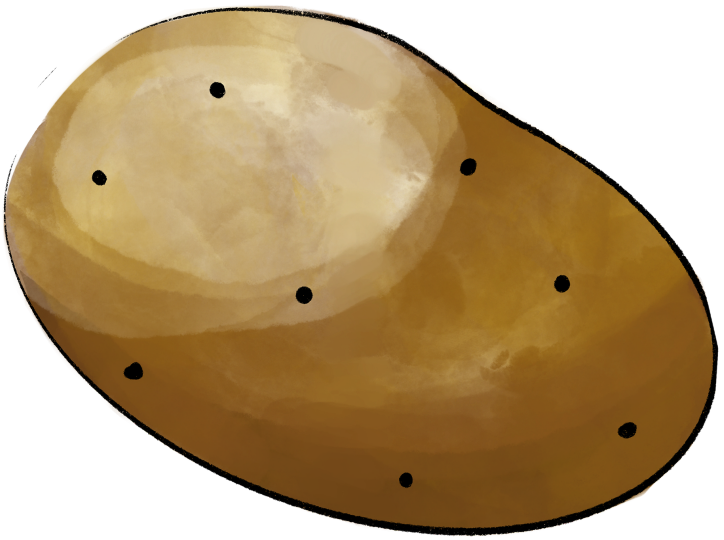
Exploring the link between the diversity of the potato microbiome and potato plant vitality

A contribution to the development of a prediction model for potato plant vitality according to its microbiome



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Layperson summary

When considering changing agricultural practices for a sustainable future, potatoes would be a topic of importance as they are the world's fourth most important staple food. After observing varying levels of vitality of the same potato variety, it was hypothesised that the microbiome (the microbial community that lives with its host) of the plants could be responsible, as an optimal microbiome can contribute to plant vitality and fitness. This is why Utrecht University and its collaborating partners are working on a prediction model that could predict the potato plant's vitality by looking at the microbiome of a batch of seed potatoes. This study hopes to contribute to this project by looking at the diversity of the potato microbiome. The diversity of the plant's microbiome is suggested to be a key factor for good plant performance and can be influenced by various variables. Therefore, the vitality of six different potato varieties, two soil types and two harvest years were compared in relation to their respective microbial diversities. The statistical analysis was performed focussing on the bacterial data of microbiome species present in the eye compartment of the seed potato tuber. In this study, it was found that the different variables relate differently to the microbial diversity of the potato microbiome and potato plant vitality. Soil type showed no influence on the alpha diversity but it did show to influence the beta diversity greatly. The variety of the potato and its harvest year showed to influence both alpha and beta diversity (two ways to describe species diversity) of the tuber's microbiome, though there was no link found between the alpha diversity of these variables and the potato plant vitality. Further research is necessary to find out what underlying factors are essential for high plant vitality in order to be able to develop the prediction model.

Abstract

This study hopes to contribute to the research on the design of a prediction model that uses biomarkers in the seed potato tuber microbiome to predict the plant's performance by exploring the link between the diversity of the potato microbiome and potato plant vitality. A 16S amplicon library was made of the tuber's eye compartment to look into the influence of environmental conditions, genotype and type of soil type on the link between microbiome diversity and plant performance. Additional assays were performed to add depth to the existing protocols. The harvest year of the potato tubers and the variety showed to influence both alpha and beta diversity of the tuber's microbiome, though these variables did not show a correlation between the alpha diversity of the microbiome and the potato plant vitality. Soil type showed no influence on the alpha diversity but it influenced the microbial community composition (beta diversity) greatly. Further research is necessary to elucidate the relationship between the diversity of the potato microbiome and the potato plant vitality for the development of a prediction model.

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Introduction

Climate change and demographic development are putting crop production under pressure with increasing demand and need for sustainable agriculture (Compant et al., 2019). In terms of sustainable agriculture, microorganisms have shown to be potential biofertilizers and biopesticides and there is increased interest to integrate them as alternatives for chemical products in agricultural practices (Compant et al., 2019). Moreover, awareness is growing within the scientific community as well as policymakers of the importance of soil biodiversity for ecosystem goods and services (Bardgett & Van Der Putten, 2014). More research on microbes is therefore important as they can potentially contribute to improved food production and quality (Barnett et al., 2015; Buchholz et al., 2019; Akimbekov et al., 2020; Jeanne, Parent & Hogue, 2019; Hao & Ashley, 2021).

Some micro-organisms can positively influence plants and because plants are sessile organisms, they rely heavily on their direct environment (Figure 1). These microbes can form symbiotic relationships with their host plants and offer functions such as the uptake and storage of water and nutrients from the surrounding soil (Pascale et al., 2020), improving root architecture and supporting the host plant against abiotic and biotic stressors (Bakker et al., 2018; Van Der Heijden & Hartmann, 2016).

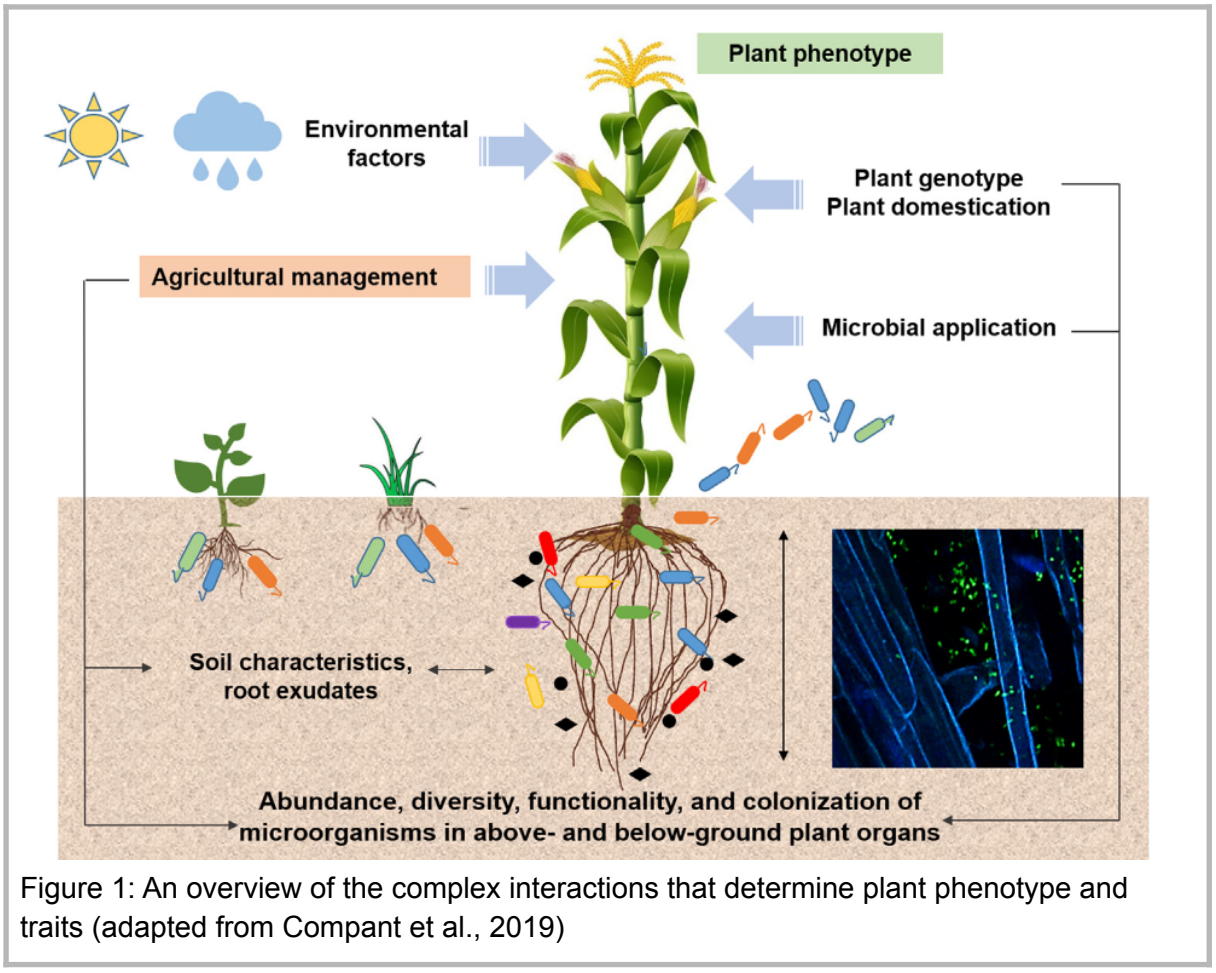


Figure 1: An overview of the complex interactions that determine plant phenotype and traits (adapted from Compant et al., 2019)

Different parts of a plant can host different compositions and levels of diversity of microbes (Dastogeer et al., 2020). For instance, plants provide ecological niches with their root system for specific microbes (Compant et al., 2019). This micro-ecosystem is called the rhizosphere, which is influenced by plant roots through rhizodeposition of secreted molecules, gum and sloughed cells (Turner, James & Poole, 2013) (Figure 1). The root exudates can consist of a variety of compounds, predominantly organic acids and sugars, and are the currency used in the symbiotic relationship between beneficial microbes and their plant host (Compant et al., 2019). With the root exudates, plants can recruit the right microbes that are present in the soil and with this specific recruitment influence the composition of their microbiome in their favour (Berendsen, Pieterse & Bakker, 2012). Although the interaction between plants and soil microbes might be below ground, its effects can extend to the plant's phenotype above ground (Zamioudis & Pieterse, 2012). Beneficial microbes can for instance promote plant growth by influencing the host plant's hormonal balance (Pascale et al., 2020). Another example is the increase in plant defence which is called induced systemic resistance (Van der Ent, Van Wees & Pieterse, 2009). An optimal microbiome can contribute to plant vitality and fitness directly and indirectly, for instance by stimulating growth.

When considering improving food production and quality, the world's staple crops should be a focus point. After rice, wheat and corn, potatoes are the world's fourth most important staple food (Buchholz et al., 2019). The Netherlands' second top production commodity is potatoes, where 2,064,784 tonnes of fresh potatoes were used for export in 2020, with an additional 1,613,784 tonnes of frozen potatoes that were exported that same year (FAOSTAT, 2020). This makes potatoes a topic of importance when considering food security and economic trade, which makes optimizing the production of potatoes of everyone's interest.

This paper contributes to a study by collaborating organisations, namely Utrecht University, Delft University of Technology and HZPC (one of the biggest companies in the seed potato trade in the world). This study investigates the presence of biomarkers, members of the potato microbiome, that could indicate the viability of the potato batches after harvesting. With this knowledge, they hope to be able to make predictions for the vitality that can be expected for this batch, regardless of geographic site. Determining biomarkers that can predict the viability of seed potatoes could contribute to sustainable agriculture as it could help reduce the use of pesticides and chemical fertilizers, since the viability can be taken into account during trade. Additionally, this could enlarge the arable land that could be used for potato production, resulting in a contribution to food security and bringing us closer to a sustainable future.

This study began after HZPC found that plants of the same potato variety showed varying levels of vitality at different farms within the Netherlands. This was a surprising observation since agricultural practices should be highly similar and the plants would be genetically the same since they are of the same cultivar. When cultivating seed potatoes from these different farms in the same plot, the differences in plant performance were observed once again. The second generation of these potato plants also showed different levels of vitality when grown on the same plot of land. Since there should be close to no genetic and environmental differences between these potato plants, it was hypothesised that the microbiome of the plants could be causing the differences in vitality since the composition can differ greatly between plants obtained from different soils (Figure 1).

The reasoning behind this hypothesis was that, apart from the soil's physicochemical properties, different sites vary in composition of the soil microbiota, which is the main reservoir of micro-organisms that can colonize plants (Buchholz et al., 2019). One gram of soil can contain thousands of individual microbial taxa, including fungi, protists, viruses, bacteria and archaea (Fierer, 2017). Even so, the type of soil has in fact shown to shape the functional communities of the microbiome of potatoes (İnceoğlu, Falcão Salles & van Elsas, 2012). When looking at wheat, it was found that different harvest years significantly affected the bacterial community structure because various environmental factors were different in each year (Bissett et al., 2013). This study was performed for wheat, but similar results could be possible for different plants as well. As aforementioned, plants are able to recruit soil microbes. How they do this and which composition of microbes forms an optimal microbiome is largely dependent on the genetics of the host plant, also in the case of potatoes (İnceoğlu, Falcão Salles & van Elsas, 2012; Dastogeer et al., 2020). Thus, plant genotype, but also the type of soil and environmental conditions, which can differ from year to year, are known factors that can influence the structural and functional diversity of the bacterial microbiota of potato plants (Dastogeer et al., 2020; İnceoğlu et al., 2010; Fierer, 2017; Buchholz et al, 2019).

The composition of microbes in the microbiome is of importance for an optimal microbiome, as well as their microbial interactions, as symbionts can complement each other (Van Der Heijden et al., 2016). A more diverse microbiome would enlarge the possibility of having the right matches of symbionts present. Furthermore, Maheraldi and Klironomos (2007) found that microbial communities with high realized species richness increased plant performance, more than those with a microbiome with low richness. Additionally, the removal of rare microbial species, which reduces diversity, has been shown to lead to an increase in aphid size and with that possibly feeding damage (Barnett et al., 2015). Taking everything into account, the diversity of the plant's microbiome is suggested to be a key factor for good plant performance (Van Der Heijden & Hartmann, 2016).

In this study, the vitality of six different potato varieties, grown of two types of soil (either sand or clay) and harvested in the years 2018-2019 and 2019-2020 were compared to their microbial diversity. The data on the potato plant vitality were acquired by image analysis on images shot by drones in the field. Taking the heritability of the seed potato into account by looking at the eye compartment of the potato, which is the direct physical link between the mother plant and the next generation, is most interesting. Statistical analysis was therefore performed focussing on the 16S sequencing data of microbiome species present in the eye compartment of the potato, as was similarly done by Buchholz et al. (2019). This study looked into the differences in mainly alpha diversity, which is the mean diversity of species in different sites, of the potato microbiome (similarly to Barnett et al., 2015) between three variables. The aim of this study is to find out whether variety, soil type and/or harvest year could influence the bacterial diversity of the potato microbiome, and whether there is a link between the diversity of the potato microbiome and potato plant vitality.
A greenhouse assay and primer performance test were performed additionally to support the experimental set-up.

Methods

Various protocols were used during this research. Most of the protocols were adapted from Casper Jongekrijg's and Ellen Mander's protocols (2020), but some are also adapted from guides provided by Qiagen. Here, the methods described are a concise version of these protocols, starting with the 16S & ITS amplicon library preparation, then the *S. ruber* and primer assay and lastly the greenhouse assay.

Cultivating *Salinibacter ruber* (*S. ruber*) and isolating its DNA

First, *Salinibacter ruber* was cultivated and its DNA was isolated. The isolate was used later on, but should be prepared in advance to maintain the right order within the protocol.

Cultivation and isolation

S. ruber was found in Spain where it lives in a very salty environment and cannot survive salt concentrations below 15%. This is why *S. ruber* requires a growth medium with high salt content and a pH of 7,2. After preparing the growth medium (DSMZ, 2007), it was inoculated with *S.ruber (*ordered from DSMZ® as an actively growing culture). Culturing took about 6 weeks in liquid culture and over 3 months on a solid culture (20g/l agar). The DNA isolation of the *S. ruber* cultures was done using the MagAttract® Microbial DNA Kit of Qiagen, which uses the Thermo Scientific™ KingFisher™ Flex Purification System.
For KingFisher-mediated PCR clean-up the same solutions are used as for the "16S Metagenomic Sequencing Library Preparation protocol". Here, 1.8 ml of liquid culture was dispensed into each required well of a 2 ml Collection Plate and centrifuged at 4500 x g for 12 min. The kit's lysis Solution MBL was incubated at 60°C for 10 minutes and 9 µl RNase A was added per mL solution MBL. Now, 350 µl of this mixture was added to each well with culture and the plate was vortexed. The cell suspensions were then transferred to a PowerBead DNA Plate, Glass 0.1 mm, which was placed in the TissueLyser II machine (QIAGEN) twice at 20 Hz for 5 min at different orientations. The plate was then centrifuged and the supernatant was put in a clean 1 ml Collection Plate or a KingFisher™ deep well plate with 100 µl of Solution IRS per well. The plate was vortexed for 5 sec and incubated at 2–8 °C for 10 min, then centrifuged at 4500 x g for 9 min. Now, 450 µl of supernatant was transferred on a KingFisher deep well, together with 500 µl of SwiftMag Beads/ethanol (1:9). This plate was placed into the KingFisher machine, together with 1 Kingfisher deep well plate with a tipcomb, another 3 with 1 ml 96% alcohol and 1 KingFisher elution plate with 100 µl of Solution EB. This was all done following the instructions of the KingFisher machine with the PowerMag® Microbial DNA Isolation protocol program initiated.

Sanger sequencing of *S. ruber* isolate

To check whether the DNA isolate was pure, Sanger sequencing was used. After DNA isolation, the DNA concentration needed to be measured so normalisation was possible for the PCR amplification of specific regions for Sanger sequencing. The dsDNA BR (BR=broad range) kit for the QUBIT machine was used for measuring DNA concentrations. The results were then used to calculate how much buffer needed to be added for the normalisation. The formula that was used is explained in Appendix 1. For the first PCR cycle, the DNA concentration had to be adjusted to 5 ng/µl. As a buffer, 10 mM Tris-HCl (pH = 8,00) was

used. Hypervariable regions V3 - V4 were amplified using 16S primers B341F and B806R, as well as 5 µl sterile Milli-Q together with 12.5 µl Kapa to a total of 25 µl per reaction. The following PCR program was used: (lid temperature 105 °C, total volume 25 µL) 1) 95 °C for 3 min, 2) 95 °C for 30 sec, 3) 55 °C for 30 sec, 4) 72 °C for 30 sec, 5) go to 2 x 24, 6) 72 °C for 5 min, 7) 10 °C forever.
For Sanger sequencing, the amplified DNA concentration had to be adjusted to 50 ng/µl if the fragments are between 300 and 700 base pairs in length. The DNA concentration was measured again and normalised. Then, 5 µl of 50 ng/µl amplified DNA was combined together with 5 µl of 2 µM forward primer (the same that were used for the PCR step) and sent to The Macrogen Sanger facility.

16S and ITS amplicon library preparation

This part was adapted from Casper Jongekrijg's protocol. With the amplicon library, the microbial composition of the potato microbiome can be examined. HZPC supplied the potato samples for this part of the study. The potato samples were collected from two harvest years (2018-2019 and 2019-2020) and processed into four replicates per sample compartment (like the eye compartment).

Grinding

The potato samples, which were delivered freeze-dried and had a flaky consistency, were processed into a powder. This is a necessary step for DNA isolation since the volume-surface ratio influences the isolation process. Sterile metal beads (3 beads of 5-6 mm diameter) were added to every sample tube under sterile conditions and the tubes were placed in the paint shaker machine SK350 of Fast & Fluid for 3 minutes. The samples were kept cool for most of the time with dry ice. The beads were well rinsed, disinfected with 70% alcohol and dried under sterile conditions before they were to be used again (Appendix 2).

Preparation

At this point, the original amount of potato samples was halved by combining replicates 1 & 2 and 3 & 4. Using a small scooping tool, a rough 1:1 mix of two samples was made in an Eppendorf tube under sterile conditions. The pooled samples were shaken and mixed thoroughly before continuing with the next step where the samples were loaded into the KingFisher bead plates. Each well was filled with 75 mg (pooled) sample powder using a scoop and the exact weights were documented in an Excel sheet.

DNA isolation

For the actual DNA isolation of the samples, bead solution and solutions C1, C5 and C6 were prepared according to Appendix 3. Here, 750 µl of solution A (400 µl RNAse & 75 ml bead solution) and 60 µl C1 were added to each well, whereafter the bead plate was put into the TissueLyser II machine (QIAGEN) twice for 10 minutes at 20 Hz at different orientations. Afterwards, the plate was placed in the centrifuge and spun for 6 minutes at 4500 g. 400 µl of supernatant was transferred into a deep well KingFisher plate, together with 470 µl of solution B (2 ml beads & 45 ml binding solution). Now, 3 more KingFisher deep well plates were filled with 500 µl C5 per well and another one was used to place the tip comb inside. Then a KingFisher standard 96 well plate was necessary for the elution step, with 100 µl C6

in every required well. The KingFisher DNA isolation protocol was chosen for the machine and the plates were loaded into the machine as was indicated by the KingFisher machine. Before continuing to the first PCR cycle, it was necessary that the DNA concentrations were the same for all samples. This is why the DNA concentration needed to be measured and normalised. This was done the same way as for Sanger sequencing.

First PCR

During the first PCR, the amplicons were amplified. For fungi, the ITS region was targeted and for bacteria the 16S region. Both regions need their own primers and PCR settings, so it is not possible to amplify both 16S and ITS at the same time. For the first PCR cycle, Illumina primers were used (see Appendix 4.). These primers have a specific sequence at the end that allows for index PCR. 2.5 µl was transferred from every sample to a PCR plate together with 2.5 µl of the forward and reverse primers, as well as the forward and reverse blocking primers. When 12.5 µl KAPA was added, the PCR plate was put in the PCR machine with the following settings: 1) 95 °C for 3 min, 2) 95 °C for 30 sec, 3) 75 °C for 10 sec (blocking step, only if 16S), 4) 55 °C for 30 sec, 5) 72 °C for 30 sec, 6) go to 2 for (24 times if 16S / 9 times if ITS), 6) 72 °C for 5 min, 7) forever 10 °C. PCR clean-up is a necessary step in the process of library preparation, for which the KingFisher machine was used. For KingFisher-mediated PCR clean-up the same solutions were used as for the “16S Metagenomic Sequencing Library Preparation protocol”. One KingFisher deep well plate was filled with PCR product and 20 µl AMPure beads was added to each well. Two KingFisher deep well plates were filled with 200 µl with 80% alcohol and another plate with the KingFisher tip comb. Finally, a KingFisher elution plate was filled with 30 µl 10 mM Tris-HCl (C6) and all the plates were placed into the Kingfisher machine.

Second PCR

The second PCR is also called index PCR because of the use of index primers, which end up giving each sample its individual label/code. For the index PCR, 20 primers total were used to create 96 unique combinations for every well. The index primers bind to the end of the Illumina primer sequence from the first PCR to amplify the target DNA even more. The same 20 primers were used for either 16S and ITS, but since the first set of primers were different it was again not possible to combine both 16S and ITS samples in this step. For each well, 2,5 µl was used for both index primers, whereafter 12.5 µl KAPA and 5 µl sterile Milli-Q H2O were added. The settings for the PCR machine were this time: 1) 95 °C for 3 min, 2) 95 °C for 30 sec, 3) 55 °C for 30 sec, 4) 72 °C for 30 sec, 5) go to 2 for (9 times if 16S / 24 times if ITS), 6) 72 °C for 5 min, 7) forever 10 °C. The cleanup step after the PCR was the same as after the first PCR, except only 15 µl 10 mM Tris-HCl (C6) was added to the elution plate.

Gel electrophoresis

To check whether the DNA was amplified correctly, an agarose gel was run. 3 µl of cleaned second PCR product was added to its corresponding well, as well as 3 µl Orange loading dye. At the start and end of each row of samples in the gel, 5 µl of DNA ladder was loaded into the gel. Here, the 1KB plus ladder was used showing many bands from 75 to 20000 base pairs long. For this experiment, the amplified samples were expected to be 650 base pairs. After the gel electrophoresis, the gel was placed under a UV lamp to reveal the location of the DNA (in the shape of a horizontal band).

Sending the samples

Before sending the samples to Useq to be sequenced, the samples first needed to be normalised again. The same protocol for Sanger sequencing was used again for the measuring of the DNA concentration, normalisation and formula, except that desired concentration was now 2 ng/µl: C = ((A*B)/2 ng/µl)-B. When sending in the samples, 96 had to be pooled into 1 tube. Preferably, 16S and ITS samples were combined in one run to minimize the mistakes of the sequencing machine. This is why, per tube, both 16S and ITS samples were combined in such a way that the index primers of each sample in one sequencing run were still unique. The tubes were then delivered to the USEQ pick-up point.

S. ruber and primer assay

In this assay, the performance of the blocking primers used to make the amplicon library was tested in combination with the internal standard (*S. ruber*). For the test, the same procedures were followed as for the 16S and ITS amplicon library preparation except for a few steps. There were samples of 6 potato varieties of 5 compartments (heel end, flesh, peel, eye and soil), which got one of the 6 treatments (16S primers, 16S blocking primers, 16S blocking primers & *S. ruber* DNA isolate, ITS primers, ITS blocking primers or ITS blocking primers & *S. ruber* DNA). Replicates of the same samples were pooled here as well, so that there were 6 pooled samples in the end per compartment per treatment. To limit costs, full use was made of each run, whilst still consisting of half ITS and half 16S samples as was done during the 16S library preparation. This made the compilation of all the samples per run quite complicated and therefore prone to errors. So, randomisation was limited, having two clusters of the 6 varieties per compartment and 2 different treatments per row, for example:

Peel 1 *S.ruber* 16S BP	Peel 2 *S.ruber* 16S BP	Peel 3 *S.ruber* 16S BP	Peel 4 *S.ruber* 16S BP	Peel 5 *S.ruber* 16S BP	Peel 6 *S.ruber* 16S BP	Soil 1 16S	Soil 2 16S	Soil 3 16S	Soil 4 16S	Soil 5 16S	Soil 6 16S

In this example, the first 6 wells were filled with the peel compartment (Peel) of the 6 varieties (1-6) with the 16S blocking primers (16S BP) & *S. ruber* DNA isolate (*S.ruber*) and the last 6 wells contained the soil compartment (Soil) of the 6 varieties with 16S primers (16S).

A final alteration of the 16S and ITS amplicon library preparation protocol was that at the DNA isolation step 750 µl of solution A (400 µl RNAse & 75 ml bead solution) and 60 µl C1, as well as 5 ng *S. ruber* were added to the wells with this treatment.

Greenhouse assay

When a promising microbe is found in the search for biomarkers, it is of interest to test it's effects on potato plant growth in a controlled environment. This is why the greenhouse assay was performed. Here, a pre-existing protocol was upscaled from 50 potatoes to 100 potatoes. In advance was soil, in this case river sand, autoclaved twice at 120°C for 45 min, with a 48 hours interval to be properly sterilised. King's B (KB) medium, here used to grow WCS358r, and 1% methylcellulose, to make the bacteria adhere better to the potatoes, was prepared in advance as well. The potatoes of two varieties (Colomba and Innovator) were marked with a permanent marker and weighed before any of the treatments.

Culturing WCS358r

In the case of *Pseudomonas putida* isolate WCS358r (Geels, 1988), a new KB plate without rifampicin was prepared. From this plate, liquid cultures were incubated at 27°C at 150 rpm for 48 hours in a shaking incubator. Afterwards, the cells were harvested and the OD600 was measured. A dilution with an OD600 value between 0.1 and 1.0 was selected. This dilution was then used to calculate the needed original volume from the bacterial suspension, which was centrifuged to harvest the bacterial cells. Here, the following formula was used: $V_1 C_1 = V_2 C_2$ in which V_1 is the final volume, C_1 is the final concentration, V_2 is the original volume and C_2 is the original concentration. The harvested cells were then added to 1.5 L 1% methylcelluloses to get a 10^9 cfu/mL bacterial concentration for the inoculation treatment of the potatoes.

Planting and harvesting

On the first day of the greenhouse assay, pots were filled halfway with the autoclaved river sand and the potatoes were dipped in the methylcellulose of either treatment, with the beneficial WCS358r or the mock, whereafter they were covered with sand. They were then placed on trays in the greenhouse, where the conditions were arranged at 20°C day temperature, 15°C night temperature, ±70% relative humidity and 14h light/day (06:00-20:00, with curtains open). The potatoes were harvested in two batches at two different time points at a seven-day interval. The tubers were gently removed from their pots and the adhering sand was removed as much as possible without causing damage whereafter the potatoes were weighed. The roots and shoots were, when present, cut off the tuber and all three compartments were weighed and the measurements were recorded in Excel.

Statistical analysis

The sequencing data from the amplicon library received by Useq were first processed by Dr Yang Song in preparation for analysis. She provided the table for the 16S data from the eye compartment and the corresponding metadata table. To make the ASV (Amplicon Sequence Variant) table that was used for the statistical analysis, first, the raw sequencing data were denoised, joined and delineated whereafter Qiime (version v.2019.7) was used to assign the taxonomy (Bolyen et al., 2019). The DADA2 pipeline in Qiime was used for filtering after the dataset was demultiplexed (Callahan et al., 2016). To minimize potential sequencing errors, the ASVs that had less than 30 reads or were hardly present (less than 3 samples) were removed. A classifier, that was trained with the 99% threshold SILVA database, was used to classify the representative sequences taxonomically (Quast et al., 2013). In order to have the dataset purely represent the bacterial reads, the mitochondrial and chloroplastic 16S reads that had remained were removed. For the statistical analysis of the amplicon library, RStudio (R version 3.4.1) was used. The packages vegan, ggplot2, patchwork & phyloseq were used to calculate the diversity indices, and their p-values (< 0.05) and to produce the figures. These diversity indices consisted of richness, Shannon's diversity index, Simpson's diversity index and evenness. Pearson's and Spearman's correlation coefficients were later calculated to test the correlations between diversity and vitality.
To process the results from the greenhouse assay, the analysis function from Excel (version 2203) was used to calculate the significance using the unpaired student's t-test.

Results

The results of this study were mainly acquired by analyzing the bacterial DNA sequences of the eye compartment of the potato samples. The data produced with potato samples that were harvested in 2019-2020 were supplemented with data from 2018-2019 and the plant vitality data was provided.

Comparing alpha diversity

Like other studies that investigated the diversity of the plant microbiome, like Buchholz et al. (2019), this study started with exploring the alpha diversity of three variables, calculated according to multiple indices. Boxplots were made to compare the results of the following four alpha diversity indices: richness, evenness, Shannon's diversity index and Simpson's diversity index, see Figures 2-4. The figures show the differences per alpha diversity index between the three variables that were investigated: the soil type on which the potatoes were grown (Figure 2), the harvest year 2018-2019 and 2019-2020 (Figure 3) and six different potato varieties (Figure 4). The significance ($p < 0.05$) between the results per harvest year and per soil type was calculated using the t.test function of RStudio and for the varieties, ANOVA was used.

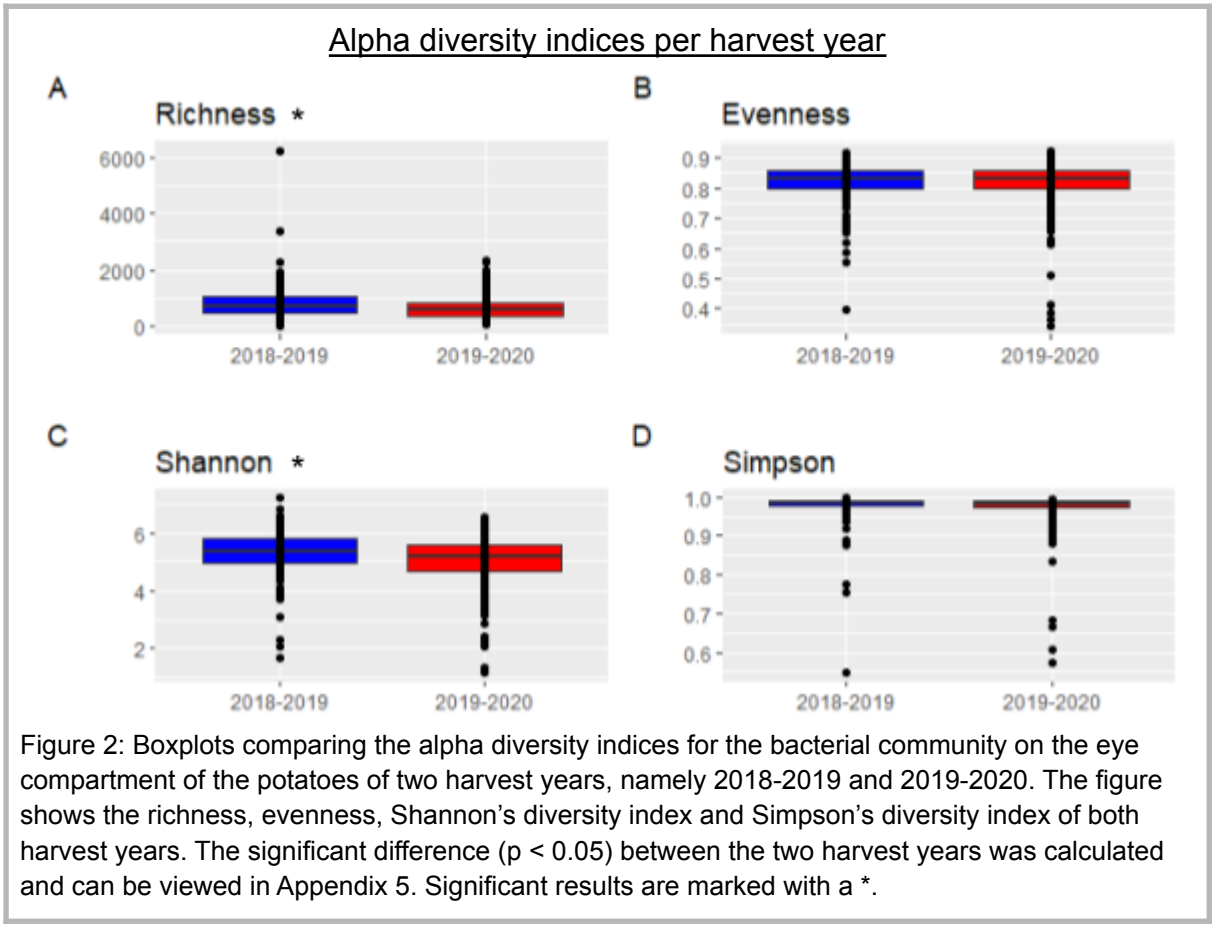


Figure 2: Boxplots comparing the alpha diversity indices for the bacterial community on the eye compartment of the potatoes of two harvest years, namely 2018-2019 and 2019-2020. The figure shows the richness, evenness, Shannon's diversity index and Simpson's diversity index of both harvest years. The significant difference ($p < 0.05$) between the two harvest years was calculated and can be viewed in Appendix 5. Significant results are marked with a *.

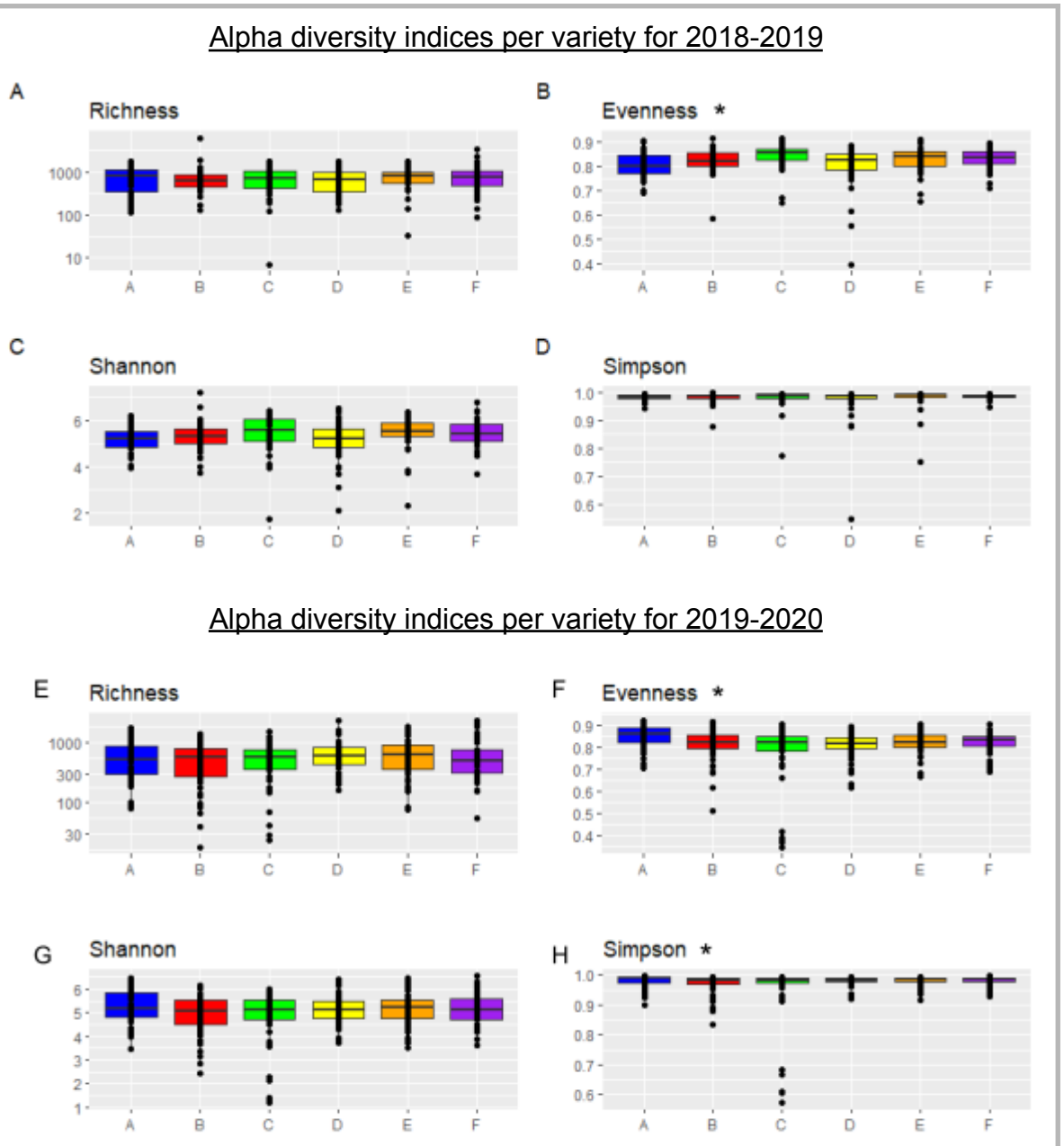


Figure 3: Boxplots comparing the alpha diversity indices for the bacterial community on the eye compartment of the potatoes of all six varieties (A-F). The figure shows the richness, evenness, Shannon's diversity index and Simpson's diversity index of all varieties of each variety. The significant difference ($p < 0.05$) between all varieties per the diversity index can be viewed in Appendix 5 and significant results are marked with a *.

When looking at the differences in diversity between the two harvest years, richness and Shannon's diversity index showed to differ significantly per year (see Figure 2). It was calculated whether any of the varieties differ significantly in terms of diversity in either harvest year. The separation per harvest year helps to exclude environmental differences since the focus is on whether genotype influences diversity. Significant differences were found between the evenness of the varieties collected in both harvest years (see Figure 3). Specifically for the batch of 2019-2020, Simpson's diversity index showed to differ significantly between the varieties. There were no significant differences between the soil

types for any of the diversity indices according to this study (Figure 4). In short, just the variables of harvest year and variety showed to have significant differences in alpha diversity, but never for all calculated alpha diversity indices.

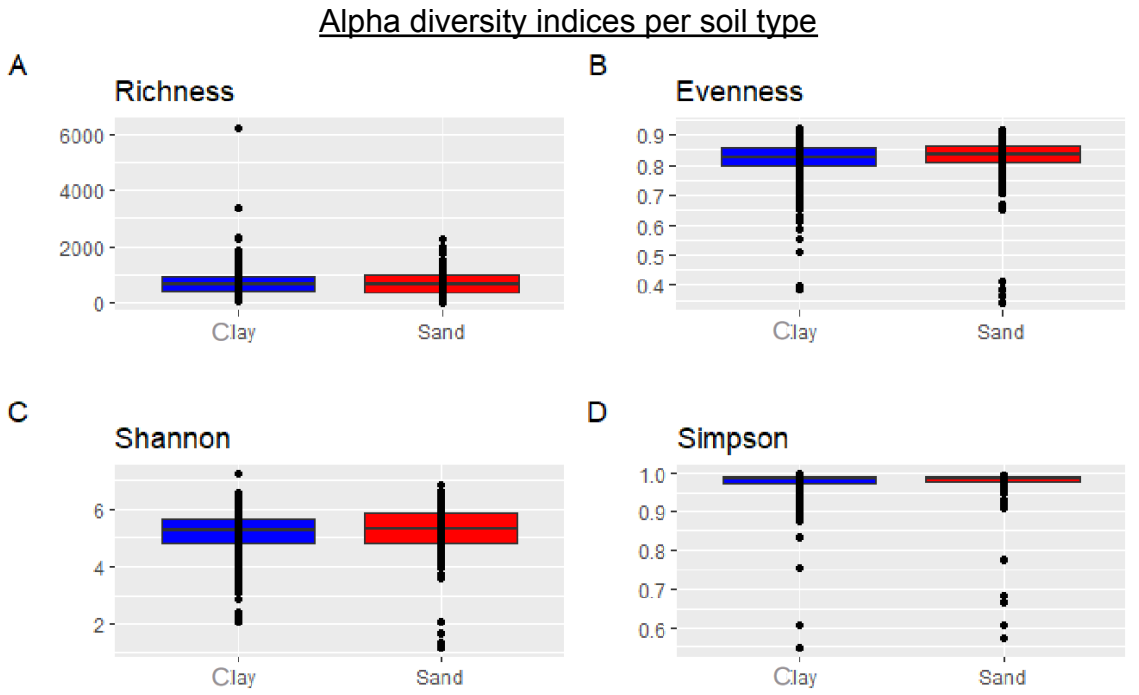


Figure 4: Boxplots comparing the alpha diversity indices for the bacterial community on the eye compartment of the potatoes that were grown on either clay or sandy soil. The figure shows the richness, evenness, Shannon's diversity index and Simpson's diversity index of both soil types. The significant difference ($p < 0.05$) between clay and sand can be viewed in Appendix 5 and significant results are marked with a *.

Correlating alpha diversity and vitality

After calculating and comparing the different diversity indices per variable, harvest year and variety showed to have an influence on the alpha diversity of the potato microbiome. Therefore, the relation between the bacterial alpha diversity of the potato tuber microbiome and the potato plant vitality of these two variables was studied (see Figures 5-7). The data for the potato plant vitality was acquired using drones (see Appendix 6 as an example). The plant vitality was then calculated using two methods and this data provided for this part of the study. For one of the two calculation methods (method 1) vitality was normalized independent of the variety, whereas the vitality calculated using the other method (method 2) was normalized depending on the variety it belongs to. Both Pearson's and Spearman's correlation coefficients were calculated to see whether there is a significant correlation between the alpha diversity and vitality of two variables, and scatterplots were created to visualise the correlation, see Figures 5-7. First, the harvest years 2018-2019 and 2019-2020 were compared (Figure 5), whereafter data on the six potato varieties were analysed, again separated by harvest year (Figures 6 & 7).

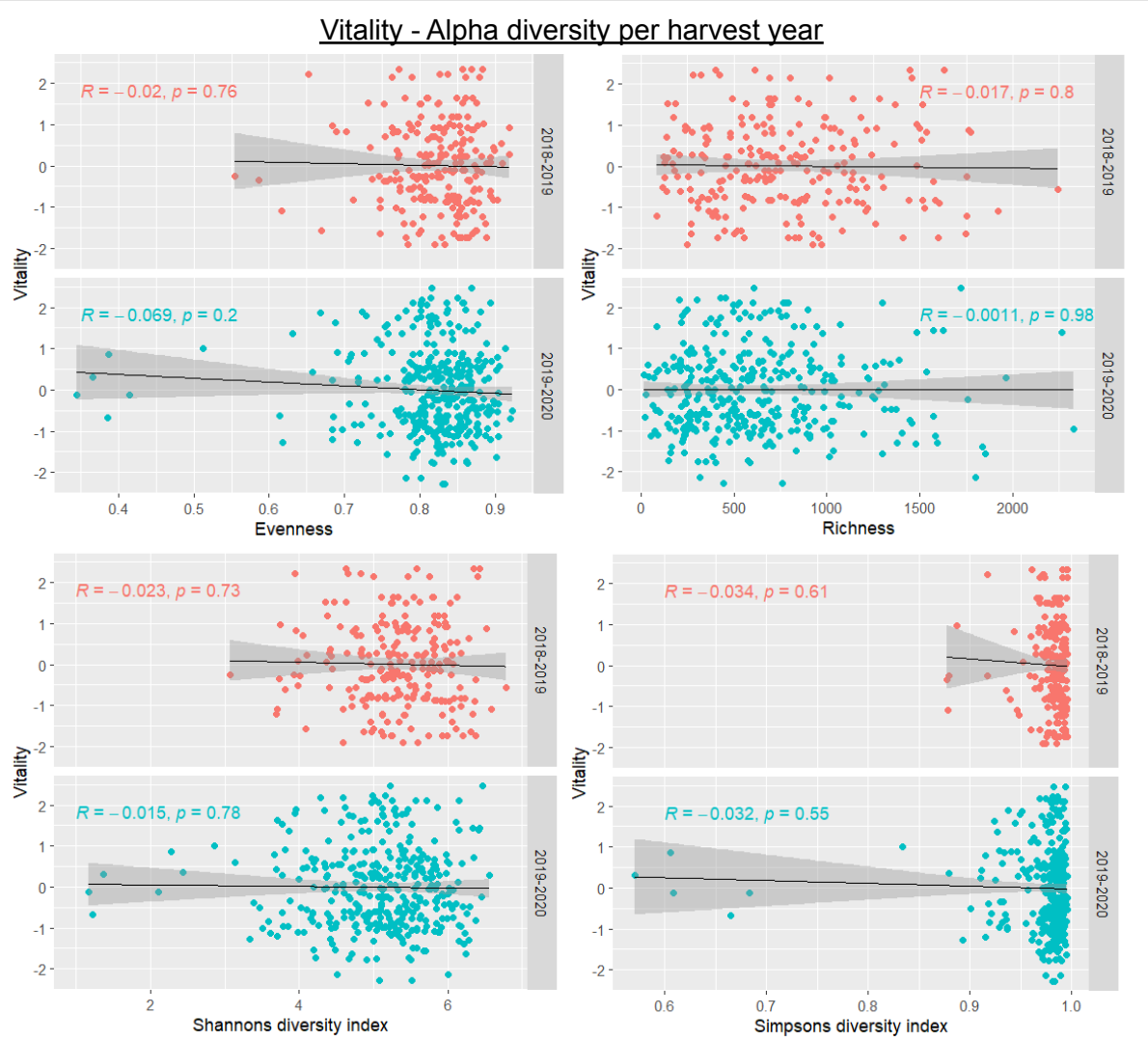


Figure 5: Scatterplots showing the plant vitality (acquired using method 1) of either harvest year against the evenness, richness, Shannon's diversity index and Simpson's diversity index. Pearson's correlation coefficient is represented by the correlation line and the "R" value, which is accompanied by its significance (the p-value).

The scatterplots show the correlation between potato plant vitality and alpha diversity per variable, each dot representing one sample. For comparing the data on the different harvest years, Figure 5, the vitality data from method 1 was used because a harvest year is an external factor and does therefore not have to consider genotype of the varieties. None of the alpha diversity indices showed a significant correlation ($p > 0.05$) with the vitality of the potato plants from either harvest year. This was the case for both Pearson's (see Figure 5) and Spearman's correlation coefficient.

Considering the variety variable (Figures 6 & 7), the vitality data of method 2 was used because this method considers genotype, which is the focus point of testing the variable of variety. The data was again separated by harvest year to exclude environmental differences and five outliers were removed from the dataset. Without the outliers, neither Pearson's nor Spearman's correlations between vitality and any of the alpha diversity indices were significant ($p > 0.05$) for either harvest year (see Figures 6 & 7). In sum, the alpha diversity of the bacterial potato tuber microbiome showed no correlations with the potato plant vitality for either harvest year or any of the six varieties.

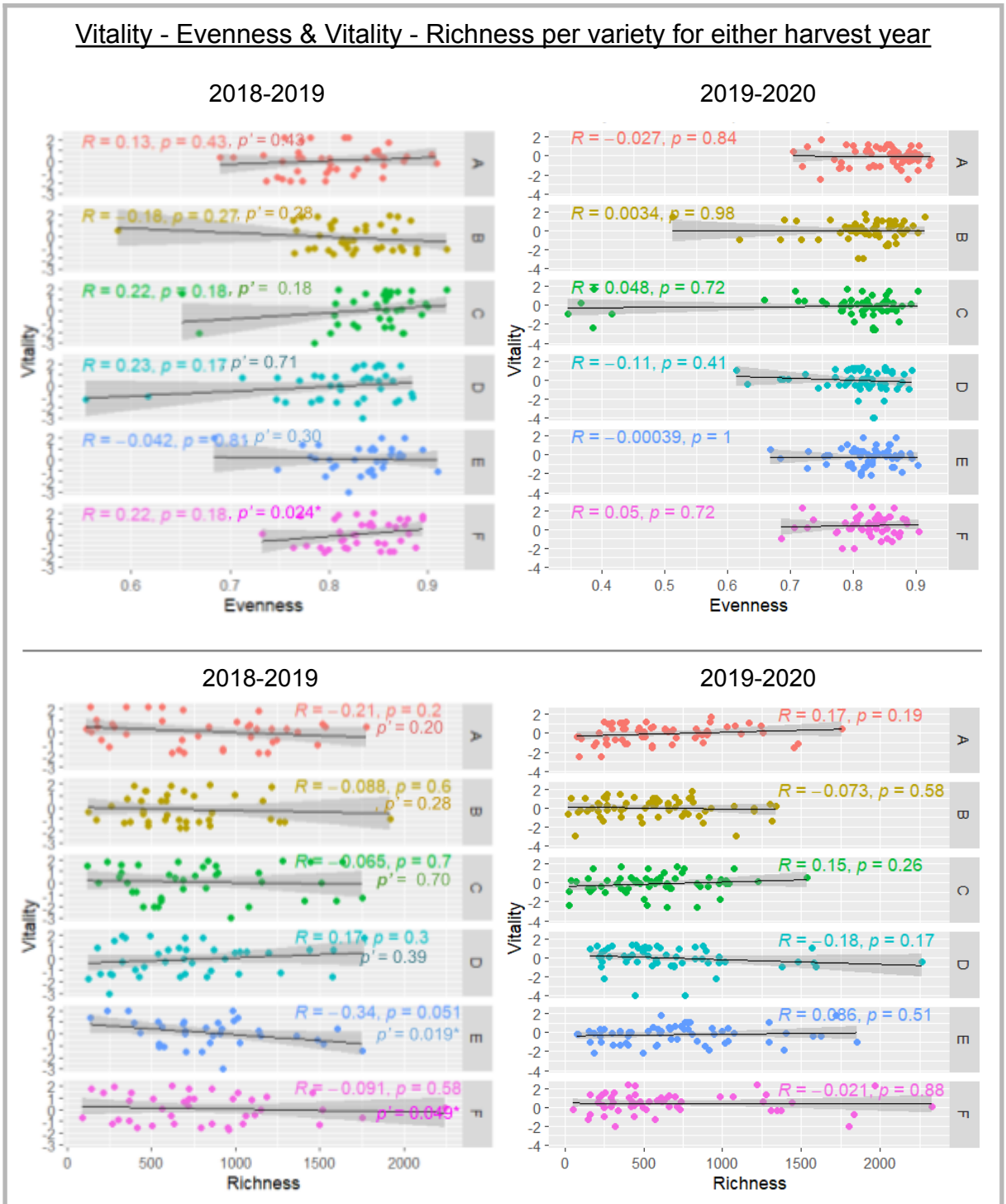


Figure 6: Scatterplots showing the plant vitality (acquired using method 2) of the six potato varieties (A-F), of both harvest years separately, against the evenness and richness of each variety. Pearson's correlation coefficient is represented by the "R" within the figure, yet both Pearson's and Spearman's correlation coefficients were calculated using the cor.test function in RStudio. The significance of the Pearson correlation is also present (*p*), as well as the significance before the removal of (5) outliers of the samples of 2018-2019 (*p'*).

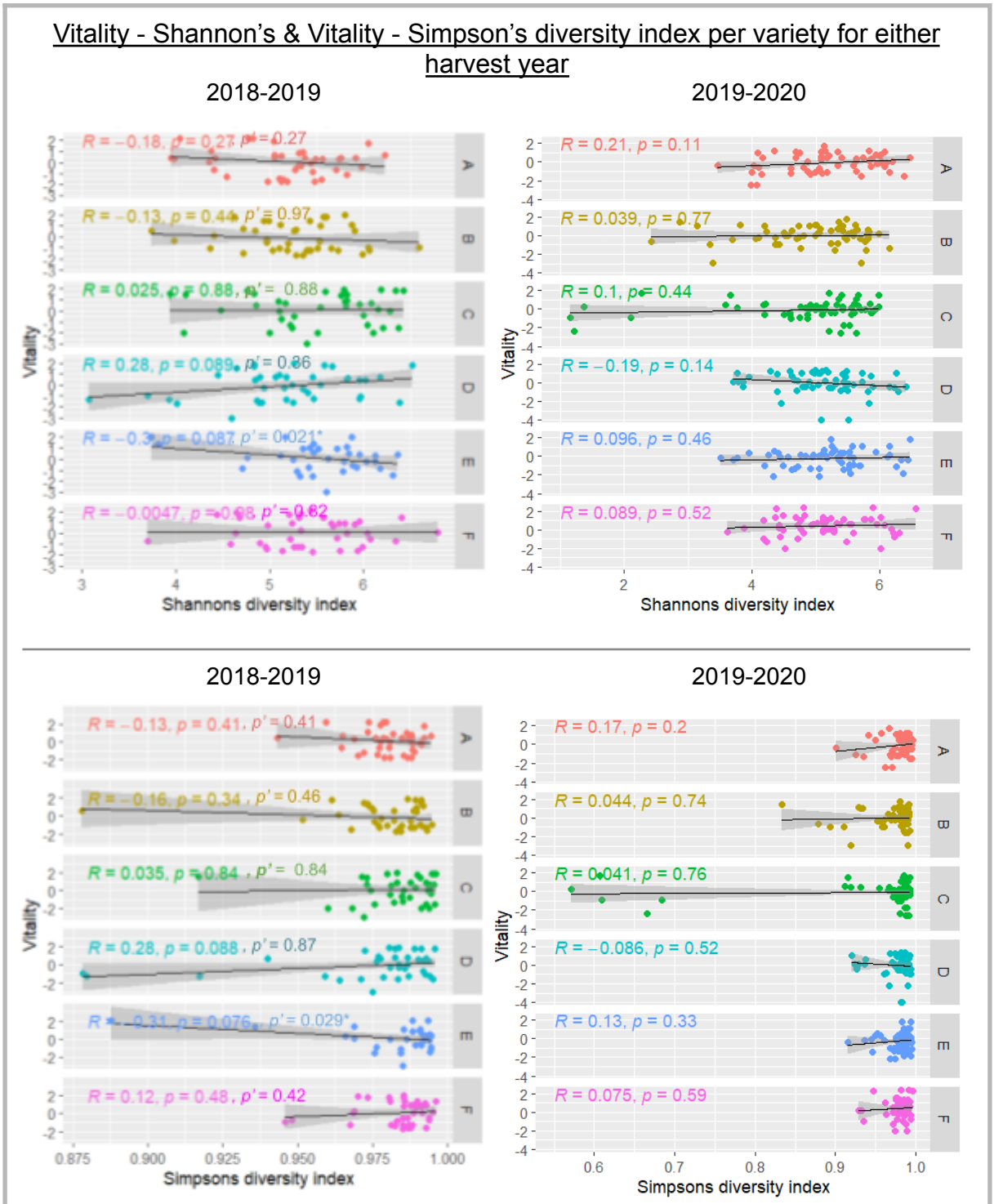


Figure 7: Scatterplots showing the plant vitality (acquired using method 2) of the six potato varieties (A-F), of both harvest years separately, against Shannon's and Simpson's diversity index of each variety. Pearson's correlation coefficient is represented by the "R" within the figure, yet both Pearson's and Spearman's correlation coefficients were calculated using the cor.test function in RStudio.The significance of the Pearson correlation is also present (*p*), as well as the significance before the removal of (5) outliers of the samples of 2018-2019 (*p'*).

Bacterial overviews

The lack of significant correlations between the alpha diversity of the potato microbiome and the plant's vitality does not rule out the possibility of a link between diversity and vitality altogether. There are other levels of diversity to consider, which is why it is interesting to look into the bacterial overview of the potato tuber microbiome. Here, principal coordinates analyses (PCoA's) represent the beta diversity of the potato microbiome for the different variables (see Figures 8-10). The code for the figures was provided by Dr. Yang Song and the figures were made in QIIME2.

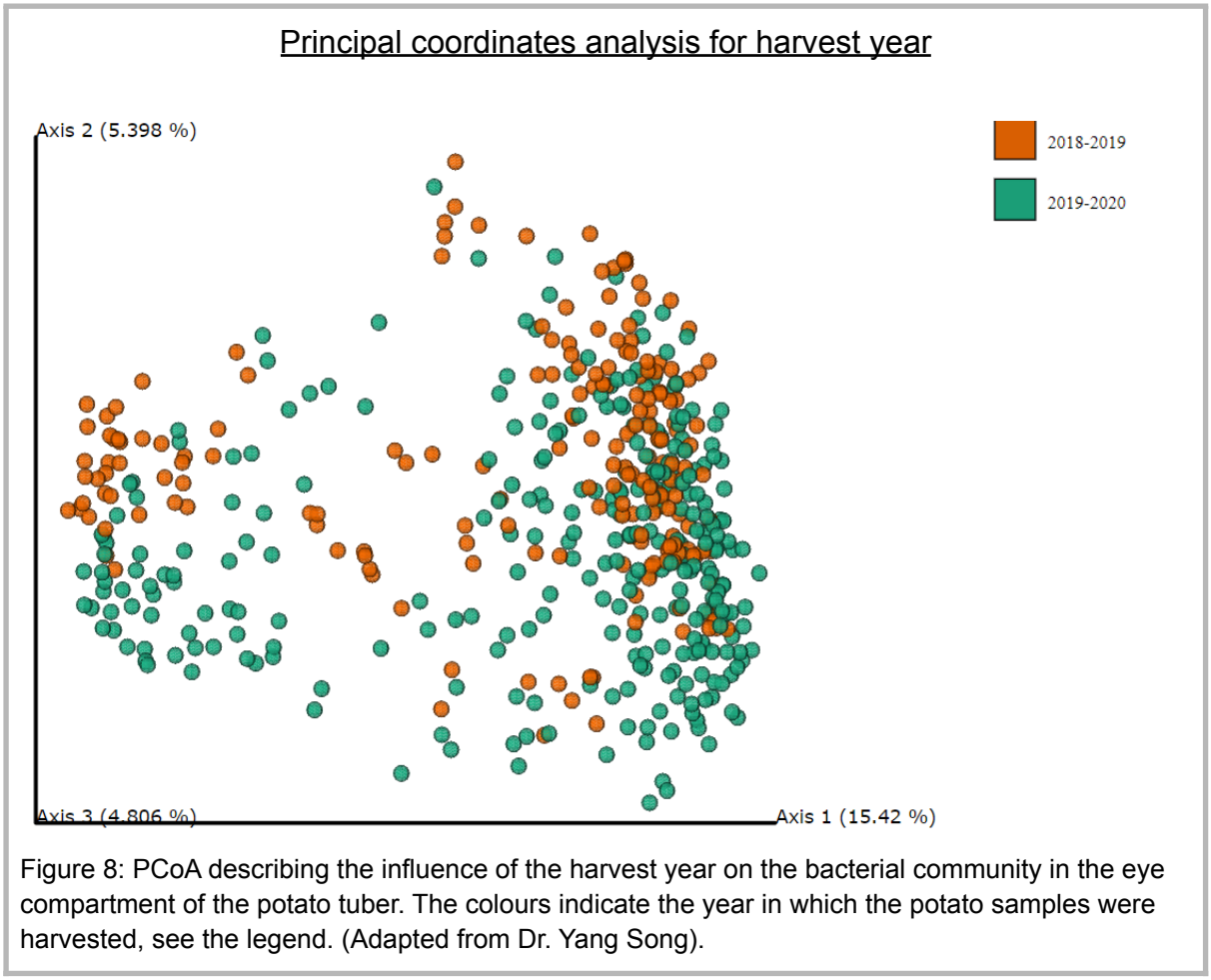


Figure 8: PCoA describing the influence of the harvest year on the bacterial community in the eye compartment of the potato tuber. The colours indicate the year in which the potato samples were harvested, see the legend. (Adapted from Dr. Yang Song).

Looking at the PCoA's, each dot represents one microbial population. The further apart the dots, the more different the populations are. In Figures 8-10 there is a clear division of the bacterial community as the dots form two distinct clusters, yet the variables seem to influence them differently. It is noteworthy that soil type shows the clearest division within the bacterial community, as the two main clusters of the bacterial community are almost completely divided by the type of soil. Even though the other two variables showed a less evident distiction, the results of all bacterial overviews were significant ($p < 0.05$). This means that, for all three variables, the microbiomial community of the potato tuber microbiome is significantly different per variable.

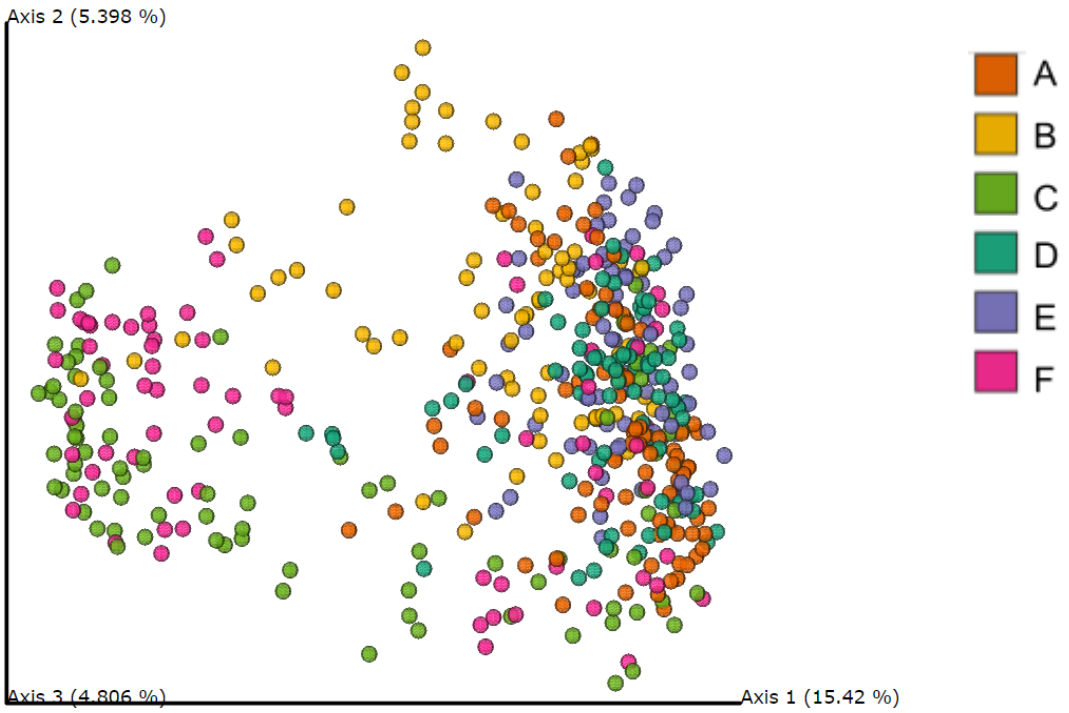


Figure 9: PCoA describing the influence of the varieties on the bacterial community in the eye compartment of the potato tuber. The colours indicate the potato varieties, see the legend.

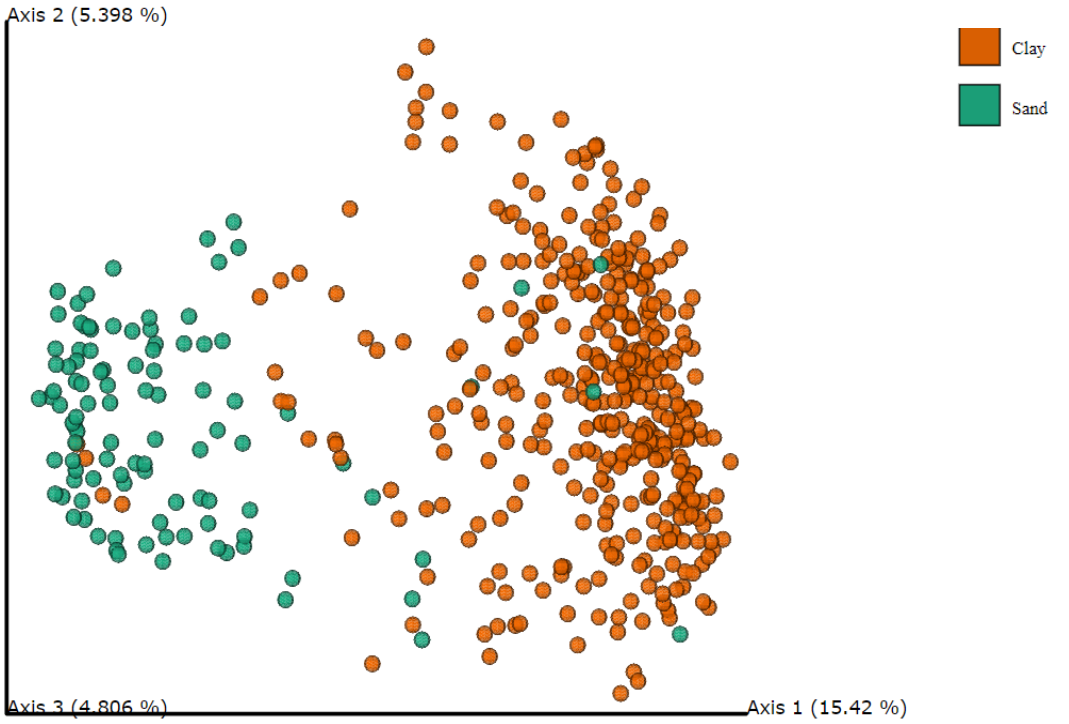


Figure 10: PCoA describing the influence of the soil type on the bacterial community in the eye compartment of the potato tuber. The colours indicate the type of agricultural soil, see the legend.

Discussion

To investigate the influences of the diversity of the potato microbiome on potato vitality, the 16S amplicon library of the eye compartment of potatoes was analyzed. Here, three variables, namely the year of harvest, the potato variety and the soil type on which they were grown, were tested on multiple diversity indices and on the relation between diversity and the observed potato plant vitality. The results were compared to see whether they were significantly different ($p < 0.05$) per variable.

Alpha diversity

To see what influence the three variables could have on the diversity of the potato microbiome, the differences per variable were looked into according to the four different alpha diversity indices (Figure 2-4).
Looking at the differences in diversity between the two harvest years, richness and Shannon's diversity index showed to differ significantly per year (see Figure 5). This suggests that harvest year influences the alpha diversity of the potato microbiome. The summers of 2018 and 2019 are both in the top 5 warmest summers in the central region of the Netherlands ever measured (KNMI, 2022). Additionally, the summer of 2018 was also extremely dry, it being the third driest summer ever measured in the same region of the Netherlands (KNMI, 2022). Considering the high temperatures of the two harvest years and the dryness of one of the two (KNMI, 2022), the results imply that humidity is a key environmental condition that influences bacterial alpha diversity of potato tubers. This is in line with other studies that claim that soil moisture is a relatively important factor that influences soil bacterial communities, more important than temperature (Fierer, 2017).

When comparing the diversity indices of the different varieties per year, significant differences ($p < 0.05$) were found between the evenness of the varieties collected in both harvest years. Additionally, Simpson's diversity index differed significantly between the varieties of one of the two harvest years. The later finding directly contradicts Buchholz et al. (2019), who found no significant differences looking at Simpson's index between different cultivars and different time points. Even so, the same study also found similar results to the ones here, where tubers of different potato varieties differed in bacterial richness and evenness. The use of different and less potato varieties in Buchholz's study could explain why results are not (completely) coherent with this study, as plant genotype could influence the alpha diversity differently since each variety could select their microbiome composition specifically. This suggests that the results in this study are in line with other literature claiming that plant genotype influences the diversity, also in the case of potatoes (İnceoğlu, Falcão Salles & van Elsas, 2012; Buchholz et al., 2019; Dastogeer et al., 2020).

In this study, there were no significant differences between the soil types for any of the diversity indices, even though studies have found that soil type can be the most determinative parameter shaping the functional communities in the potato rhizosphere (İnceoğlu, Falcão Salles & van Elsas, 2012). The results here suggest that this is not reflected in the alpha diversity of the potato microbiome, possibly because the community can be depicted by soil type without the alpha diversity numbers being significantly different.

This would explain why soil type has been found to correlate with potato yield (Jeanne et al., 2012), even though here there are no significant results considering alpha diversity.
All in all, the results of this study suggest that both genotype and environmental conditions influence the bacterial alpha diversity of the potato tuber microbiome, which is in line with the literature.

Alpha diversity and vitality

The scatterplots in Figures 5-7 were produced to show the alpha diversity in relation to the vitality for the harvest years and varieties. The correlation was calculated using both Pearson's and Spearman's correlation coefficients. None of the indices showed a significant correlation ($p < 0.05$) between the vitality of the potato plants of either harvest year or variety calculated using either correlation coefficient after the removal of 5 outliers. This suggests that environmental conditions and genotype did not influence the diversity of the potato microbiome in a way that affects the potato plant's vitality.
Still, the environmental conditions of temperature and humidity were rather unfavourable for the two harvest years (KNMI, 2022), which could have influenced the alpha diversity (Figures 2 & 3). It could be possible that the genetics of the potato plants made them resistant to these environmental conditions or that agricultural practices countered the unfavourable environmental conditions, thereby safeguarding the plant's vitality, perhaps by maintaining the beta diversity. Farming and soil and crop management have shown to influence alpha diversity (Jeanne et al., 2019), providing an explanation for the observed significant differences in alpha diversity per year, as human practices can influence microbiota of above- and below-ground plant parts (Compant et al., 2019). Extrapolating the plant vitality to potato productivity showed that alpha diversity barely correlates to potato yield and residual potato yield (Jeanne et al., 2019), even though this research did not take the varieties of the potatoes into account. This would explain why, in this study, there were significant differences found in alpha diversity of the potato microbiome, but not in correlation to potato plant vitality.

Beta diversity

The principal coordinate analyses show the bacterial overview of the data and can be considered a representation of beta diversity. Figures 8-10 show a clear division within the bacterial communities with all results being significant ($p < 0.05$), yet the variables seem to influence them differently.
Looking at Figure 8, the division of the bacterial community is significant between the two harvest years, though not extreme, which is desirable for the development of the prediction model. This is because, if the two harvest years differed much more, the predictions are not usable across different years. A clear difference between the environmental conditions of these 2018 and 2019 would again be humidity (KNMI, 2022), which could have influenced the observed beta diversity between these two harvest years.

When looking at the effects of the variety on the bacterial community, there was a significant difference ($p < 0.05$) (Figure 9). This is in line with other studies that also found that the beta diversity was affected by potato genotype, where different potato varieties hosted distinct bacterial assemblages (Buchholz et al., 2019). This shows that different potato plant

genotypes tend to select different microbes to colonize the potato tubers (Berendsen, Pieterse & Bakker, 2012; İnceoğlu, Falcão Salles & van Elsas, 2012; Buchholz et al., 2019; Dastogeer et al., 2020).
The principal coordinate analysis of Figure 10 shows an apparent (and significant) division in microbial composition between the sample that were grown on clay or sandy soil. This is in line with the claim of Buchholz et al (2019) that the bacterial community composition (beta diversity) was affected by both the plant's genotype and cultivation in potting soil. The results of this study suggest that soil type is a great influencer of the beta diversity of the potato microbiome, also compared to the other variables which showed less distinction in the microbial community. This would be in line with other literature that found that the rhizosphere microbial community structure is determined more by soil type than the plant species cultivated in that soil (Liu et al., 2020). The effects of environmental conditions could also be influenced by the soil type in this case, since drainage is different between clay and sandy soil, making the drought of 2018 more intense for the plants grown in sandy soil. All in all, beta diversity seems to be influenced greatly by the studied variables. In particular, soil type seems to be of interest considering the evident division in microbial composition of the potato microbiome between clay and sandy soil used to grow the potatoes on.

Conclusion

The aim of this study was to find out whether variety, soil type and/or harvest year could influence the bacterial diversity of the potato microbiome, and whether there is a link between the diversity of the potato microbiome and potato plant vitality. In this study, it was found that different variables relate differently to the microbial diversity of the potato microbiome and potato plant vitality. The harvest year of the potato tubers and the type of variety showed to influence the alpha and beta diversity of the tuber’s microbiome, though these variables did not show a correlation between the alpha diversity of the microbiome and the potato plant vitality. The type of soil on which the tubers were grown was found to have not influenced the alpha diversity but showed to influence the microbial community composition (beta diversity) greatly. All in all, variety, soil type and/or harvest year showed to influence the bacterial diversity of the potato microbiome, but on different levels of diversity, and there is was no link found between the alpha diversity of the potato microbiome and potato plant vitality. The possible link between other levels of microbial diversity and potato plant vitality was not studied here, making further research necessary to find out whether there is a link between diversity and vitality and what underlying factors might be essential to this link in order to develop the prediction model.

Recommendations

This study focussed on the alpha and beta diversity of the potato microbiome and its link with potato plant vitality as a contribution to a greater study that is tempting to design a prediction model for potato plant vitality according to biomarkers. The contributions to this big project are limited due to limited time and resources, which is why the following recommendations are made for follow-up research.

In this study, only the bacterial composition of the eye compartment of the potato plant was studied, as this seemed the most promising. Evenso, this study could benefit from the addition of using different parts of the potato, studied separately or pooled together for a more accurate representation of the whole seed. The data on the fungal presence is already available, but analysing it does not seem like a very promising effort as 99% of the microbial community of the potato microbiome are bacteria (İnceoğlu et al., 2011)

This study is mainly focused on alpha diversity, which is just a small part of the research that is done on the potato microbiome. For the alpha diversity, relative abundance would be a logical addition to this particular set-up (Barnett et al., 2015). The results of this study suggest that beta diversity could be a more important factor for potato plant vitality than alpha diversity, which can be taken into account for the development of the prediction model. Therefore, one could assess the beta diversity of the same variables to expand the assessment additionally to the principal coordinate analysis, and more variables can be considered (Buchholz et al., 2019).

In general, looking at diversity is just one side of the story, as this is still to be linked to vitality. The vitality of the samples could be used as a variable in the before-mentioned diversity analysis, but since we are looking for biomarkers, the microbes present should be identified and analyzed. Looking at the microbial composition of the plants with varying vitality could possibly help find the golden egg of the potato microbiome, or maybe identify the culprit for low vitality. More sophisticated tools are available to examine soil (Bardgett & Van Der Putten, 2014) and its inhabitants.

Microbial interactions is another aspect that should be taken into consideration in reaching the end goal of this research in finding a predictive method, where machine learning could be applied (Weiss et al., 2016; Xia, 2020), for determining the vitality of a potato batch.

Additional assays

This study consists of the main research on the diversity of the potato microbiome and the link between this diversity and the plant's vitality, as well as two additional assays. The two assays consisted of a greenhouse assay and a primer test. The primer assay was performed by analyzing the bacterial DNA sequences of potato samples with different combinations of blocking and non-blocking primers. For the greenhouse assay, the parameter to investigate growth was the weight of different potato parts after being treated with either a mock or a promising microbe solution.

S. ruber and primer assay

To investigate the influence of the addition of an internal standard on (blocking) primers, potato samples used in the amplicon library were re-sequenced with the addition of an internal standard, for which the *S. ruber* DNA isolate was used. In this *S. ruber* assay, a set amount of DNA was added to the potato samples, of which was known that it was not present in the original samples. With the addition of this fixed amount of DNA, the relative abundance could be shown when looking at the original amount of added DNA. The addition of this internal standard was tested in combination with blocking and non-blocking primers. When making the 16S/ITS amplicon library, blocking primers were added to block plant DNA from amplifying during the library prep. With the addition of *S. ruber,* it was of interest to test if using regular 16S and ITS primers or the blocking primers would be best when using an internal standard.

In the diversity study, the focus was on the 16S amplicon library results. To stay consistent, the focus of the primer assay was also on the bacterial results of the assay. Even so, the results of the sequencing by Useq were not useful, since there were hardly any reads yielded per sample, which suggested something had gone wrong. Because there was no proper data to work with, analysis was not possible.

The results were probably lacking due to failed sequencing caused by either a protocol error or a faulty sequencing machine. Apparently, the former is not uncommon and the protocol could be tweaked if these problems keep occurring, like adding a mixture of multiple internal standards (Petersen & Dahllöf, 2005; Harrison et al., 2021).

Without the problems that resulted in the lack of sequencing data, little influence of *S. ruber* on either set of primers would have been expected. The *S. ruber* DNA isolate should have been amplified like any other bacterial DNA by the primers. The blocking primers block out the plant material and, since *S. ruber* is neither more nor less similar to plant DNA than other bacteria, the blocking primers should therefore not be affected by the addition of the internal standard. Evenso, the concept of using an internal standard has been used in other studies (Regalado et al., 2020) with other internal standards and therefore should be confirmed experimentally in the case of *S. ruber*, which is why it is recommended to redo the assay.

Greenhouse assay

If a promising biomarker was found within the study, it was of interest to test its influence on potatoes in a greenhouse set-up. Protocols were already available but were made for small scale experiments. Therefore, this assay was an attempt to upscale the existing greenhouse protocol to accommodate at least 100 potato samples. At the start of this assay, there were a total of 100 potatoes with 50 per variety. The potatoes were harvested at two different moments. The first batch was harvested after 64 days, and the second batch after 71. When harvested, the total weight and that of the tuber, roots and sprouts separately were measured and the number of shoots was noted down. Some potatoes had started to rot and were therefore subtracted from the number of samples. There were other potatoes had not grown shoots (yet), but were still added to the data. The four different weights of the total plant, tuber, shoot and roots were compared between variety and treatment.

The results were processed in Excel and there turned out to be no significant differences ($p > 0.05$) between the WCS358r (bacterial) and the mock treatment when looking at the total weight as well as the separate compartments. Also, there was no difference found between the two harvest time points ($p > 0.05$). When comparing the results between the two varieties there were significant differences ($p < 0.05$) (see Appendix 7), though the harvesting moment did not show this significance ($p > 0.05$). Except for the tuber weight, both other compartments as well as the total weight of variety A were found to be significantly heavier than that of the other variety B, as is shown in Appendix 7.

The lack of significant differences ($p < 0.05$) found between the WCS358r (bacterial) and the mock treatment when looking at the total weight or weight of the separate compartments was surprising, as WCS358r has been proven to promote potato growth, especially of the root system (Bakker, Weisbeek & Schippers, 1988). This lack of significant results considering the inoculation could be explained if WCS358r was not able to survive the malfunctioning greenhouse room. WCS358 strains have shown to grow well at temperatures between 15 and 30 ℃ (Marugg et al.,1985), so the temperatures of the malfunctioning greenhouse probably did not kill the WCS358r bacteria (see Appendix 8). Other studies had set relative humidity levels of 70-93% in their protocol (Ran et al., 2005) and since this was not safeguarded in this greenhouse, the lack of humidity could be responsible for killing WCS358r. In the case of the specific potato breeds used in the assay, variety A supposedly has high drought resistance whereas they advise watering variety B in dry conditions (HPZC, 2022). There were significant differences ($p < 0.05$) when comparing the results between the two varieties (see Appendix 7), which could be explained by the resilience to the environmental conditions instead of the response of the variety to either treatment. The greenhouse assay could be redone to be able to conclude whether the protocol can indeed be upscaled. In this case, more varieties could be tested as well. Greenhouse conditions should be monitored throughout the experiment to prevent the complications that were faced this time.

References

Akimbekov, N., Qiao, X., Digel, I., Abdieva, G., Ualieva, P., & Zhubanova, A. (2020). The effect of leonardite-derived amendments on soil microbiome structure and potato yield. *Agriculture*, *10*(5), 147.

Atza, E., & Budko, N. (2022). High-Throughput Analysis of Potato Vitality. In *European Consortium for Mathematics in Industry* (pp. 273-279). Springer, Cham.

Bakker, P. A., Pieterse, C. M., de Jonge, R., & Berendsen, R. L. (2018). The soil-borne legacy. *Cell*, *172*(6), 1178-1180.

Bakker, P. A., Weisbeek, P. J., & Schippers, B. (1988). Siderophore production by plant growth-promoting pseudomonas SPP. *Journal of plant nutrition*, *11*(6-11), 925-933.

Bardgett, R. D., & Van Der Putten, W. H. (2014). Belowground biodiversity and ecosystem functioning. *Nature*, *515*(7528), 505-511.

Barnett, B. A., Holm, D. G., Koym, J. W., Wilson, R. G., & Manter, D. K. (2015). Site and clone effects on the potato root-associated core microbiome and its relationship to tuber yield and nutrients. *American Journal of Potato Research*, *92*(1), 1-9.

Berendsen, R. L., Pieterse, C. M., & Bakker, P. A. (2012). The rhizosphere microbiome and plant health. *Trends in plant science*, *17*(8), 478-486.

Bissett, A., Richardson, A. E., Baker, G., Kirkegaard, J., & Thrall, P. H. (2013). Bacterial community response to tillage and nutrient additions in a long-term wheat cropping experiment. *Soil Biology and Biochemistry*, *58*, 281-292.

Bolyen, E., Rideout, J. R., Dillon, M. R., Bokulich, N. A., Abnet, C. C., Al-Ghalith, G. A., ... & Caporaso, J. G. (2019). Reproducible, interactive, scalable and extensible microbiome data science using QIIME 2. *Nature biotechnology*, *37*(8), 852-857.

Buchholz, F., Antonielli, L., Kostić, T., Sessitsch, A., & Mitter, B. (2019). The bacterial community in potato is recruited from soil and partly inherited across generations. *PloS one*, *14*(11), e0223691.

Callahan, B. J., McMurdie, P. J., Rosen, M. J., Han, A. W., Johnson, A. J. A., & Holmes, S. P. (2016). DADA2: High-resolution sample inference from Illumina amplicon data. *Nature methods*, *13*(7), 581-583.

Compant, S., Samad, A., Faist, H., & Sessitsch, A. (2019). A review on the plant microbiome: ecology, functions, and emerging trends in microbial application. *Journal of advanced research*, *19*, 29-37.

Dastogeer, K. M., Tumpa, F. H., Sultana, A., Akter, M. A., & Chakraborty, A. (2020). Plant microbiome–an account of the factors that shape community composition and diversity. *Current Plant Biology*, *23*, 100161.

FAOSTAT, Top 20 Commodities Production in Netherlands 2020. Viewed on 05-04-2022. https://www.fao.org/faostat/en/#rankings/commodities_by_country

FAOSTAT, Top 20 Commodities, Export Quantity in Netherlands 2020. Viewed on 05-04-2022. https://www.fao.org/faostat/en/#rankings/commodities_by_country_exports

Fierer, N. (2017). Embracing the unknown: disentangling the complexities of the soil microbiome. *Nature Reviews Microbiology*, *15*(10), 579-590.

Hao, J., & Ashley, K. (2021). Irreplaceable role of amendment-based strategies to enhance soil health and disease suppression in potato production. *Microorganisms*, *9*(8), 1660.

Harrison, J. G., John Calder, W., Shuman, B., & Alex Buerkle, C. (2021). The quest for absolute abundance: The use of internal standards for DNA-based community ecology. *Molecular Ecology Resources*, *21*(1), 30-43.

HZPC (2022). https://www.hzpc.com/nl (22 April 2022).

HZPC (2022). *Onze aardappel rassen.* https://www.hzpc.com/nl/onze-aardappel-rassen (22 April 2022).

İnceoğlu, Ö., Falcão Salles, J., & van Elsas, J. D. (2012). Soil and cultivar type shape the bacterial community in the potato rhizosphere. *Microbial ecology*, *63*(2), 460-470.

İnceoğlu, Ö., Salles, J. F., van Overbeek, L., & van Elsas, J. D. (2010). Effects of plant genotype and growth stage on the betaproteobacterial communities associated with different potato cultivars in two fields. *Applied and environmental microbiology*, *76*(11), 3675-3684.

Jeanne, T., Parent, S. É., & Hogue, R. (2019). Using a soil bacterial species balance index to estimate potato crop productivity. *PLoS One*, *14*(3), e0214089.

KNMI (06-09-2022). *Seizonesextremen zomer - extremen KNMI-station De Bilt vanaf 1901* https://www.knmi.nl/nederland-nu/klimatologie/lijsten/seizoensextremen/zomer (11-11-2022)

Liu, L., Huang, X., Zhang, J., Cai, Z., Jiang, K., & Chang, Y. (2020). Deciphering the relative importance of soil and plant traits on the development of rhizosphere microbial communities. *Soil Biology and Biochemistry*, *148*, 107909.

Maherali, H., & Klironomos, J. N. (2007). Influence of phylogeny on fungal community assembly and ecosystem functioning. *science*, *316*(5832), 1746-1748.

Marugg, J. D., Van Spanje, M. H. W. P., Hoekstra, W. P., Schippers, B., & Weisbeek, P. J. (1985). Isolation and analysis of genes involved in siderophore biosynthesis in plant-growth-stimulating Pseudomonas putida WCS358. *Journal of Bacteriology*, *164*(2), 563-570.

Pascale, A., Proietti, S., Pantelides, I. S., & Stringlis, I. A. (2020). Modulation of the root microbiome by plant molecules: the basis for targeted disease suppression and plant growth promotion. *Frontiers in Plant Science*, *10*, 1741.

Petersen, D. G., & Dahllöf, I. (2005). Improvements for comparative analysis of changes in diversity of microbial communities using internal standards in PCR-DGGE. *FEMS Microbiology Ecology*, *53*(3), 339-348.

Quast, C., Pruesse, E., Yilmaz, P., Gerken, J., & Schweer, T. (2013). yarza, P., Peplies, J. and Glockner, FO (2013). The SILVA ribosomal RNA gene database project: improved data processing and web-based tools. *Nucleic Acids Research*, *41*.

Ran, L. X., Li, Z. N., Wu, G. J., Van Loon, L. C., & Bakker, P. A. H. M. (2005). Induction of systemic resistance against bacterial wilt in Eucalyptus urophylla by fluorescent Pseudomonas spp. *European Journal of Plant Pathology*, *113*(1), 59-70.

Regalado, J., Lundberg, D. S., Deusch, O., Kersten, S., Karasov, T., Poersch, K., ... & Weigel, D. (2020). Combining whole-genome shotgun sequencing and rRNA gene amplicon analyses to improve detection of microbe–microbe interaction networks in plant leaves. *The ISME journal*, *14*(8), 2116-2130.

Turner, T. R., James, E. K., & Poole, P. S. (2013). The plant microbiome. *Genome biology*, *14*(6), 1-10.

Van der Ent, S., Van Wees, S. C., & Pieterse, C. M. (2009). Jasmonate signaling in plant interactions with resistance-inducing beneficial microbes. *Phytochemistry*, *70*(13-14), 1581-1588.

Van Der Heijden, M. G., & Hartmann, M. (2016). Networking in the plant microbiome. *PLoS Biology*, *14*(2), e1002378.

Van Der Heijden, M. G., Bruin, S. D., Luckerhoff, L., Van Logtestijn, R. S., & Schlaeppi, K. (2016). A widespread plant-fungal-bacterial symbiosis promotes plant biodiversity, plant nutrition and seedling recruitment. *The ISME journal*, *10*(2), 389-399.

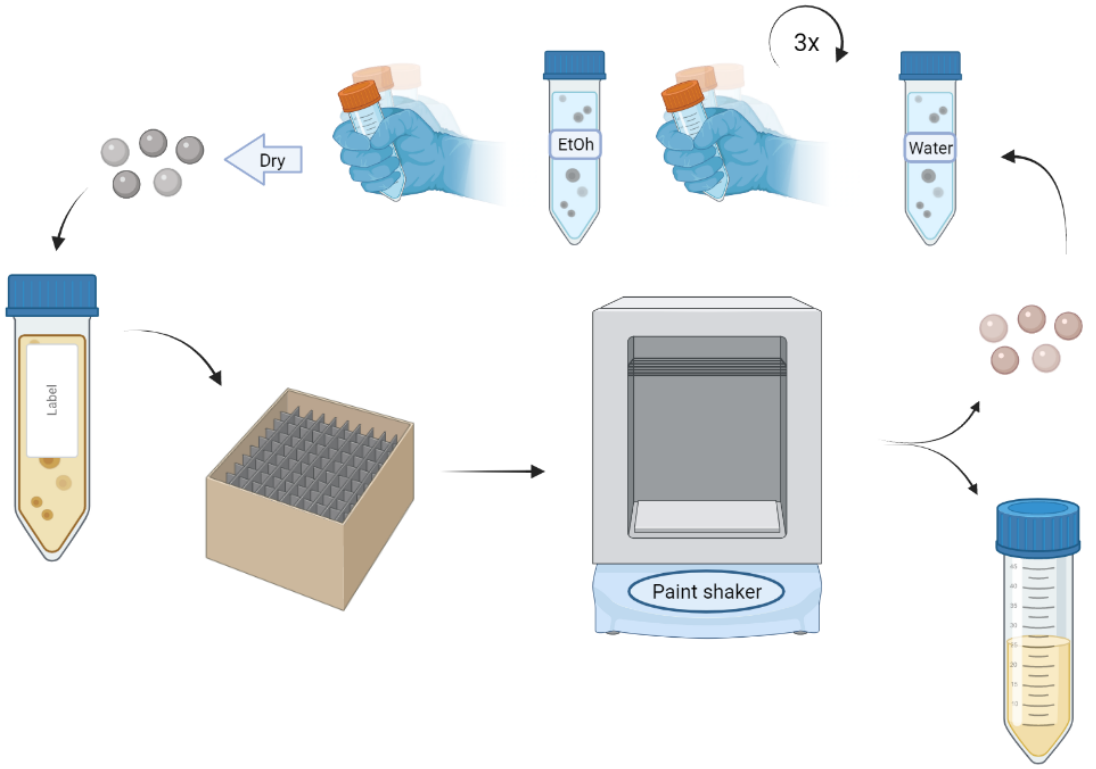
Weiss, S., Van Treuren, W., Lozupone, C., Faust, K., Friedman, J., Deng, Y., ... & Knight, R. (2016). Correlation detection strategies in microbial data sets vary widely in sensitivity and precision. *The ISME journal*, *10*(7), 1669-1681.

Xia, Y. (2020). Correlation and association analyses in microbiome study integrating multiomics in health and disease. *Progress in Molecular Biology and Translational Science*, *171*, 309-491.

Zamioudis, C., & Pieterse, C. M. (2012). Modulation of host immunity by beneficial microbes. *Molecular Plant-Microbe Interactions*, *25*(2), 139-150.

Appendix

Appendix 1 : Normalization formula used for multiple normalization steps within the protocol.		
C = ((A*B)/5 ng/µl)-B		
A	B	C
Measured DNA concentration (ng/µl)	Original DNA sample to be pipetted to the new solution in µl	Amount of buffer needed to add to new solution in µl



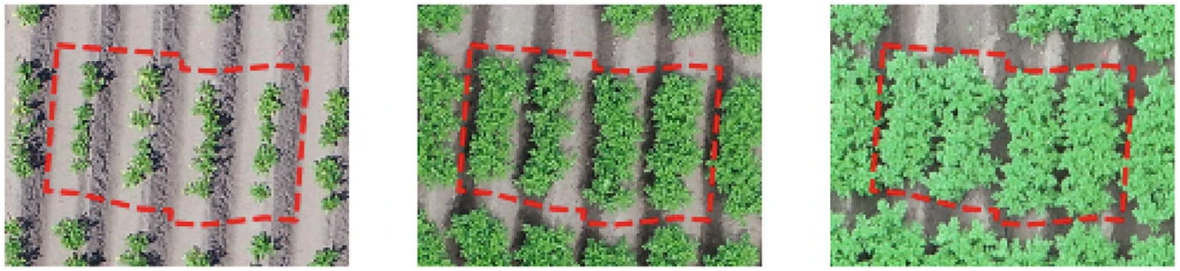
Appendix 2 : workflow of the grinding process in the DNA extraction protocol. Clean and dry beads were added to the original freeze-dried samples, whereafter they were placed in a special tray for the paint shaker machine. When finished, the samples were taken out of the paint shaker machine and were ready for the next step in the protocol after the metal beads were removed using a magnet. The beads were then rinsed, first once with alcohol and then trice with ethanol, whereafter they were left to dry in a sterile environment. When dry, they were used again. During the grinding process, the sample tubes were only opened under sterile conditions.

Appendix 3 : Working solutions for the 16S/ITS amplicon library prep				
Working solution	**For 100 reactions (ml)**	**Chemicals**	**Concentration working solution (mM)**	**Volume stock solution (ml)**
Bead solution	75	NaH2PO4 Guanidinium-isothiocyanate H2O	181 121	37,5 15 22,5
C1 pH = 10	6	NaCl SDS Tris H2O	150 4% 500	0,6 1,2 3 1,2
C5 pH = 7	150	Tris NaCl EtOH H2O	10 100 50%	1,5 10 75,8 62,7
C6 pH = 8	6	Tris H2O	10	0,06 5,94

Appendix 4 : First PCR cycle primer list for 16S and ITS with quantities	
Primers for 16S	**Primers for ITS**
V3/V4 Fw primer 16S-B341F-ill (2 µM)	ITS2 Fw primer fITS7-ill primer (2 µM)
V3/V4 Rv primer 16S-B806R-ill (2 µM)	ITS2 Rv primer ITS4-ill primer (2 µM)
pPNA blocking primer (2,5 µM)	cl1ITS2-F blocking primer (2 µM)
mPNA blocking primer (2,5 µM)	clITS2-R blocking primer (2 µM)

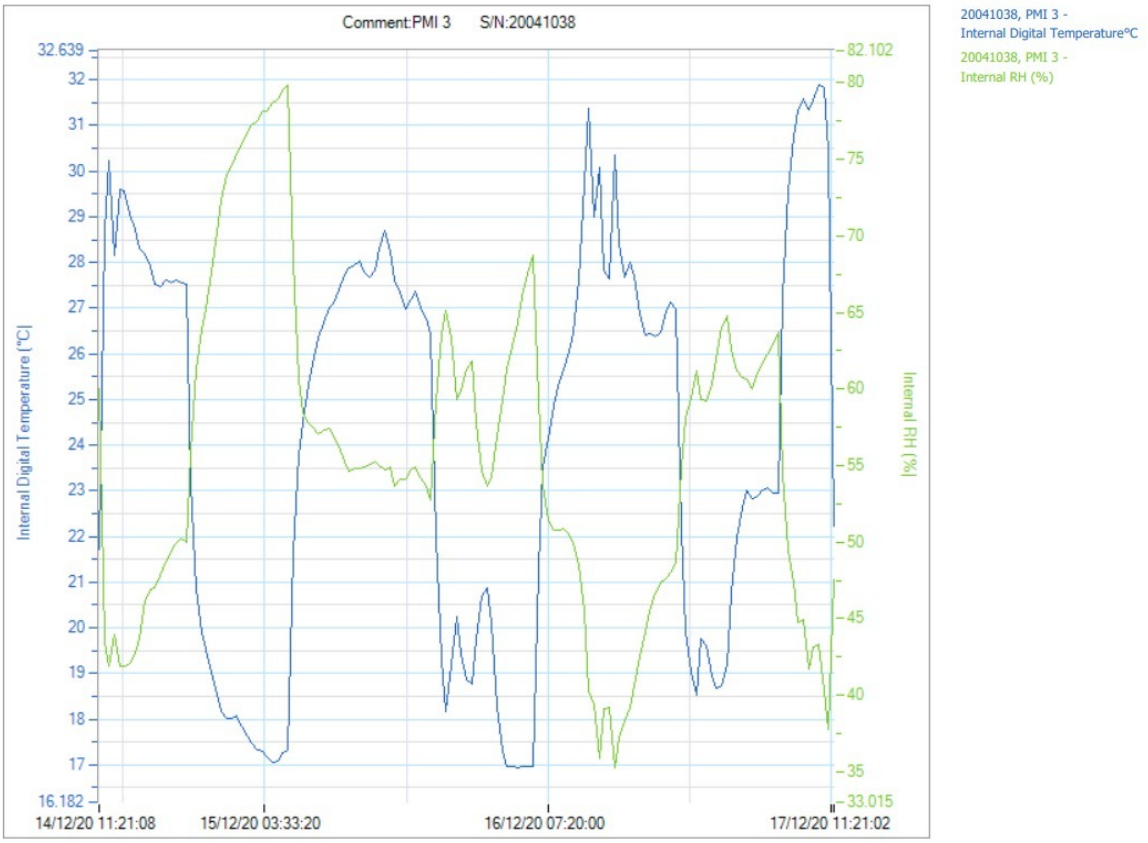
Appendix 5 : Significance per figure. P-values < 0.05 are marked with an *.			
Figure	**Significance**	**Figure**	**Significance**
4.A	0.670	6.A	0.871
4.B	0.981	6.B	0.00468 *
4.C	0.202	6.C	0.221
4.D	0.905	6.D	0.352
5.A	7.856e-05 *	6.E	0.347
5.B	0.373	6.F	1.96e-04 *
5.C	0.001 *	6.G	0.0512
5.D	0.205	6.H	9.26e-04 *

Appendix 6: Exemplary drones images of the canopy of potato batches with different levels of vitality. Adapted from Atza & Budko (2022).



Appendix 7: Results of the unpaired t-tests that compared the weight differences between the two potato varieties.

T-test	Total	Tuber	Roots	Shoot
Mean	98,95793	84,82625	10,85923	0,568841
Variance	1799,43	1171,449	17,90565	0,134879
Observations	44	44	44	44
Pooled Variance	83	79	73	65
Hypothesized Mean Difference	0	0	0	0
t Stat	2,343489	1,4707	9,427504	5,571008
P(T<=t) one-tail	0,010748 *	0,072672	1,48E-14 *	2,61E-07 *
t Critical one-tail	1,66342	1,664371	1,665996	1,668636
P(T<=t) two-tail	0,021496 *	0,145344	2,95E-14 *	5,21E-07 *
t Critical two-tail	1,98896	1,99045	1,992997	1,997138



Appendix 8: results provided by the thermometer in the first greenhouse room for both the measured temperature in ℃ and relative humidity (RH) in % over two days. Measurements were taken every half hour by the thermometer.