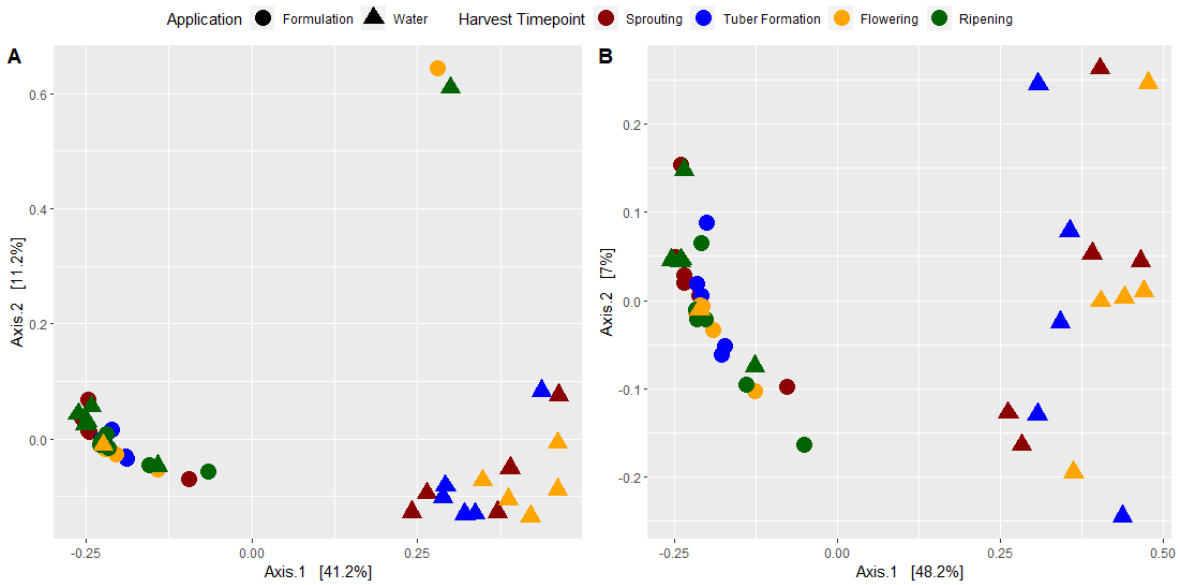



Figure 43: **Fungal community composition resulting from Bray-Curtis distance matrix (A) and with exclusion of two outliers (B).**

In addition to the microbial diversity, we observed significant differences in the microbial composition between the treatments and harvest time points for both bacterial (treatment: $R^2$=0.115, p=0.001, harvest: $R^2$=0.157, p=0.001 and treatment*harvest: $R^2$=0.109, p=0.001) and fungal (treatment: $R^2$=0.205, p=0.001, harvest: $R^2$=0.097, p=0.015 and treatment*harvest: $R^2$=0.111, p=0.004) communities. The adonis-based comparisons of the microbial communities revealed that the bacterial community is stronger influenced by the sampling time than by the treatment. In contrast for the fungal community, we observed a stronger influence of the treatment than the sampling time point. These differences were also observable in a NMDS and PCoA plot for bacterial and fungal communities, respectively (Fig 43, 44). Especially for the bacterial community composition the treated samples form a very narrow cluster, and the untreated ones are more scattered and diffused. Interestingly, the water control samples from the last sampling time point tend to cluster with the formulation treated samples. For the fungal community a similar pattern was observed. Again, the treated samples form a confined cluster, while the untreated samples are more dispersed and the control samples from the last sampling time point group with the formulation samples.

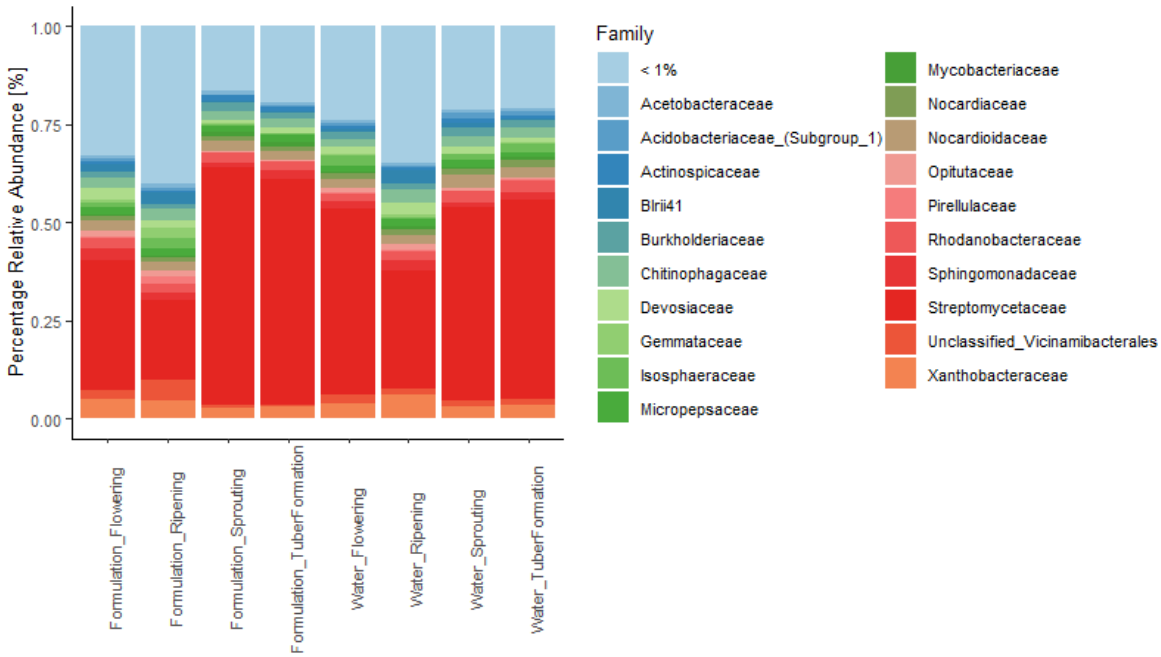



Figure 44: **Bacterial taxonomic composition on family level.**

*Actinobacteria* was the dominating phylum (54.5%), followed by *Proteobacteria* (22.5%), and together they made up almost 80% of the relative abundance. On family level *Strepto-*

*mycetaceae* was the most abundant taxon (43.6%), followed by the ASVs with low abundance below 1% (Fig. 45). However, the abundance of the *Streptomyceae* varied and increased over time, especially for the formulation treated samples.

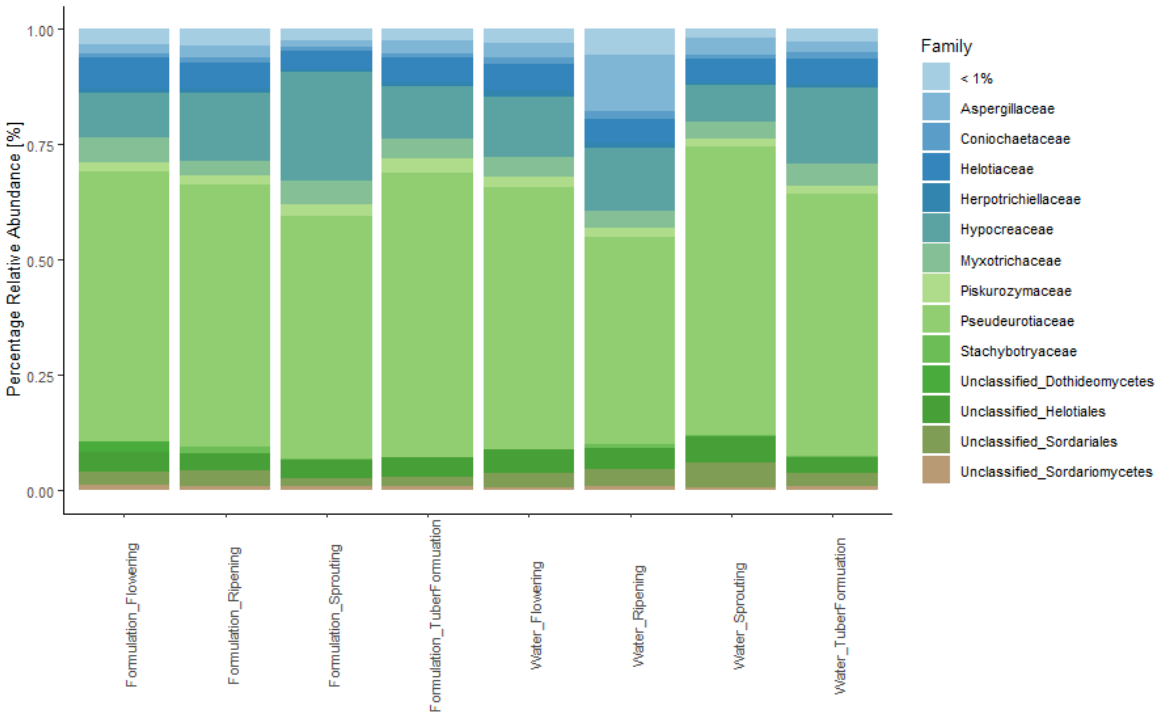



Figure 45: **Fungal taxonomic composition on family level.**

The fungal dataset was dominated by *Ascomycota* with a relative abundance of 96.3%. On family level the *Pseudoeurotiaceae* (56.3%) were the most abundant group, followed by the *Hypocreaceae* (13.8%) (Fig. 46). The water control showed higher abundance of *Aspergillaceae* (5.3%) than the formulation treated samples (2.3%).

### 3.2.11 Effect of the beneficial consortium 'Patricia' containing 2-methyl-1-butanol on different potato genotypes

Due to the fact that the first greenhouse experiment, in which the formulation efficiency with different potato genotypes had to be evaluated, was not successful, a second greenhouse experiment was conducted during summer 2021. The aim of this experiment was to identify the most fitting potato genotypes, which showed the best interaction effects with the beneficial microbial consortia against *R. solani* and *V. dahliae*, out of the eleven that were preselected by the international project partners. The experiments were conducted with the same soil as used by the project partner in Munich in order to increase the comparability. Planting pots were filled with soil (delivered from Bavaria) and watered for one

week. After one week the plantlets were planted into the pots. The plantlets were acclimatized for two weeks via addition of more water than the specific water holding capacity (WHC) of 60%. After acclimatization, the aim was to adjust the soil to the WHC 60% and to grow the plantlets for three additional weeks. As the greenhouse was not air-conditioned, it was not possible to keep the WHC at 60%. In the fifth week, the stress induction of the plantlets via infection with the pathogen was conducted. In the treatment groups, the plantlets were inoculated with the beneficial consortia. In the seventh week, leaves were harvested for the detection of plant volatiles and sent to the partner MPI in Potsdam. The final harvesting was conducted in week eight; here, the DNA sampling of the rhizosphere and further amplicon sequencing will be conducted. Additionally, the biomass was evaluated and the roots were sampled and sent to the partner UPPA in Pau for detection of endophytes.

The assessment of the effect of the beneficial consortia resulted in genotype specific effects. Differences in the number of tubers after infection with *V. dahliae* were observed for the genotypes 'Atol' and 'Krab'. Consortia 'Patricia' supplemented with 2-methyl-1-butanol (booster) increased the number of tubers, whereby the contrary effect of this formulation was observed for the genotype 'Krab' (Fig. 47). Furthermore, consortium 'Patricia' decreased the growth of the shoots of potato breed 'Bihoro' (Fig. 48). The same effect of consortium 'Patricia' was observed for the length of the roots of potato breed 'Rudawa'. Consortium 'Patricia' decreased the length of the roots, but if the consortium was supplemented with the boosting substance the growth of the roots increased (Fig. 49).

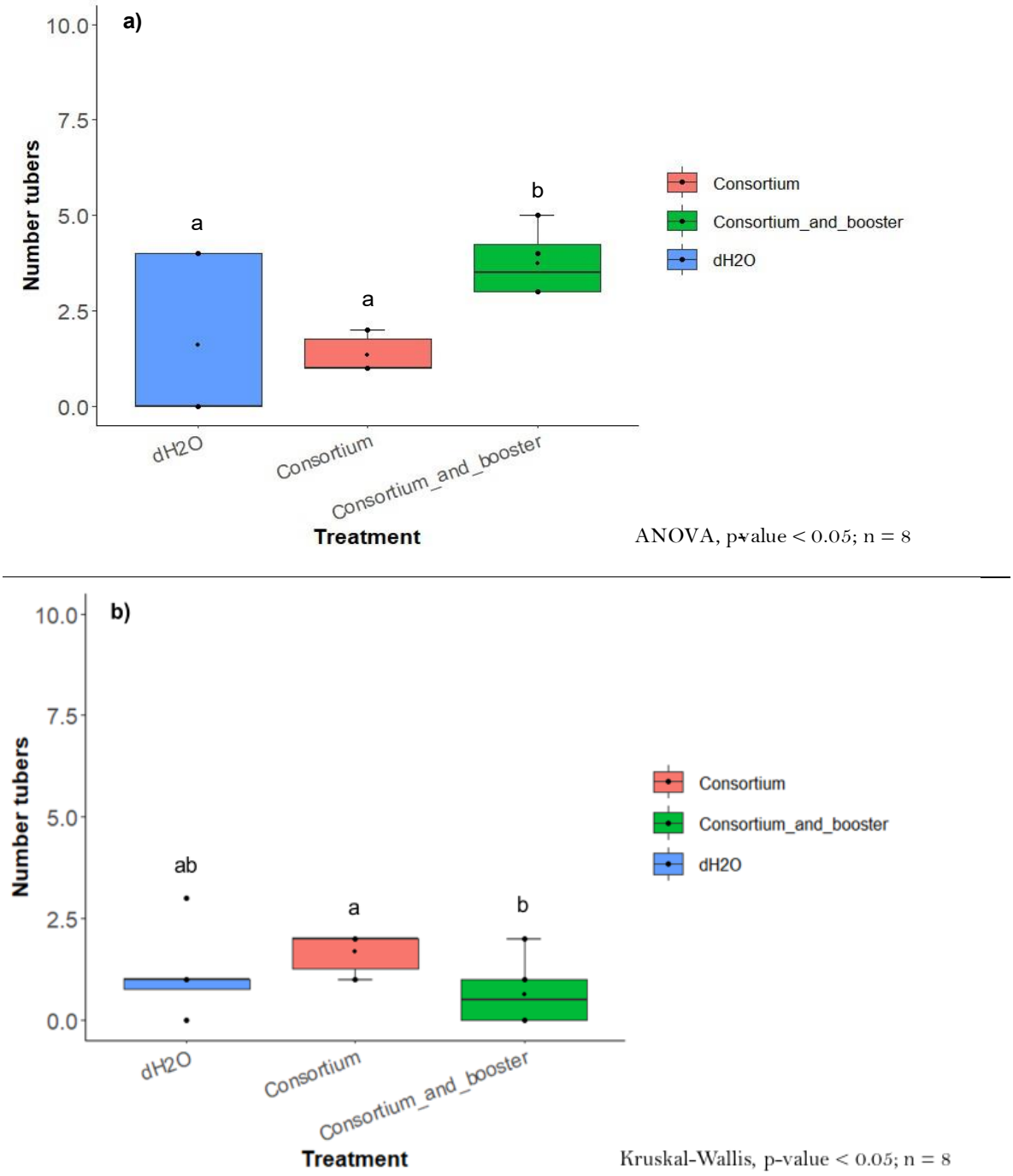



Figure 46: **Assessment of the tuber number of potato breed 'Atol' and 'Krab' after infection with the pathogen** *V. dahliae* **ELV16**. The plantlets were treated with water, consortium 'Patricia' or consortium 'Patricia' supplemented with 2-methyl-1-butanol. (a) Potato breed 'Atol'. (b) Potato breed 'Krab'. The sample size was n=8.

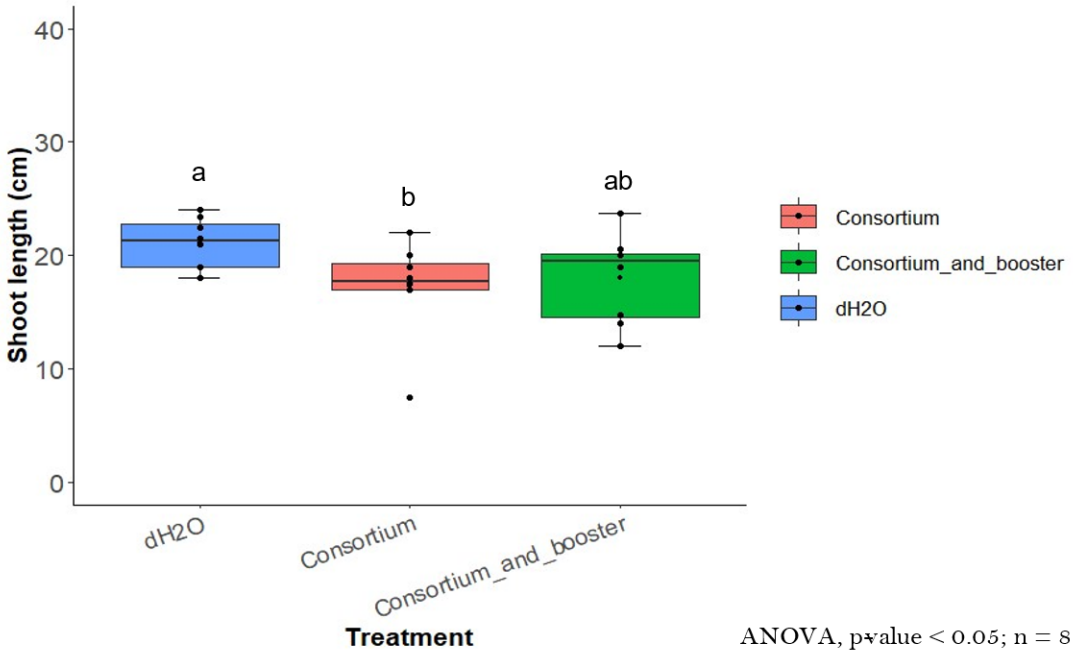



Figure 47: **Assessment of the length of shoots of potato breed 'Bihoro' after infection with the pathogen** *V. dahliae* **ELV16**. The plantlets were treated with water, consortium 'Patricia' or consortium 'Patricia' supplemented with 2-methyl-1-butanol. The sample size was n=8.

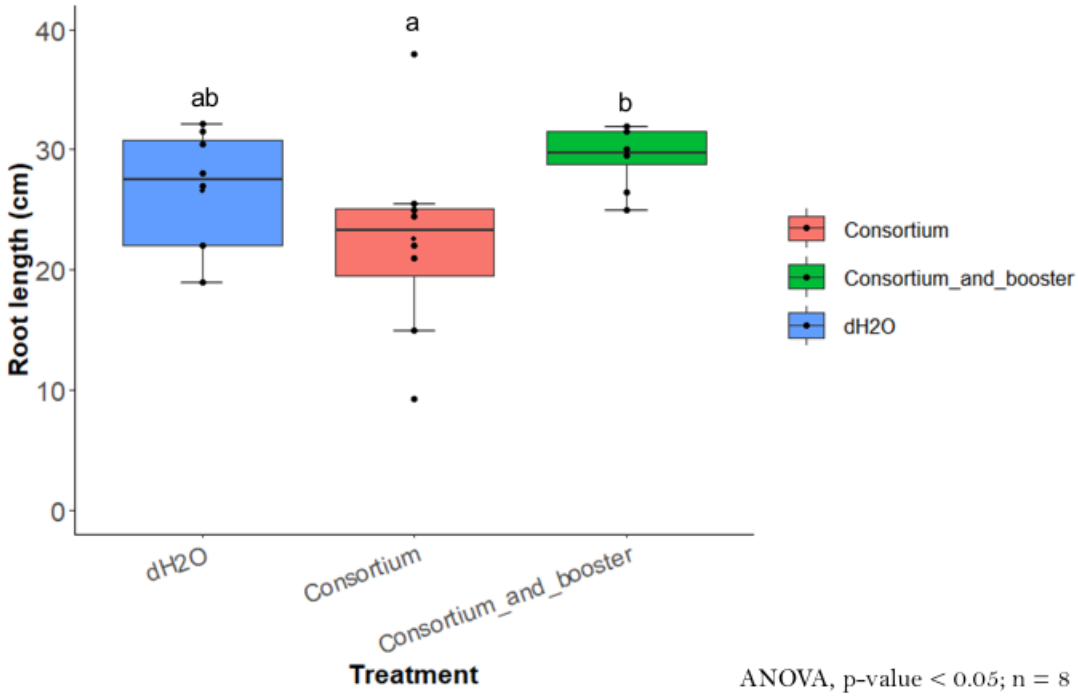



Figure 48: **Assessment of the length of the roots of potato breed 'Rudawa' after infection with the pathogen** *V. dahlia* **ELV16**. The plantlets were treated with water, consortium 'Patricia' or consortium 'Patricia' supplemented with 2-methyl-1-butanol. The sample size was n=8.

For the assessment of the effects of the formulation on the genotypes after infection with *R. solani*, different genotypes were shown to be differentially affected compared to the experiment with the pathogen *V. dahliae*. The growth of the shoots of potato breed 'Brda Stara' and 'Desiree' increased after inoculation with consortium 'Patricia' (Fig. 50).

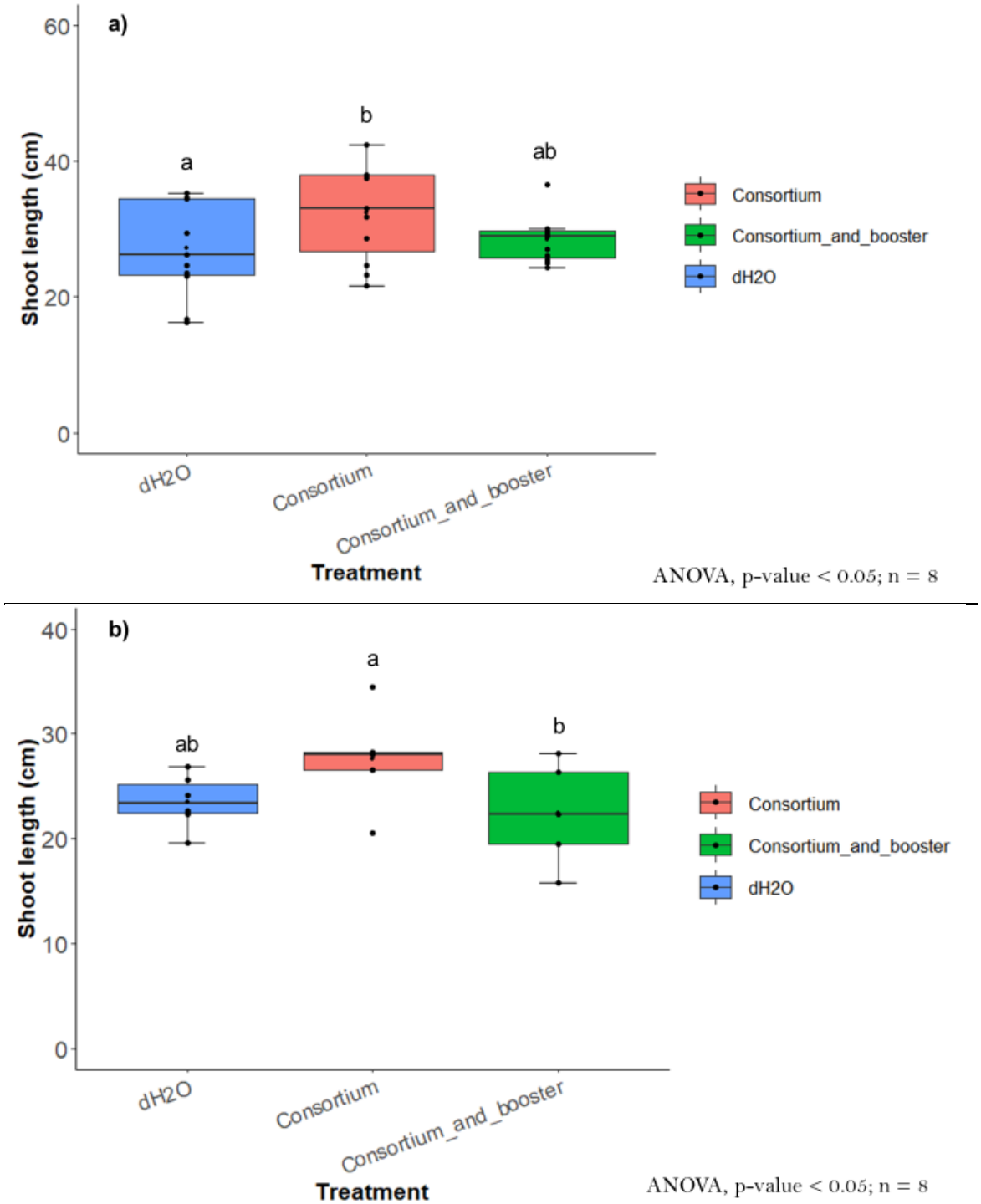



Figure 49: **Assessment of the length of the potato shoots of breed 'Brda Stara' and 'Desiree' after infection with the pathogen** *R. solani* **AG-3.** The plantlets were treated with water, consortium 'Patricia' or consortium 'Patricia' supplemented with 2-methyl-1- butanol. (a) Potato breed 'Brda Stara'. (b) Potato breed 'Desiree'. The sample size was n=8.

Furthermore, the shoot weight of potato breed 'Pasja Pomorska' (Fig. 51) and the dry weight of the shoot of potato breed 'Salto' (Fig. 52) decreased after the application of consortium 'Patricia' supplemented with 2-methyl-1-butanol.

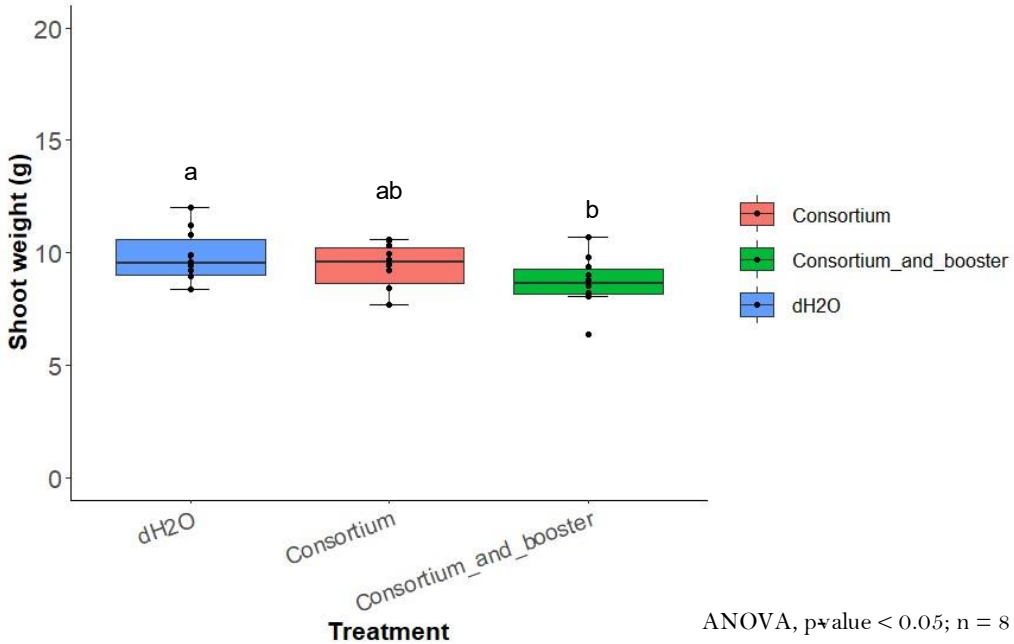



Figure 50: **Assessment of the weight of the shoots of potato breed 'Pasja Pomorska' after infection with the pathogen** *R. solani* **AG-3.** The plantlets were treated with water, consortium 'Patricia' or consortium 'Patricia' supplemented with 2-methyl-1-butanol. The sample size was n=8.

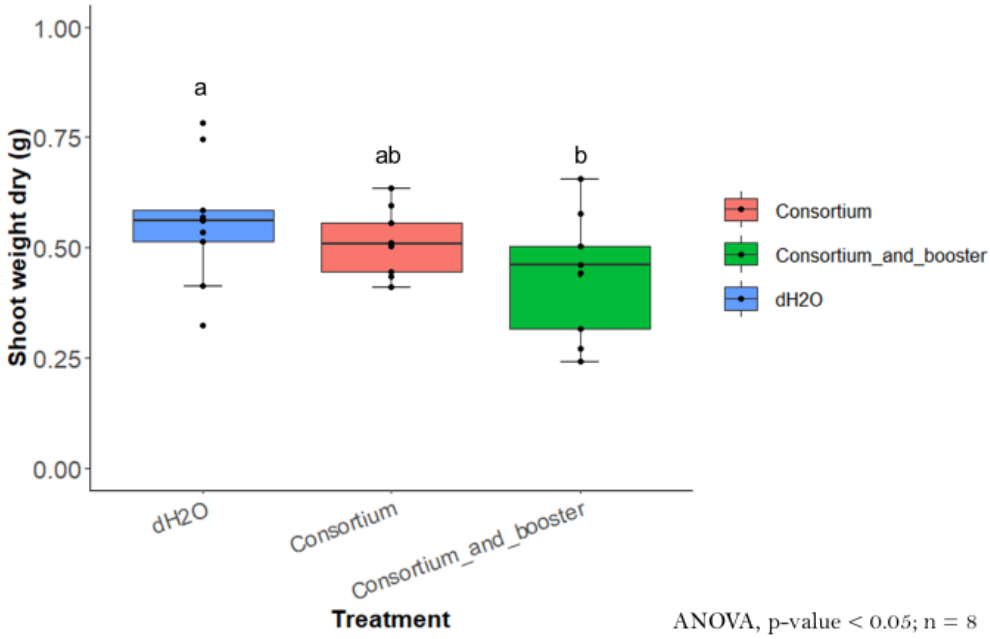



Figure 51: **Assessment of the dry weight of the shoots of potato breed 'Salto' after infection with the pathogen** *R. solani* **AG-3**. The plantlets were treated with water, consortium 'Patricia' or consortium 'Patricia' supplemented with 2-methyl-1-butanol. The sample size was n=8.

Since the formulation did not significantly improve the shoot growth of several potato cultivars, it was decided to analyse the microbiome composition of the rhizosphere from the field trial, descibed in chapter 3.2.7, instead.

### 3.2.12 Effect of 2-methyl-1-butanol on the production of volatiles on the beneficial consortium 'Patricia'

A gas chromatography – solid phase mass spectrometry (GC-MS) headspace analysis was performed to detect volatiles produced by the bacterial consortium 'Patricia'. The aim of this experiment was the detection of volatiles which are produced or consumed by the consortium after the supplementation of the boosting substance 2-methyl-1-butanol. For the preparation of the GC-MS analysis the consortium was streaked either on Nutrition Broth II agar or on Nutrition Broth II agar containing 2-methyl-1-butanol (0.5% v/v). 2-methyl-1-butanol increased the production of '1-butanol, 2-methyl, acetate', '1-Butanol, 2-methyl-, propanoate', 'Propanoic acid, 2-methyl-, 2-methylbutyl ester', 'Butanoic acid, 2-methyl-, 2-methylbutyl ester' and 'Butanoic acid, 3-methyl-, 2-methylbutyl ester' and the consumption of 'Dimethyl trisulfide' of the consortium 'Patricia'. 'Butanoic acid, 2-methylbutyl ester' was

only produced by consortium 'Patricia' supplemented with the boosting substance. In contrast, 1-Undecene was only produced by consortium 'Patricia' itself. Benzaldehyde was consumed via both formulations in the same amounts.

Table 11: **Evaluation of the boosting effect of volatile production by consortium 'Patricia' supplemented with 2-methyl-1-butanol**. In order to obtain insights in the volatile production or consumption through the formulations a GC-MS headspace measurement was performed. 50 µl of the bacterial consortium with an OD600 of 0.8 were streaked either on Nutrition Broth II agar or Nutrition Broth II agar containing 2-methyl-1-butanol (0.5% v/v).

| Volatile | Patricia | Patricia and Booster |
|---|---|---|
| **1-butanol, 2-methyl, acetate** | Constant production | Production increased |
| **Benzaldehyde** | Consumption | Consumption |
| **Dimethyl trisulfide** | Consumption | Consumption increased |
| **1-Butanol, 2-methyl-, propanoate** | Constant production | Production increased |
| **Propanoic acid, 2-methyl-, 2-methylbutyl ester** | Constant production | Production increased |
| **Butanoic acid, 2-methylbutyl ester** | Not produced | New production |
| **1-Undecene** | New production | Not produced |
| **Butanoic acid, 2-methyl-, 2-methylbutyl ester** | New production | Production increased |
| **Butanoic acid, 3-methyl-, 2-methylbutyl ester** | New production | Production increased |

## Tabellenverzeichnis

## Abbildungsverzeichnis

**Literaturverzeichnis**

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## Abkürzungen

| | |
|---|---|
| ANOVA | Analysis of variance |
| ASV | Amplicon sequence variant |
| °C | Degree Celsius |
| CFU | Colony forming unit |
| cm | centimeter |
| DCA | Dual culture assay |
| DNA | Deoxyribonucleic acid |
| fdr | False discovery rate |
| Fig | Figure |
| g | gram |
| Ha | Hectar |
| ITS | Internal transcribed region |
| Kg | Kilogram |
| m | Meter |
| min | Minute |
| ml | Mililiter |
| mm | Millimeter |
| NMDS | Non-metric multidimensional scaling |
| PCR | Polymerase-Chain-Reaction |
| PDVA | Petri dish VOCs assay |
| rRNA | Ribosomal ribonucleic acid |
| SCAM | Strain collection of antagonist microorganisms |
| sp. | Species |
| TCVA | Two clamp VOCs assay |
| VOC | Volatile organic component |
| WP | Work package |
| µg | microgram |

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Institut für Umweltbiotechnologie
https://www.tugraz.at/institute/ubt/home