Report: Resistance of potato tubers against soft rot Pectobacteriaceae (SRP)

Disease resistance in potato tubers against soft rot Pectobacteriaceae

Viola Kurm[1] & Jan van der Wolf[1]

1 Wageningen University & Research

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Summary

Soft rot Pectobacteriaceae (SRP) continue to cause blackleg disease and tuber soft rot during cultivation of seed tubers. Currently, there are no chemical compounds that are effective against SRP and no resistant cultivars. Past studies have indicated differences in disease incidence between tuber lots of the same cultivar. The goal of the present study was to determine if there are indeed differences in disease incidence between different lots inoculated with the currently most frequently occurring SRP species *Dickeya solani* and *Pectobacterium brasiliense*. Subsequently, we assessed if these differences were associated with differences in the tuber microbiome (bacteria and fungi) and/or the set of secondary compounds in the tuber.

In two consecutive growing seasons, different lots of the cultivars Kondor and Spunta, originating from different locations, were planted in two common fields with an equal load of the two pathogens. Differences in disease incidence were assessed and in both years three lots with a high disease incidence and three lots with a low disease incidence were selected for microbiome and metabolome analysis. These lots belonged to the cultivar Kondor, as disease incidence in cultivar Spunta was generally low and no large differences could be detected. In addition, a pot experiment was conducted, in which the different lots were infected with the *Rhizoctonia solani* and *Colletotrichum coccodes* in order to determine if potential suppressiveness in the lots would also be effective against these fungal pathogens. In the second year, microbial community composition was additionally measured in the different soils that the lots originated from to compare taxa present in the tuber and in the soil. Abiotic soil parameters were measured as well to determine their potential influence on suppressiveness.

In both years, differences between lots in blackleg disease incidence were found, although differences were larger in year 1 than in year 2 and disease incidence varied with location and pathogen. No differences were found in the disease incidences with *R. solani* and *C. coccodes*. Both the bacterial and the fungal community composition differed between lots with a low and a high disease incidence. In 2018, the bacterial taxa *Pseudomonas* sp., *Curtobacterium* sp., *Pantoea* sp. and *Rhodococcus* sp., as well as the fungal taxa *Vishniacozyma heimaeyensis*, *Penicillium brevicompactum, Debaryomyces hansenii*, and *Rhodotorula babjevae* were among the taxa that were significantly increased in tubers with a lower disease incidence, i.e. higher suppressiveness. In 2019, the bacterial taxa *Staphylococcus* spp., *Pseudarthrobacter* sp., *Glutamicibacter* sp., *Paenarthrobacter* sp., *Brevibacterium* sp., Candidatus *Udaeobacter*, and members of the Bacillacae, and the fungal taxa Fusarium oxysporum, *Debaryomyces hansenii, Plectosphaerella niemejerarum, Vishniacozyma heimaeyensis*, and *Mycosphaerella tassiana* showed most association with a low disease incidence. In soil, taxa from the genera Bacillaceae and Planoccocaceae, and the genera Candidatus *Udaeobacter* and *Bradyrhizobium*, as well as the fungi *Cladosporium cladosporides, Saitozyma podzolica, Fusarium oxysporum* and two species of the genus *Solicoccozyma* were correlated with a low disease incidence in the tubers originating from this soil. Several of these taxa have previously been described as being involved in plant growth promotion and disease suppressiveness, indicating that they might have showed antagonism against SRP. Moreover, there was a high variation between the two years and between individual lots in microbial community composition. This indicates that different taxa can increase resistance against the SRPs rather than a specific set of species. It was also found that most of the taxa that were associated with suppressiveness in the tuber were also present in the soil of origin, indicating that these taxa might have colonized the plants from the soil as endophytes. However, abundance in soil was not correlated with abundance in the tuber, meaning that also soils with a low abundance of the respective taxa can yield tubers with a high abundance and vice versa. Which factors influence recruitment and final abundance in the tubers, is poorly understood and should be subject to further investigation. In addition, no clear correlation was found between abiotic soil parameters and disease suppressiveness, indicating that suppressiveness is not dependent on soil type or mineral status within the range of soils that was tested in this study.

The results of this study suggest that bacterial and fungal taxa in the soil can colonize tubers growing in the respective soil and contribute to disease suppressiveness against SRP in the following field generation. In order confirm this hypothesis, the respective taxa will have to be added to infected tubers to prove an effect in disease incidence.

1 Introduction

Worldwide, soft rot Pectobacteriaceae (SRP), are a major problem to potato cultivation (Czajkowski et al., 2011; Toth et al., 2011). In the Netherlands, especially the species *Dickeya solani* and *Pectobacterium brasiliense* have emerged as the dominant causative agents of soft rot in storage and blackleg in the field (van der Wolf et al., 2017). The pathogens are spread mainly by seed. Latently infected mother tubers can result in blackleg inflicted plants and infection of the daughter tubers, which can result in high disease incidences in later field generations (Pérombelon, 1992). There are currently no effective treatments against SRP. Control measures include the planting of disease-free minitubers from sterile culture, the testing of seed lots for the presence of SRPs, and dry storage conditions (Czajkowski et al., 2011; Toth et al., 2011). In addition, some potato varieties show lower disease incidences than others, although there are no varieties that are resistant. Nevertheless, both pathogens are still prevalent in Dutch potato cultivation and fundamental knowledge about origin, epidemiology and control measures is still scarce.

Since no chemical compounds are known to be effective against SRPs, there has been increasing attention to biological factors that influence disease resistance, such as antagonistic bacteria and bacteriophages (Czajkowski et al., 2012; Czajkowski, 2016). Moreover, a recent study indicated differences in disease incidence between different lots from the same variety loaded with the same inoculum level and planted in the same soil (van der Wolf et al, unpublished). While differences between varieties can be attributed to genetic differences, this is not the case for genetically identical tubers from the same variety. While it is yet unknown what causes these differences, it can be assumed that the soil at the location the tubers were grown plays a significant role in disease suppressiveness. Both abiotic and biotic soil factors have frequently been described to alter plant growth and resistance to different kinds of stress. Specific abiotic factors, such as the concentration of macro- and micronutrients or the presence of humic acids have been reported to influence disease resistance in different crops (Van Gijsegem et al., 2021). In addition, biotic factors, such as the soil microbial community, have been demonstrated to influence plant growth and resistance to below- as well as above-ground diseases and pests (Pineda et al., 2010). In the present study we investigate both biotic and abiotic factors and their role in disease resistance.

Several abiotic soil parameters have been associated with increased resistance against SRP. These include low moisture levels, low nitrogen, but high calcium and magnesium concentrations (Charkowski, 2015). Several studies reported increased calcium concentrations in the tubers and reduced soft rot in response to calcium-amendment of calcium deficient soils (McGuire and Kelman, 1984; Bain et al., 1996). This is likely due to strengthening of the cell wall and cell membrane. In addition, calcium may have an effect on the production of defensive compounds, such as phenols, in the tuber itself (Ngadze et al., 2014). In contrast, high nitrogen fertilization is assumed to increase the plant vegetative growth and decrease the production of phenols (Ali et al., 2014). Other compounds that are known to protect potato tubers from SRPs are phytoalexins and pathogenesis related proteins (Lyon et al., 1992). However, there are no studies that show that abiotic soil parameters influence the production of these compounds. Still, soil physiological and chemical soil parameters could indirectly influence tuber resistance through their effects on the soil microbiome (Diallo et al., 2011).

The role of the microbiome in mitigating plant disease has been frequently reported. Especially the soil, rhizosphere and endophytic microbiome are known for their various effects on plant resistance (Berendsen et al., 2012). One mechanism is the induction of systemic resistance resulting in more rapid and increased production of defensive compounds in response to pathogen attack (Pieterse et al., 2014). This effect might be transferred to the next generation, as systemic acquired resistance has been found to be transferable (Floryszak-Wieczorek et al., 2015). Other mechanisms of protection involve competition with the pathogen for essential nutrients, but also direct antagonism through the production of antibiotic compounds (Compant et al., 2005). Actions of the soil and rhizosphere microbiome are dependent on the microbial community at the current location of growth. Still, the soil microbiome is known to be one of the major sources of endophytic bacteria in the plant and consequentially in the seed (Compant et al., 2012). Thus, plant resistance might not only be influenced by current soil microbiome, but also by the microbiome associated with the previous field generation.

Endophytic bacteria have been found to play a role in potato disease resistance as well. Sessitsch et al. (2004) isolated several endophytic bacterial taxa from potato stems that showed antagonism towards a range of fungal and bacterial pathogens. Lahlali and Hijri (2010) found endophytic fungal taxa that were antagonistic against *Rhizoctonia solani*. In addition, endophytes could be isolated from tubers that showed *in vitro* inhibition against *Pectobacterium atrosepticum* (Liu et al., 2020b). It can be expected that the microbiome associated with the seed tuber will be affected by the environment in which the seed was grown in the previous year, and the microbiome of the environment in which the seed is planted. Together, these sources of microbial species will influence the endophytic microbial community and thus the resistance to plant pathogens. However, it is still poorly understood how the soil, the tubers were grown in, in the previous generation, affects the endophytic microbiome in potato and subsequently resistance.

The aim of the present study is to investigate the causes of differences in disease resistance between genetically identical potato lots grown in different soils. We hypothesize that tuber lots from different origins show differences in disease incidence if loaded with the same inoculum and planted in the same field and that these differences are correlated with differences in the tuber microbiome and metabolome. Further, we expect to find a correlation between abiotic or biotic parameters or both of the original soil with metabolome and/or microbiome composition in the seed tubers.

Seed tubers from different lots were grown at a common location after inoculation with SRP. Disease incidence was assessed during the growing season. Subsequently we determined the microbiome and metabolome composition of lots expressing different disease incidences. In order to establish a link with the location of origin, abiotic soil parameters and the microbiome composition of the original soil were assessed as well. Moreover, it was tested whether different lots of tubers of the same cultivar also express differences in resistance against *Rhizoctonia solani*, the causative agent of black scurf, and *Colletotrichum coccoides*, the causative agent of black dot, in order to investigate if non-genetic resistance is a more general trait and effective against various diseases.

2 Material and Methods

2.1 Deltaplan Erwinia 2015 and 2016

In a previous project, Deltaplan Erwinia, a similar study has been conducted, which can be viewed as a pilot to the presented project and the methods and results will briefly be described.
In both years, seed lots of the cultivars Kondor (16 lots in 2015 and 19 lots in 2016) and Spunta (21 lots in 2015 and 20 lots in 2016) were used. After vacuum inoculation with 10^6 cells/ml of Dickeya solani (IPO 2222) or water as a control, tubers were planted in sandy soil (location Buitenpost in 2015 and location Veenklooster in 2016). Per lot, 4 replicate plots of 24 tubers were planted. Disease development was monitored throughout the growing season.

2.2 Study 2018

Fourteen lots each from the two cultivars Kondor and Spunta were used. Spunta is known to be less susceptible to blackleg than Kondor. For origin of the tubers see Table S1. After inoculation the tubers were planted at two different locations, here called Driezum (sandy soil) and Munnekezijl (clay soil). Per treatment, lot and location 4 replicate plots of 16 tubers were planted.

2.2.1 Treatment

Before planting at the location Munnekezijl, lots were vacuum-inoculated either with water as a negative control or with a solution of *Pectobacterium brasiliense* (IPO 3469) at a concentration of 10^6 cells/ml (=Pbras High) or a solution of *Dickeya solani* (IPO 2222) at a concentration of 10^6 cells/ml (=Dsol High), which resulted in a total of 336 plots of 16 tubers each. At the location Driezum lots were inoculated either with water, a solution of *P. brasiliense* at a concentration of 10^4 cells/ml (=Pbras Low) or 10^6 cells/ml or a solution of *D. solani* at a concentration of 10^4 cells/ml (=Dsol Low) or 10^6 cells/ml, resulting in 560 plots with 16 tubers each. In addition, of every lot, 16 tubers were planted that were inoculated with Dsol High. Before planting, a third was removed from each of these tuber from the stolon-end and a thick peel was taken, finely chopped, flash-frozen in liquid nitrogen and stored at -80°C for later analysis of the microbiome and metabolome. The remaining tuber was dipped in talcum powder and planted. Each of the tubers received an identification number to be able to link disease in the field to the sample.
All inoculations in this study were done by HZPC (Metslawier, The Netherlands). After inoculation 10 tubers of 3 lots were used to assess inoculation efficiency. Two pieces of potato peel from each tuber were transferred to a plastic bag (Bioreba) with the addition of Ringer's solution and crushed with a mallet. 50 µl of the undiluted, 10x or 100x diluted extracts were plated on DCVP medium (first layer: 5.5 g/l $CaCl_2$ x $2H_2O$, 1 g/l tryptone, 1.5 ml 0.1% Crystal violet, 1.6 g/l $NaNO_3$, 15g/l agar, 1 ml cycloheximide (200 mg/ml stock); second layer: 5.5 ml EDTA (5.5%, pH=8), 6 ml NaOH 5M, 25 g dipecta pectin) . Cavity forming colonies were counted.

2.2.2 Disease assessment

Tubers were planted at the end of April in a randomized set-up and non-emergence (number of not emerged plants) and disease-incidence were assessed once per week until mid-June. In addition, a stand-number was given by visual observations of the same person during the two field experiments, which represents the overall vigour of the plants. Based on these results three lots of cultivar Kondor with a high disease incidence (K9, K13, K14) and three with a low disease incidence (K6, K8, K10) were selected for further analysis. In addition three lots with a relative high disease incidence (S4, S5 S7) and a relatively low disease incidence (S8, S9, S12) were selected from cultivar Spunta. For cultivar Spunta the progeny tubers of these lots were tested for the presence of SRP (see 2.2.3.), but no further

microbiome and metabolome analyses were conducted as only little differences between lots with respect to disease incidence were observed.

2.2.3 Progeny tubers

At the location Driezum, from the 12 chosen lots and the treatments water, Pbras High and Dsol High 200 progeny tubers were analysed per lot and treatment by the NAK for the presence of generic *Dickeya*, *P. parmentieri, P. atrosepticum* and *P. brasiliense*. Per lot, ten subsamples of 20 tubers were tested.

2.2.4 Sequencing of the tuber microbiome

Of each of the six selected lots, ten tuber peel samples that had been frozen previously were analysed. The frozen samples were transferred to 15 ml bead beating tubes filled each with two ceramic beads (2.8 mm) and two metal beads (2.8mm) (Precellys, Bertin, Montigny-le-Bretonneux, France). Each sample was beaten 2-3 times for 10s at 6000 rpm and 0°C in a Precellys Evolution bead beater with a Cryolys cooling system. In between rounds, samples were cooled in liquid nitrogen to prevent thawing. 250 mg of the resulting frozen powder was used for DNA extraction with the MagAttract PowerSoil DNA KF kit (Qiagen) according to manufacturer's instructions.
The amplification of the bacterial 16S rDNA sequences was carried out using the primers MSAf-B-515f (5'-TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGGTGCCAGCMGCCGCGGTAA-3') and MSAr-B-806r (5'-GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGGGACTACHVGGGTWTCTAAT-3') including MiSeq-adapters, a pPNA clamp (5- GGCTCAACCCTGGACAG-3') and a mPNA clamp (5'- GGCAAGTGTTCTTCGGA-3'). The following protocol was used: 5.75 µl water, 1 µl dNTPs (5mM), 5 µl 5xQ5 reaction buffer, 1.25 µl of each primer, 4 µl each of each PNA, 0.25 µl Q5 HF DNA polymerase and 2.5 µl DNA resulting in a final volume of 25 µl. The samples were amplified with a starting temperature of 98°C for 30s, followed by 30 cycles of 98°C for 10 s, 75°C for 10 s, 50°C for 30 s, 72°C for 30 s and a final 2 min at 72°C.
The amplification of ITS sequences was carried out using the primers MSAf-F-gITS7 (5'-TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGGTGARTCATCGARTCTTTG-3') and MSAr-F-ITS4 (5'-GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGTCCTCCGCTTATTGATATGC-3') using the following protocol: 16 µl water, 1 µl dNTPs (5mM), 5 µl 5xQ5 reaction buffer, 0.125 µl of each primers, 0.25 µl Q5 HF DNA polymerase and 2.5 µl DNA. The samples were amplified with a starting temperature of 98°C for 30 s, followed by 30 cycles of 10 s of 98°C, 30 s of 50°C, 30 s of 72°C and a final 2 min at 72°C. Illumina MiSeq sequencing was carried out at the Bioscience group of WUR with 2x 250 nt paired end reading.

2.2.5 Resistance against fungal pathogens

For this experiment, ten lots of cultivar Spunta were tested for their resistance against *Rhizoctonia solani* and *Colletotrichum coccodes*. For *R. solani*, the strain *R. solani* AG3 was cultivated on oat kernels. After emergence of the plant, 40 kernels were placed per 10 l pot together with one tuber per pot. For de C. coccodes treatment 10 ml spore-suspension (10^5 spores/ml) were added to the stem base. Per treatment, six plants were set up. After harvest, the tubers were immediately scored for *Rhizoctonia* symptoms, disease categories being none, light, moderate and strong. The tubers for C. coccodes disease assessment were stored for 3 month at 20°C and a high humidity. Afterwards the tubers were scored again for disease symptoms of C. coccodes. The disease index was calculated as follows: $index = \frac{\frac{light+2x\ moderate+3x\ strong}{total\ number\ tubers} x50}{1.5}$.
In the following the final index was calculated with 8.6118-(index ×0.1048).
The R. solani sclerotia index was calculated as follows: SI=$(0\ x\ no.tubers\ healthy + 1x\ no.tubers\ very\ light + 2x\ no.tubers\ light + 3\ x\ no.tubers\ moderate + \frac{4x\ no.tubers\ strong}{4x\ no.tubers\ total})$ x 100.

2.3 Study 2019

In the second year only one cultivar, Kondor, was used because the disease incidence was generally low in Spunta, not allowing detection of differences in disease incidence between the lots. Twenty lots (Table

S1) were planted on two locations, Kolummerzwaag (sandy soil) Munnekezijl (clay soil). Tubers were also planted at a third location at Sevilla, Spain. But due to *Phytophtora* infections, no results could be obtained from this location. Per lot, location and treatment of four replicate plots of 24 tubers were planted.

2.3.1 Treatments

Before planting at both locations, lots were either vacuum inoculated with water as a negative control, with *Pectobacterium brasiliense* (IPO 3469) at a concentration of 10^6 cells/ml (=Pbras High) or a solution of *Dickeya solani* (IPO 2222) at a concentration of 10^6 cells/ml (=Dsol High), resulting in a total of 240 plots per location, with 24 tubers each. In addition, at the location Kolummerzwaag, per lot 24 tubers were inoculated with Dsol high and a third of each tuber was cut off for later analysis as described in year 2018.

2.3.2 Disease assessment

Tubers were planted at the end of April in a randomized set-up. Once a week, non-emergence and disease incidence were assessed and a stand-number was given until begin of July. Based on these results, again three lots were chosen with a high disease incidence (K18, K19, K20) and three with a low disease incidence (K13, K14, K17).

2.3.3 Soil parameter analysis

After harvest of the potato plants in 2018 soil samples were taken from each field. The fields were separated into eight blocks. From each block, twenty samples of 200 g were taken randomly at a depth of 5-25 meter, pooled and frozen. Measurement of soil parameters was carried out at Eurofins (Wageningen, The Netherlands). For all measured parameters see Table S2.

2.3.4 Sequencing of the tuber and soil microbiome

Analysis of the microbiome of the six selected lots was done according to the procedure in 2018. In addition, from 10 tubers peel was taken separately from the stolon-end, rose-end and the tuber middle to assess if microbial communities differ between the tuber parts. Furthermore, the microbial community was assessed in eight soil samples from the fields that the selected lots originated from. DNA extraction was done in a similar way for soil, but without percellys bead-beating.

2.3.5 Resistance against fungal pathogens

All twenty lots of Kondor were tested for their resistance against *Rhizoctonia solani* AG3. R. solani was grown on oat kernels and 40 kernels were added to 10 l pots with one tuber after emergence of the plant. Per lot, ten replicates were set up, together with four water controls per lot, resulting in 280 pots. Disease assessment was done similar to the year 2018.

2.3.6 Microbiome data analysis

All analyses were done in R version 3.6.1.
Filtering, error removal, dereplication, merging of paired end reads and chimera removal were done using the package DADA2 (Callahan et al., 2016). For taxonomic identification of 16S rDNA sequences the silva train set version 128 was used, for fungal ITS sequences the sh general release dataset from 02.02.2019 was used. OTU contingency tables, sample data and taxonomic trees were stored in phyloseq objects (McMurdie and Holmes, 2013).
In the 16S rDNA dataset, mitochondrial and chloroplast sequences were removed. Subsequently OTUs that showed an overall abundance below twenty and which fell below the prevalence threshold of 0.05 were also removed from the dataset. Sample counts were transformed to relative abundance. Non metric multidimensional scaling was applied using the built-in ordinate function with weighted unifrac

dissimilarity. The vegan package (Oksanen et al., 2013) was used to assess differences in community composition between different lots and lots with high or low disease incidence by means of a permutational multivariate analysis of variance using the adonis function. In addition a splsda was done using the mixOmics package and the OTUs contributing to the difference between lots and disease incidence were extracted. In addition the package DESeq2 (Love et al., 2014) was used to detect OTUs with differing abundances between treatments.
Both 16S rRNA and ITS sequencing data from 2018 and 2019 were merged after the error correction and dereplication steps in DADA2. The same statistical analysis as for the unmerged datasets was carried out and the package microeco was used for calculating the relative abundance if phyla in the different lots (Liu et al., 2020a)

2.3.7 Measurement and statistical analysis of the tuber microbiome

200 mg of the frozen powder from each tuber that was used for microbiome analysis was also used for assessing the metabolome. As a quality control a pool of powder from each analysed tuber was used and measured in five replicates. The samples were extracted in acidified 75% methanol and analysed by liquid chromatography coupled to high resolution mass spectrometry (LCMS), a platform that is specifically suitable to analyse plant secondary metabolites. Raw data files were processed in a completely unbiased manner, taking all detected compounds into account, using the dedicated workflow at WPR-Bioscience (De Vos et al., 2007; Jeon et al., 2021).
The metabolome data set from 2018 and 2019 consisted of relative intensity data of 548 and 1621 variables (metabolites), respectively. Any variable with more than 40% missing values was omitted from the data set. The intensity data were log-transformed. For PCA missing values were replaced by half of the minimum before pareto-scaling. Linear models were used for determining the association between disease incidence and metabolite presence and quantity. Presence-absence analysis was carried out with a generalized linear model with a logit link and a binomial variance function. The significance effect of disease incidence was assessed with a t-test. A threshold was applied to the p-values to control the false discovery rate at 5% using the Benjamini-Hochberg procedure.
To compare metabolite quantities a linear model mixed model was used with a fixed disease incidence effect and random lot effect. The random effect was removed from the model when the corresponding variance was close to zero. The significance of the fixed effect was assessed by the Kenward-Rogers approximate F-test. The significance of the random effect was tested for using a likelihood ratio test. For each test, thresholds were applied to the p-values (across the metabolites) to control the false discovery rate at 5%. This analysis was carried out with disease incidence both as a categorical variable (high, low) and a continuous variable (disease incidence of the respective lot in %). Several models were simplified by removing the random effect when the likelihood ratio was non-significant. In this case a regular F-test was uses to assess the fixed effect. The mixed model analysis was repeated including all observations by imputing values below LOD by half the minimum values. In addition, a random forest model was used to find a potential association between a number of metabolites and disease incidence. The model was trained with the log-2 transformed metabolome data to predict disease incidence within a lot.

2.3.8 Statistical analysis

Disease incidence in the field was analysed with a glm (binomial distribution), using the number of diseased plants per plot as the response variable and cultivar, bacterial species, concentration and location as the response variable. The effect of lot on disease incidence was assessed per location and cultivar. A similar analysis was performed for the number of non-emerged tubers. Disease incidence and non-emergence of tubers that were cut and numbered before planting as compared to uncut tubers was also analysed with a glm, using cutting and lot as response variable for every cultivar separately. For the progeny tuber the incidence of positive reactions for each tested pathogen was used as the response variable in four different glms. Disease incidence of the lot (high or low), cultivar and species were the explanatory variables. For fungal pathogens, glms were performed with disease index as the response variable and lot and treatment (inoculation with the pathogen or water control) as the explanatory variables.
The effect of soil parameters on disease incidence in 2019 was analysed with glms (binomial distribution) and the stepAIC function from package MASS (Venables and Ripley, 2002), yielding the final model with the parameters explaining most of the variation. The relationship between these parameters and disease incidence was tested with individual pearson-correlations. To assess if these particular parameters were associated with microbial community composition, weighted unifrac distances were calculated for both

the 16S rRNA and ITS dataset. A permutational multivariate analysis of variance using the adonis function was performed for each soil parameter. This analysis was performed excluding the location Sevilla.

3 Results

3.1 Deltaplan Erwinia 2015 and 2016

Disease incidence was dependent on cultivar, treatment with *D. solani* or water, year and the interactions between cultivar and treatment and between cultivar treatment and year Table 1).
Overall, the disease incidence of plants inoculated with *D. solani* was on average 98% higher than that of plants inoculated with water (8% vs 0.2%). In addition disease incidence was 28% higher in cultivar Kondor (7 ±14%) than in cultivar Spunta (2 ±4%) and the disease incidence was 36% higher in the year 2015 (7%±14%) than in the year 2016 (2% ±5%).

Table 1: Results of a glm analysis, with binomial distribution, disease incidence as the response variable and cultivar, *D. solani* treatment, year (which equals location) as the explanatory variables.

Variable	Chisq	df	P-value
Cultivar	1409.0	1	<0.01
D.sol	3798.4	1	<0.01
Year	1012.5	1	<0.01
Cultivar *D.sol	37.6	1	<0.01
Cultivar *Year	1.2	1	0.28
Year*D.sol	0.8	1	0.38
Cultivar *Year*D.sol	12.2	1	<0.01

In the following the results will be presented per cultivar. As lots differ per year, the effect of lot was analysed only.

3.1.1 Spunta

For the cultivar Spunta, disease incidence was low with an average of 4% in plants inoculated with *D. solani* and 0.2% in lots inoculated with water. Nevertheless this difference was significant (X^2=804.53, p<0.01). There were also significant differences between lots with respect to disease incidence (X^2=651.78, p<0.01) and an interactions with inoculation treatment (X^2=113.98, p<0.01) (Fig. 1).

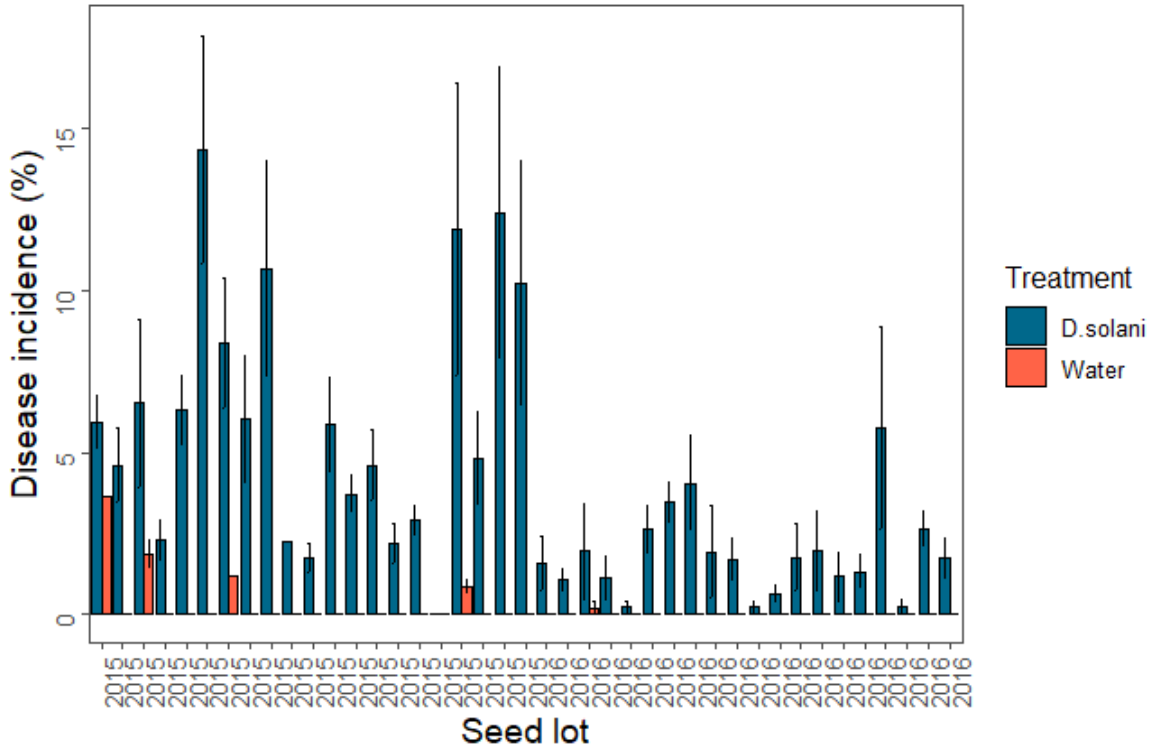


Fig. 1: This bar chart shows the disease incidence (%) of the 41 lots of cv. Spunta in 2015 and 2016 inoculated with *Dickeya solani* or water; error bars represent the standard error.

3.1.2 Kondor

Also for cultivar Kondor, the disease incidence was higher after inoculation with *D. solani* (15%) than with water (0.1%). Just as for the cultivar Spunta, disease incidence differed significantly between lots (X^2=1583.85, p<0.01) and between lots in interaction with inoculation treatment (X^2=71.75, p<0.01)(Fig. 2).

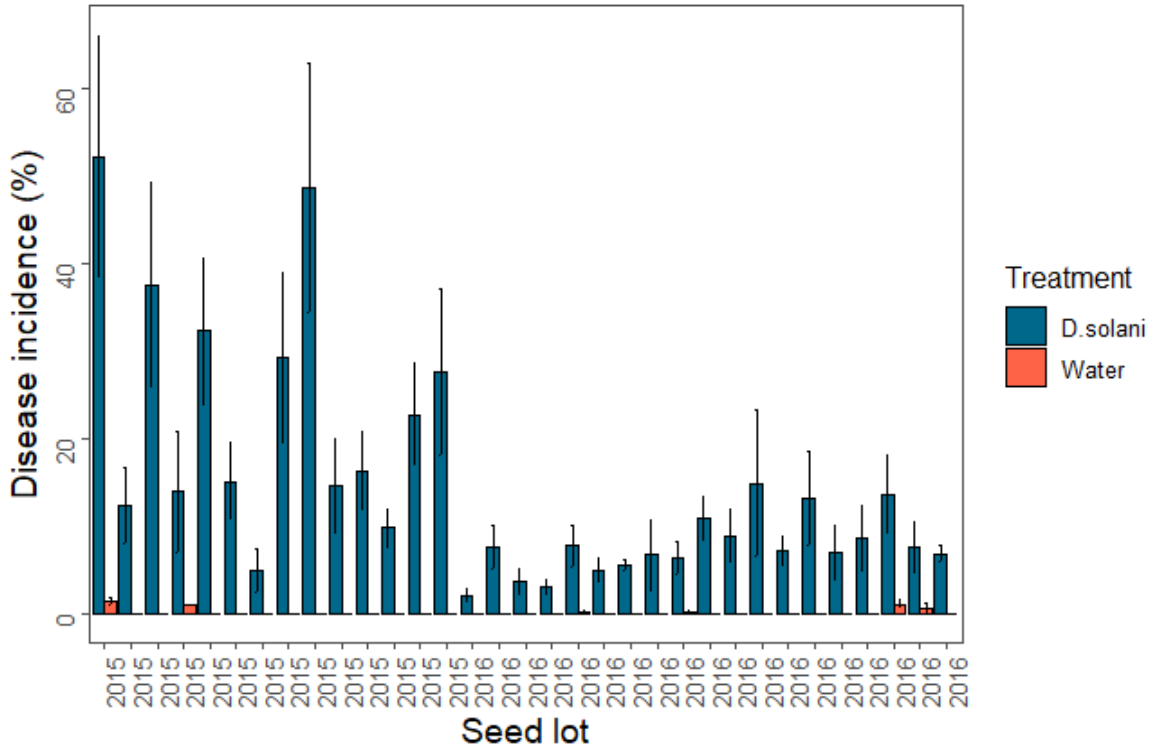


Fig. 2: This bar chart shows the disease incidence (%) of the 33 lots of cv. Kondor in 2015 and 2016 inoculated with *Dickeya solani* or water; error bars represent the standard error.

3.2 Study 2018

3.2.1 Disease incidence in the field

Disease incidence was dependent on cultivar, bacterial species, concentration, location and their interactions (Table 2). Nevertheless, several main effects could be observed.
Disease incidence was generally 32% higher at the location Driezum (sandy soil) than at Munnekezijl (clay soil). Also disease incidence was 72% higher for cultivar Kondor (on average 28 ±25% disease incidence) than for cultivar Spunta (on average 5 ±8% disease incidence). *Dickeya solani* was associated with a higher disease incidence of 25 ± 23% compared to *Pectobacterium brasiliense* (18±23%) and the negative control (1 ±4%). In addition, a higher concentration of the bacterial treatment was associated with a 50% higher disease incidence.

In the following the results will be presented per cultivar.

Table 2: Results of a glm analysis, with binomial distribution, disease incidence as the response variable, and cultivar, species, concentration and location as the explanatory variables.

Variable	Chisq	df	P-value
Cultivar	1627.6	1	<0.01
Species	90.3	1	<0.01
Concentration	444.9	1	<0.01
Location	208.6	1	<0.01
Cultivar*Species	5.7	1	0.02
Cultivar*Concentration	4.9	1	0.03
Species*Concentration	30.4	1	<0.01
Cultivar*Location	8.7	1	<0.01
Species*Location	2.1	1	0.15
Cultivar*Species*Location	1.0	1	0.32

3.2.2 Spunta

For cultivar Spunta, disease incidence is 50% higher in the sandy soil compared to the clay soil (X^2=29.9, p<0.01). An interaction effect between lot and species shows that in most lots, but not in all, disease incidence is higher after inoculation with *D. solani* compared to *P. brasiliense* (X^2=46.4, p<0.01). No difference in disease incidence between lots could be detected (X^2=12.6, p=0.48).

3.2.3 Kondor

The disease incidence of cultivar Kondor was analysed per location due to interaction effects.

3.2.3.1 Location Driezum (sandy soil)

At location Driezum, all variables (lot, species, concentration) and their interactions had an effect on disease incidence (Table 3).
The statistical interaction between lot and concentration is due to a low disease incidence found in some water control samples, but not in other. However, there is an interaction between lot and species as for some lot disease incidence was higher with *D.solani* and in others with *P. brasiliense* (Fig. 2). Disease incidence with *D. solani* was generally higher, but only significant at the lower concentration (data not shown).

Table 3: Results of a glm analysis, with biomial distribution, disease incidence as the response variable, and lot species and concentration as the explanatory variables; for cultivar Kondor at location Driezum.

Variable	Chisq	df	p-value
Lot	253.6	13	<0.01
Species	191.7	1	<0.01
Concentration	345.3	1	<0.01
Lot*Species	51.6	13	<0.01
Lot*Concentration	40.0	13	<0.01
Species*Concentration	38.4	1	<0.01
Lot*Species*Concentration	39.0	13	<0.01

3.2.3.2 Location Munnekezijl (clay soil)

Also at location Munnekezijl there is an effect of lot (X^2=152.3, p<0.01), but also an interaction of lot and species(X^2=127.8, p<0.01) (Fig. 2).

Based on these results, the lots of cultivar Kondor K9, K13 and K14 as well as K6, K8 and K10 were chosen for further analysis.

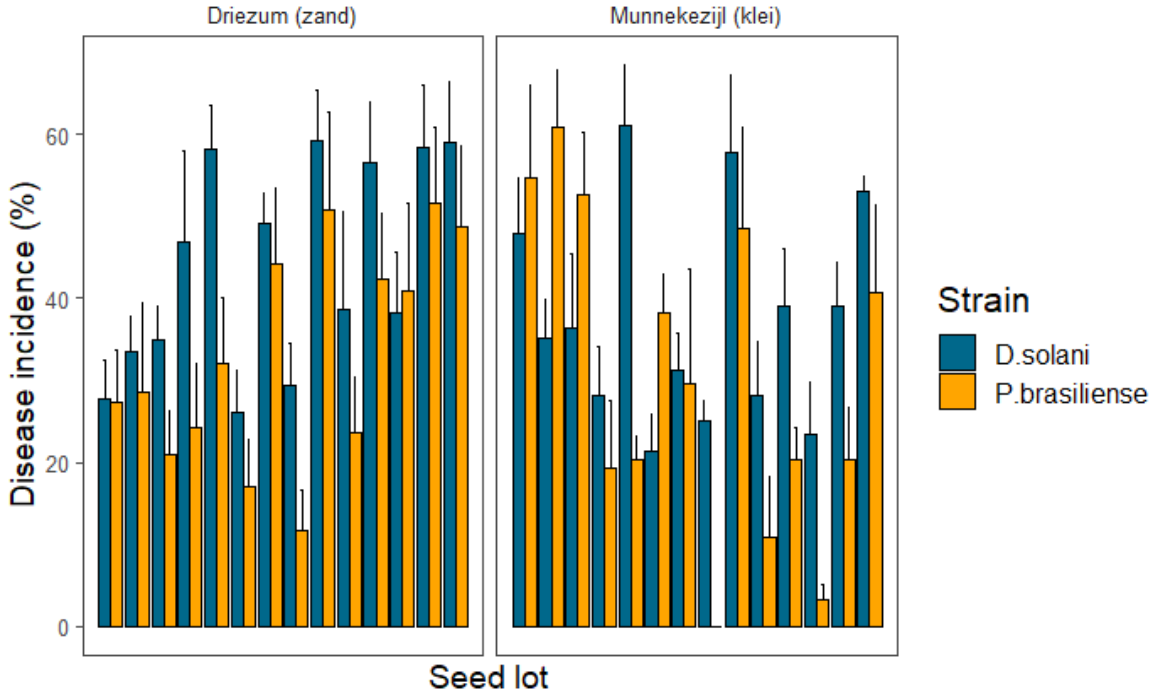


Fig. 2: This bar chart shows the disease incidence (%) of the 14 lots of Kondor inoculated with *Dickeya solani* and *Pectobacterium brasiliense* at the location Driezum and Munnekezijl; blue bars represent the disease incidence of plants inoculated with *D. solani* and yellow bars represent disease incidence with *P. brasiliense*; results were averaged over concentration; error bars represent the standard error.

3.2.4 Emergence in the field

Emergence was generally high (90-99%). Emergence was also affected by cultivar, species, concentration and location. In addition there was an interaction effect between cultivar and species and between cultivar and location (Table 4).
As with disease incidence, non-emergence is higher at the sandy than at the clay soil, although this effect is less pronounced for Spunta than for Kondor. Non-emergence was 50% higher for Kondor than for Spunta, and about 25% higher for tubers inoculated with *D. solani* than with *P. brasiliense*.
For both cultivars there are also differences between lots (Kondor: X^2=132.3, p>0.01, Spunta: X^2=29.0, p>0.01) (Fig.3). Differences between lots differed slightly between locations and species.

Table 4: Table 1: Results of a glm analysis, with binomial distribution, emergence as the response variable, and cultivar, species, concentration and location as the explanatory variables.

Variable	Chisq	df	p-value

Cultivar	44.2	1	<0.01
Species	9.1	1	<0.01
Concentration	4.0	1	0.04
Location	21.1	1	<0.01
Cultivar*Species	10.3	1	<0.01
Cultivar*Concentration	0.4	1	0.53
Species*Concentration	0.4	1	0.51
Cultivar*Location	7.3	1	<0.01
Species*Location	2.3	1	0.13
Cultivar*Species*Location	0.4	1	0.53

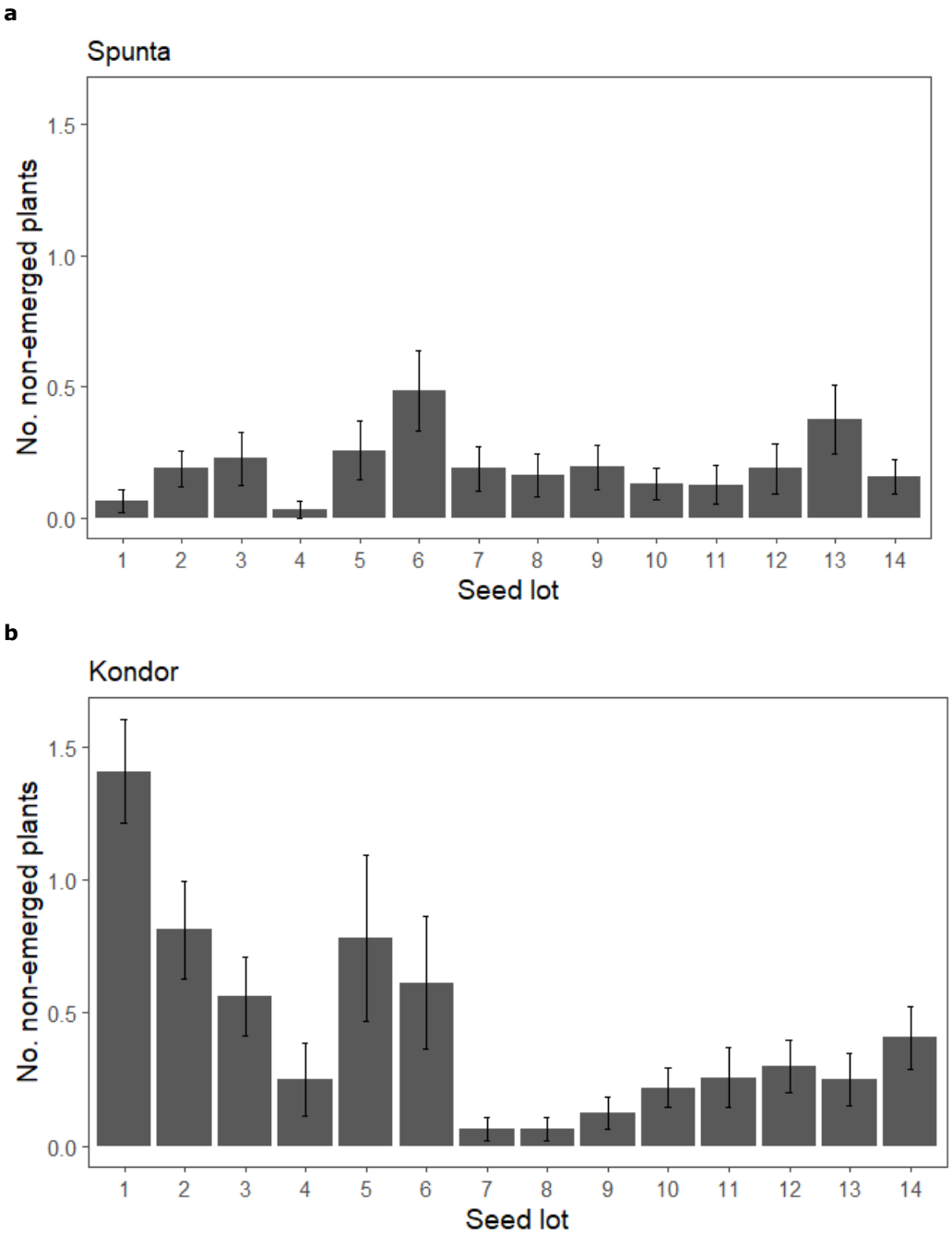


Fig.3: Average number of non-emerged plants for each seed lot of a) Spunta and b) Kondor; error bars represent the standard error.

3.2.5 Cut tubers

3.2.5.1 Kondor

For cultivar Kondor disease incidence was 18% higher in cut tubers than uncut tubers (X^2=18.8, p<0.01). Disease incidence also differed between lots of the cut tubers (X^2=200.1, p<0.01), but there was no interaction between lot and tuber-cutting. Non-emergence did not differ between cut and uncut tubers (X^2=1.2, p=0.28), but differed between lots (X^2=54.1, p<0.01).

3.2.5.2 Spunta

For Spunta disease incidence was 43% higher in cut tubers compared to uncut tuber (X^2=38.5, p<0.01). Again disease incidence differed between lots of the cut tubers (X^2=29.6, p<0.01). Also non-emergence was 72% higher in cut tubers (X^2=21.4, p<0.01). There were also differences between lots (X^2=47.3, p<0.01).

3.2.6 Progeny tuber test

The incidence of *Dickeya* sp. in progeny tubers showed no difference between lots, cultivars or disease incidence. However, progeny tubers from seed tubers inoculated with *D. solani* had on average a 14-fold higher incidence of *Dickeya* than from tubers inoculated with P. brasiliense (X^2=278.7, p<0.01) (Table S3). Progeny tubers from seed tubers inoculated with *P. brasiliense* had a 3-fold higher incidence of *P. brasiliense* (X^2=58.8, p<0.01). A low incidence of *P. parmentieri* was only detected in Kondor with no significant differences between species and disease incidence in the field. *P. atrosepticum* was not detected in any progeny tubers.

3.2.7 Fungal pathogens

A general linear model was used to assess the influence of the factors treatment (pathogen inoculation or water control) and lot on the disease index. For *R. solani* inoculated tubers, there was only a treatment effect as *R. solani* treated tubers had an average disease incidence of 35.34±13.50 compared to a disease incidence of 0.95±2.75 in the control treatment and 1.65±4.51 in the *C. coccodes* treatment (X^2=785.8, p<0.01). There was no difference between lots (X^2=4.1, p=0.91).
For the *C. coccodes* treatment as well, the disease index was higher in the *C. coccodes* treated tubers (26.88 ±13.74) than in the control (9.82±9.75) or *R. solani* (12.11±12.12) treated tubers (X^2=92.5, p<0.01). There was an effect of lot as well, but this disappeared when initial infection (infection in the water control) with *C. coccodes* was subtracted from the infection in the pathogen treatment.

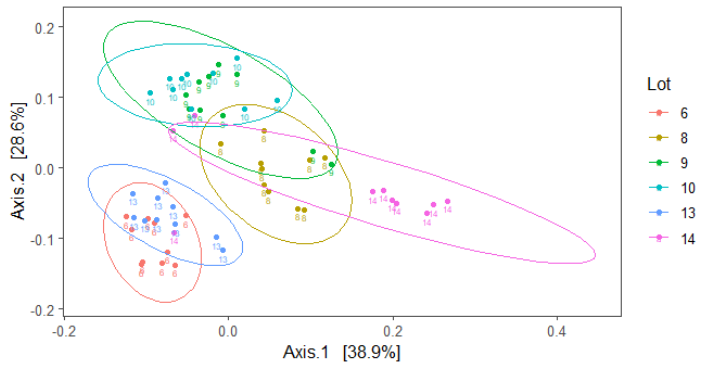
3.2.8 Microbiome data analysis

3.2.8.1 Bacterial 16S rDNA

3.2.8.1.1 Non-metric multidimensional scaling (NMDS)

The NMDS represents the pairwise dissimilarity between treatments in a two -dimensional space and thereby collapses information into two dimensions. Multidimensional scaling showed that the bacterial communities in samples in 2018 from the cultivar Kondor, are distinct from each other on the first two axes. In addition, samples within lots cluster with each other with respect to the bacterial community composition (Fig. 4a). However, the first two axis do not show a clear separation between lots with a low and a high disease incidence (Fig. 4b).
As NMDS is visual method for depicting community differences, a permanova was conducted to assess statistical differences between communities. A permanova (permutational analysis of variance) tests differences between groups with many variables using permutation. Here this analysis revealed significant differences between lots (F=29.8, p<0.01) and also between high and low disease incidence (F=4.8, p<0.01). In addition, there was a significant difference between tubers that gave rise to a diseased plant and tubers that produced healthy plants (F=3.6 , p=0.02).

a



b

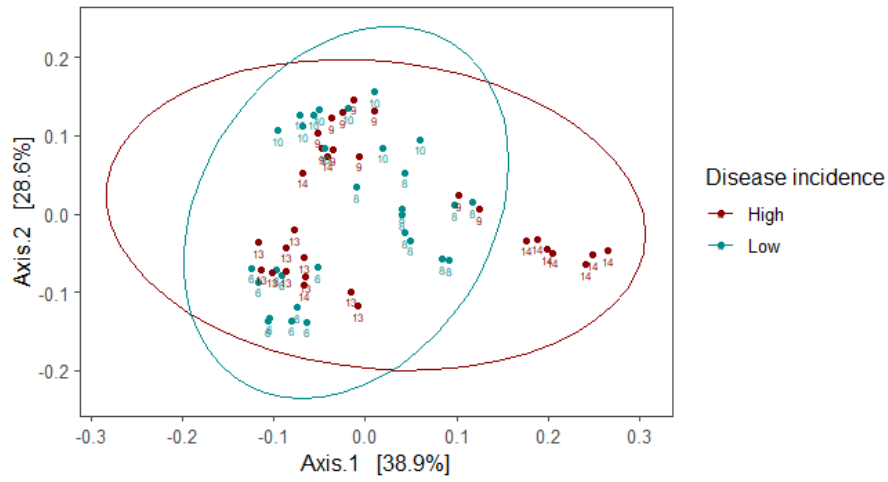


Fig. 4: Non-metric multidimensional scaling with weighted unifrac distances, colours and ellipses indicate a) lot, b) disease incidence of the lot (high, low).

3.2.8.1.2 Sparse partial least squares discriminant analysis (SPLSDA)

SPLSDA is a method to classify samples based on a priori selection of a discriminative variable, which was in this case low or high disease incidence. An SPLSDA could clearly separate lots with a high and low disease incidence (Fig. 5). For the first component 99 OTUs (Operational Taxonomic Unit, reflecting a bacterial species or strain) were selected (Table S4). 45 OTUs belonging to 29 genera were more abundant in the samples with a low disease incidence, including *Curtobacterium sp.*, *Pantoea sp*. *Pseudomonas sp.* and *Rhodococcus sp.*, with double or even tenfold higher abundances in the low incidence samples (Fig. 6). 6 OTUs could not be found at all in the samples from the high disease incidence lots. These belonged to the genera *Skermanella sp.*, *Nocardioides sp.*, *Sinomonas sp*, *Oryzihumus sp* and the families Elev-16S-1332 and Microbacteriaceae. However, these taxa were very low abundant in the whole dataset. 54 taxa were more abundant at high disease incidence. These included the genera *Brachybacterium sp.*, *Nocardioides sp.*, *Lysinibacillus sp*. and *Sanguibacter sp. .*

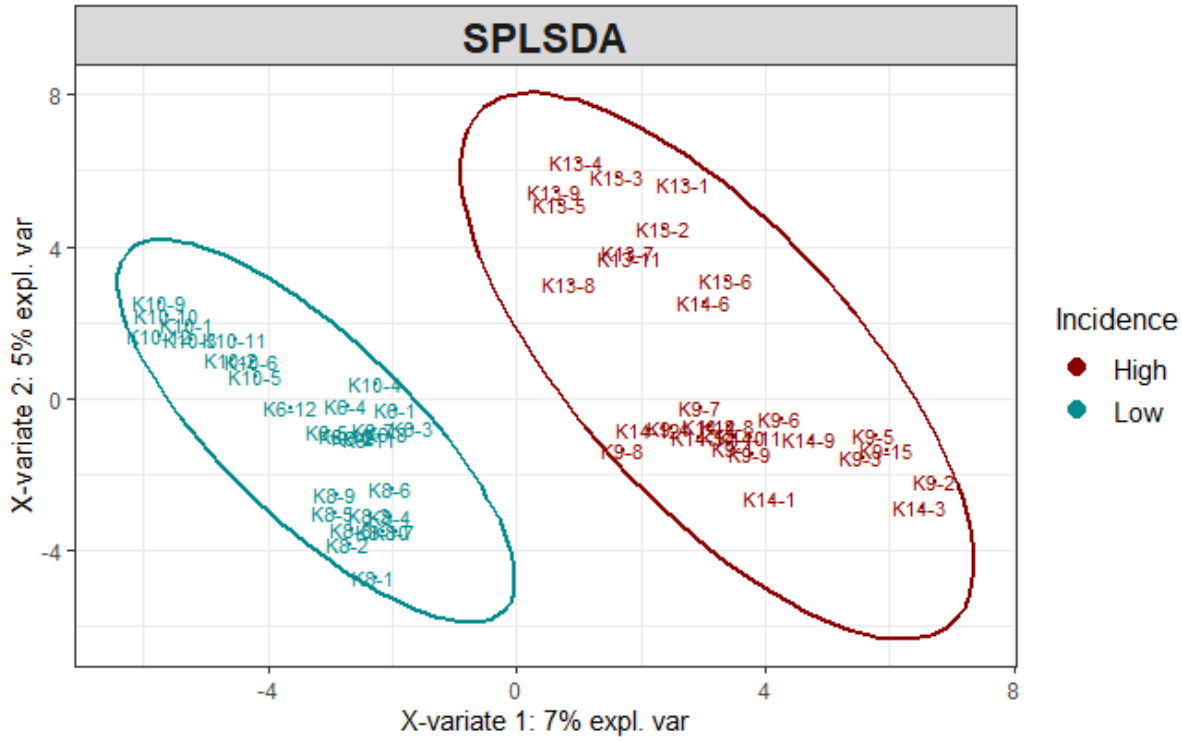


Fig. 5: SPLSDA of OTU abundances with 'Incidence' as the separating factor. Individual data points represent samples. Three components and 240 variables were chosen for the final plot.

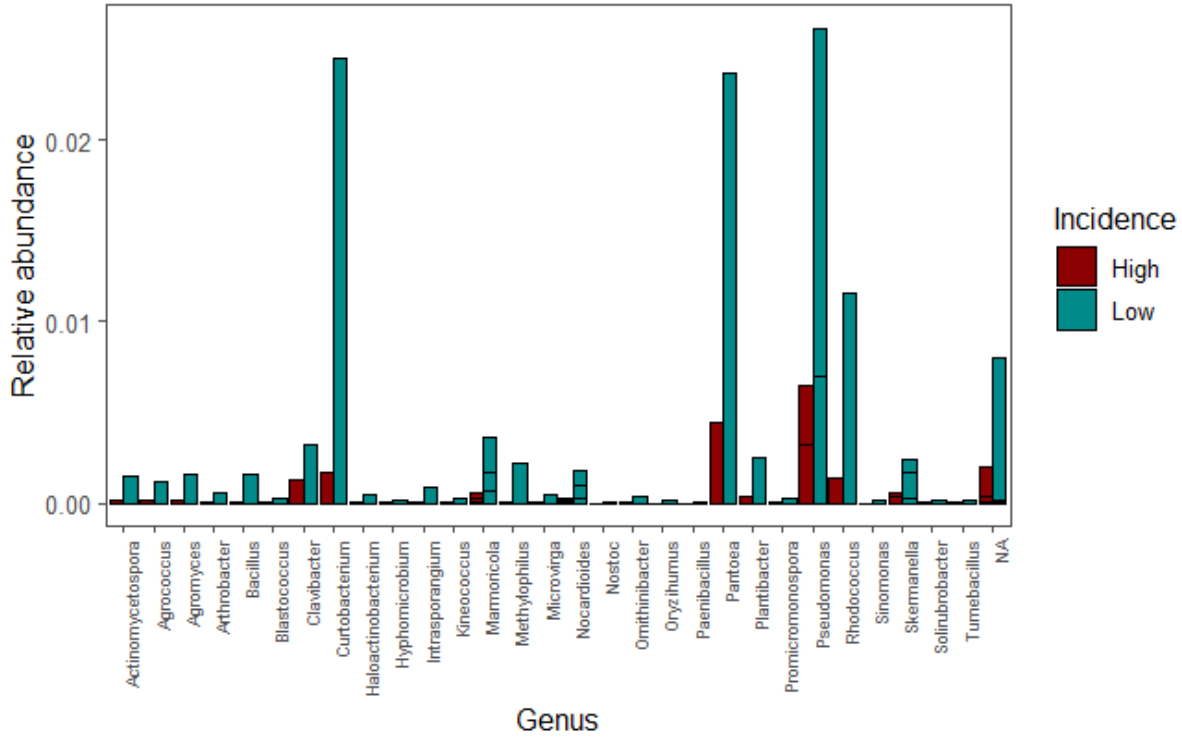


Fig. 6: Relative abundances of all 45 OTUs that were more abundant in samples with a low disease incidence and contributed to the separation in the SPLSDA; the column NA indicates taxa that could not be identified to genus level.

3.2.8.1.3 DESeq2

DESeq2 implements a differential analysis based on the negative binomial distribution. It models raw sequence counts while using normalization factors to account for sequencing depth. It tests for differential expression between two variables (here high and low disease incidence) using a negative binomial model. Using the DESeq2 analysis, 208 OTUs were detected as differing significantly in abundance between high and low disease incidence, 62 OTUs of which were more abundant in low disease incidence samples (Fig.7). Again the most abundant genera in the samples with a low disease incidence were *Pseudomonas sp.*, *Pantoea sp.*, *Rhodococcus sp.*, *Pseudomonas sp.* and *Curtobacterium*

sp. (Fig. 8), but the genera *Pedobacter sp.*, *Pseudomonas sp., Shinella sp.* and *Clavibacter sp.* showed the highest log2-fold change.

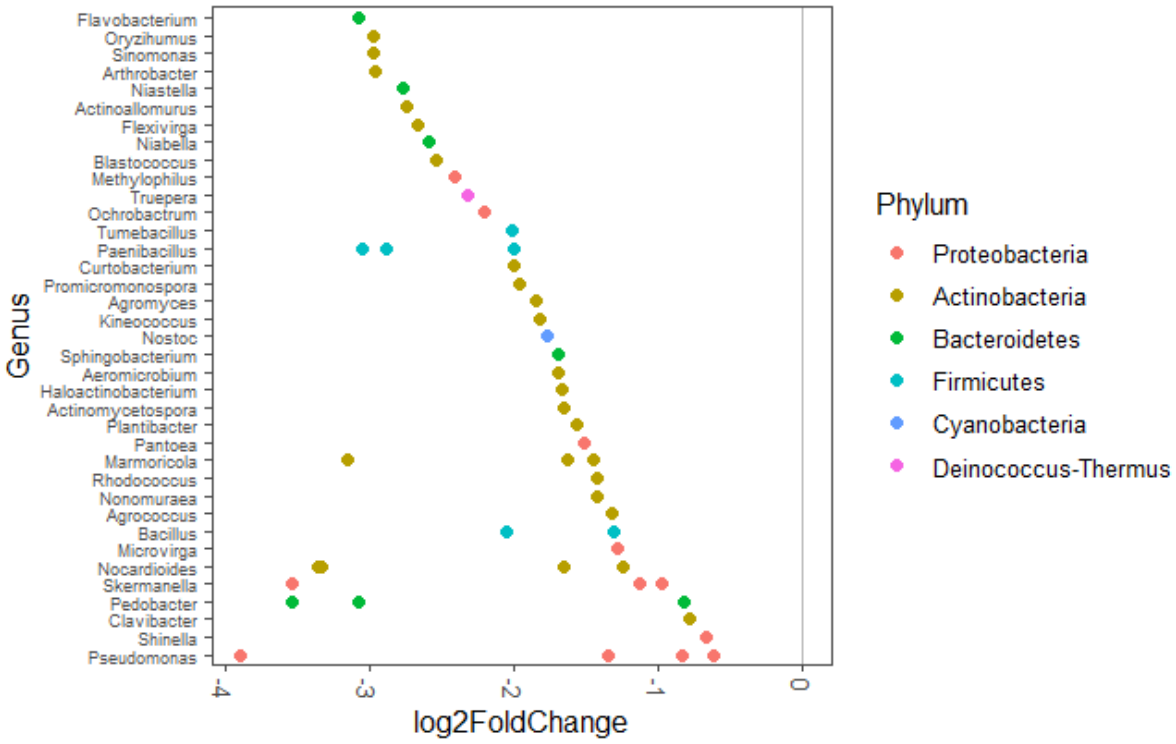


Fig. 7: log2-fold change in abundance in the low disease incidence samples compared to the high disease incidence samples of 62 taxa; each datapoint represents an OTU.

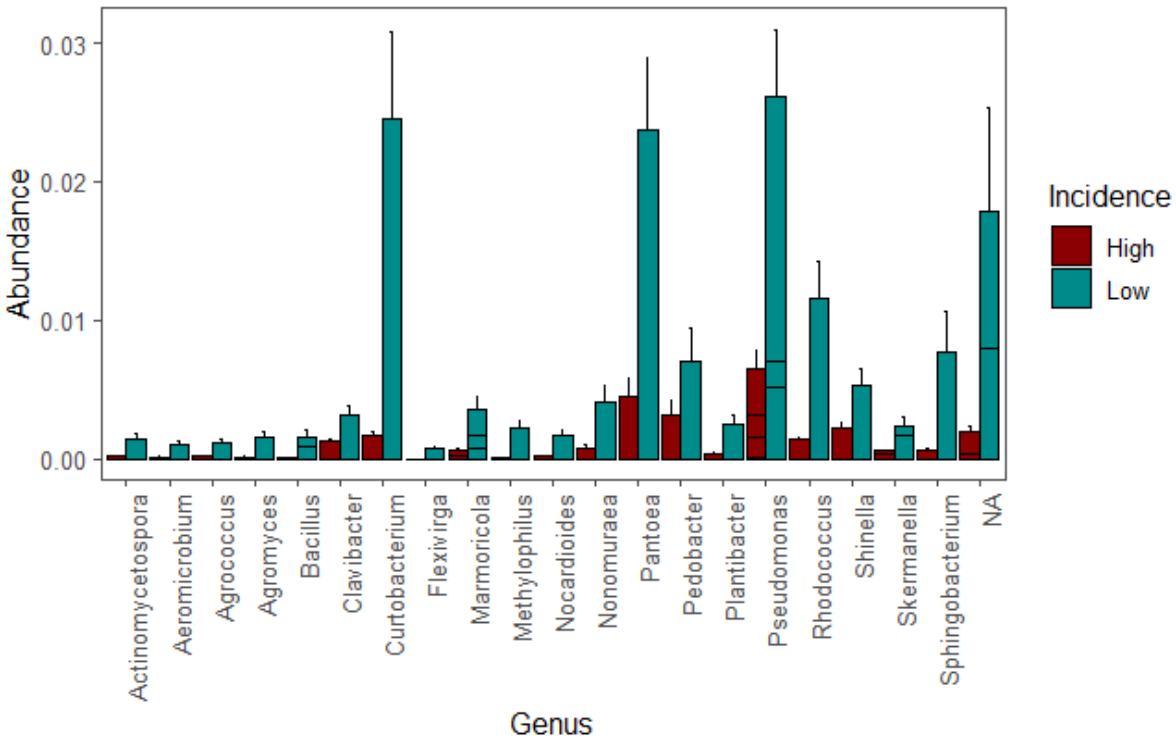


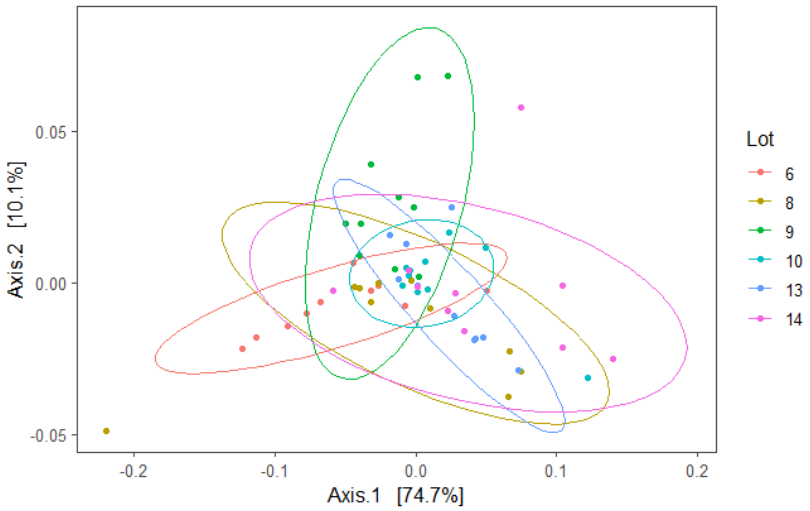
Fig. 8: Average relative abundance in lots with a high and low disease incidence, of the 30 most abundant OTUs, belonging to 10 genera, that showed a significant increase in lots with a low disease incidence according to the DESeq2 analysis; the column NA indicates taxa that could not be identified to genus level.

3.2.8.2 Fungal ITS

3.2.8.2.1 NNMDS

Multidimensional scaling did not show a distinct clustering of samples according to lot or disease incidence on the first two axes (Fig. 9). However a permanova revealed significant differences between lots (F=4.8, p=0.02), high and low disease incidence (F=11.2, p=0.01), and tubers that produced healthy or diseased plants (F=12.5, p=0.01).

a



b

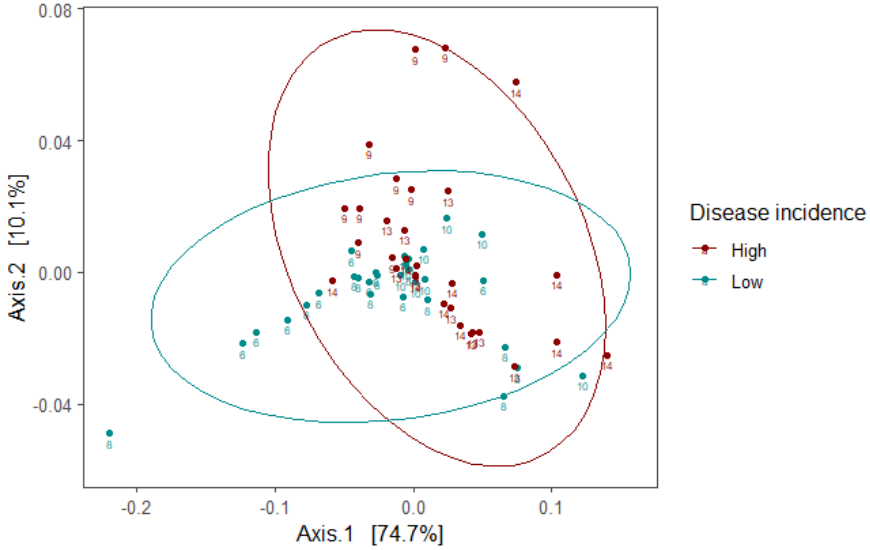


Fig. 9: Non-metric multidimensional scaling with weighted unifrac distances, colours and ellipses indicate a) lot ,b) disease incidence of the lot (high, low).

3.2.8.2.2 SPLSDA

Also an splsda could not completely separate lots with a high and a low disease incidence (Fig. 10). Still 90 OTUs were selected for the first component and 59 OTUs, belonging to 18 genera, were more abundant at low disease incidence (Fig. 11). Unfortunately, many of those taxa could only be identified to phylum level. Species that could be identified and were significantly increased in abundance in lot with a low disease incidence include *Helminthosporium solani*, *Vishniacozyma heimaeyensis*, *Penicillium brevicompactum*, *Rhodotorula babjevae, Plectosphaerella cucumerina* and *Debaryomyces hansenii*. Eight taxa could only be found in samples with a low disease incidence, identified as Leucosporidium *sp.*, *Pyrenochaeta inflorescentiae*, *Verticillium albo-atrum*, *Tetracladium sp.*, *Mucor hiemalis*, *Sloofia cresolica*, *Solicoccozyma aeria* and a taxon of the order of Xylariales. Taxa that were more abundant at high disease incidence included *Colletotrichum coccodes*, *Paraphaesosphaeria sp.*, *Aspergillus ruber*, and *Cladosporium delicatulum*.

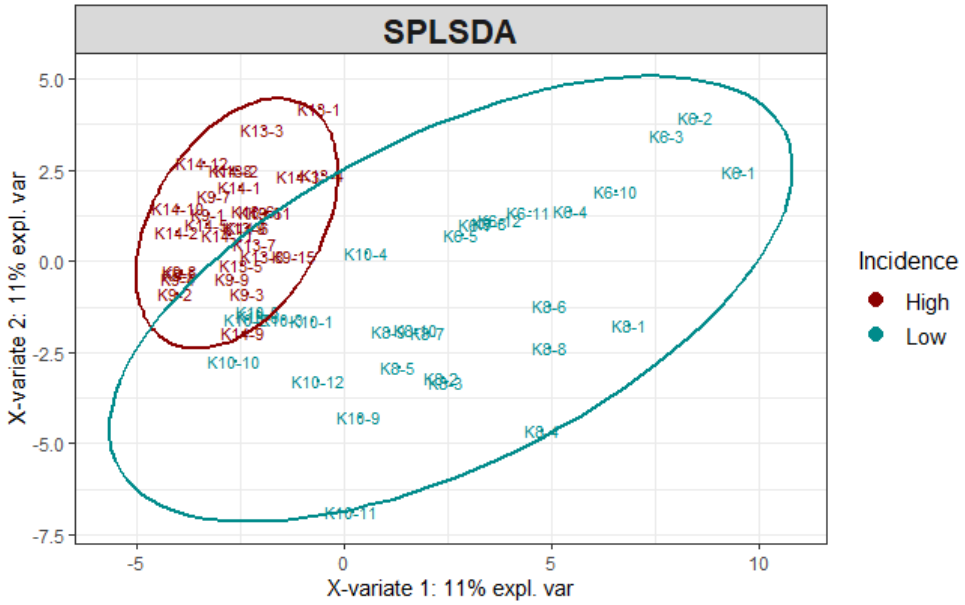


Fig. 10: SPLSDA of OTU abundances with Incidence as the separating factor. Two components and 120 variables were chosen for the final plot.

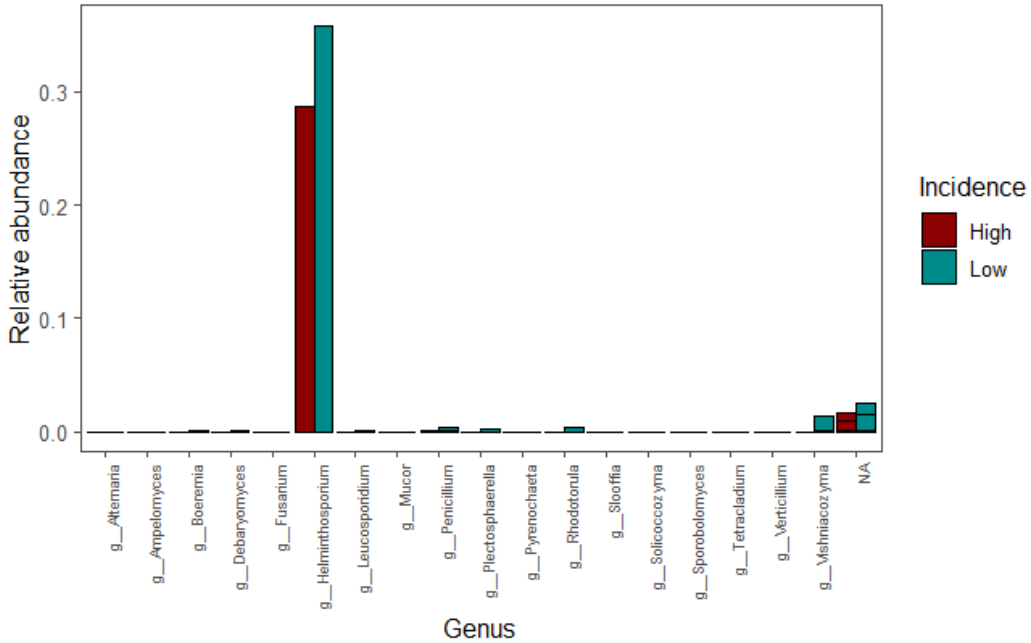


Fig. 11: Relative abundances of all 59 OTUs that were more abundant in samples with a low disease incidence and contributed to the separation in the SPLSDA; the column NA indicates taxa that could not be identified to genus level.

3.2.8.2.3 DESeq2

With the DESeq2 analysis, 40 OTUs were found to significantly differ in abundance between high and low disease incidence, of which 31 could be identified to genus level and 17, belonging to 12 genera, were increased in abundance in lots with a low disease incidence (Fig. 12). In contrast to the splsda analysis, the increase in abundance of *Helminthosporium sp.* was not significant in the Deseq2 analysis. Still the genera *Vshniacozyma sp.*, *Rhodotorula sp., Penicillium sp., Setophoma sp., Plectophaerella sp.* and *Debaryomces sp.* were significantly increased in low disease incidence lots (Fig. 13).

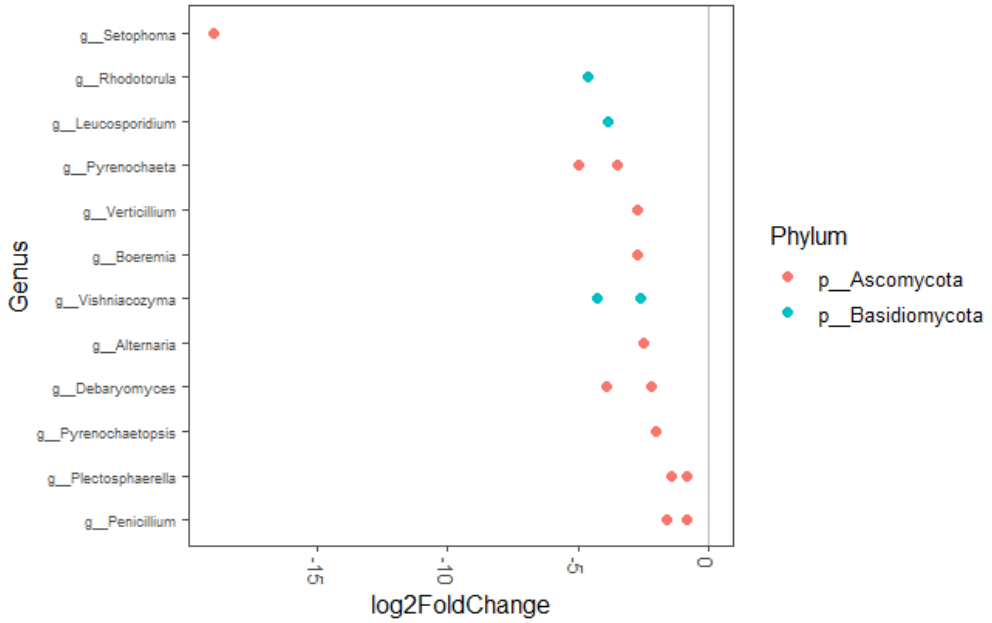


Fig. 12: log2-fold change in abundance in the low disease incidence samples compared to the high disease incidence samples of taxa; each datapoint represents an OTU.

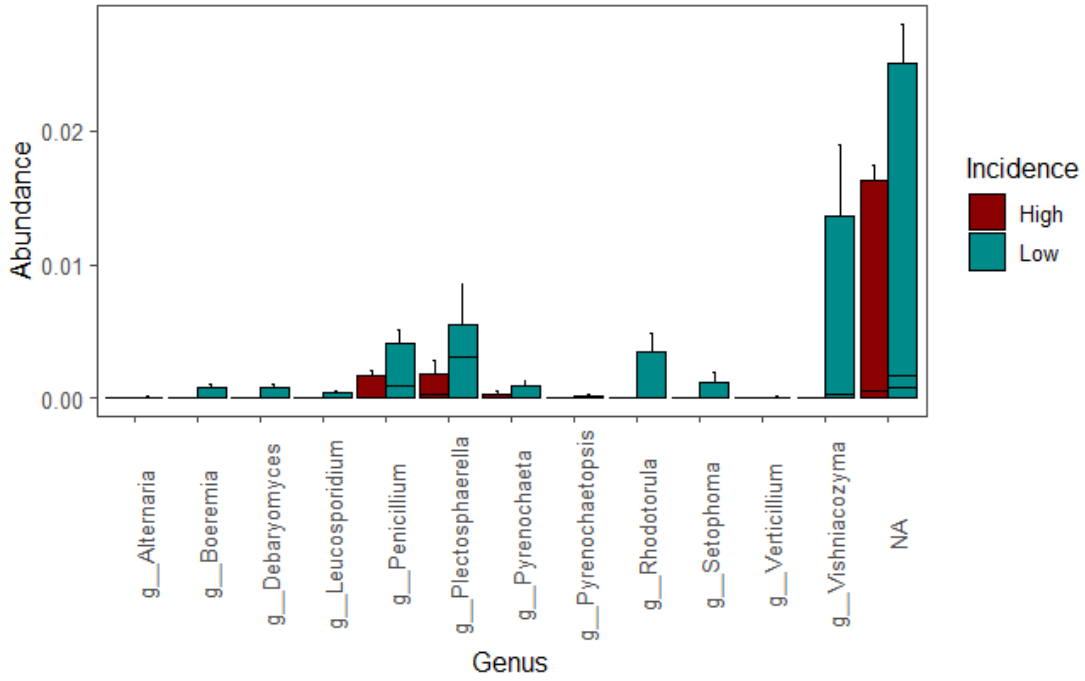


Fig. 13: Relative average abundance in lots with a high and low disease incidence, of OTUs that showed a significant increase in lots with a low disease incidence; the column NA indicates taxa that could not be identified to genus level.

3.3 Study 2019

3.3.1 Disease incidence in the field

3.3.1.1 Location Munnekezijl (clay soil)

At the clay soil location an effect of Species could be detected as disease incidence was higher in plants inoculated with *P. brasiliense* (42%) compared to *D. solani* (36%) and the negative control (1%) (X^2=1310.9, p<0.01). There was also a significant effect of lot (X^2=166.7, p<0.01) and an interaction between lot and species (X^2=151.1, p<0.01) (Fig. 15).

3.3.1.2 Location Kollumerzwaag (sandy soil)

At the sandy soil location the disease incidence differed significantly between treatment with *D. solani* (67%) and *P. brasiliense* (64%) and with the water control (2%) (X^2=2512.4, p<0.01. There was also a significant effect of lot (X^2=88.9, p<0.01) and an interaction between lot and species (X^2=189.7, p<0.01) (Fig. 15).

Although differences between lots were not as pronounced as in the previous year, the lots K13, K14 and K17 with a relatively low disease incidence as well as K18, K19 and K20 with a relatively high disease incidence were chosen for further analysis.

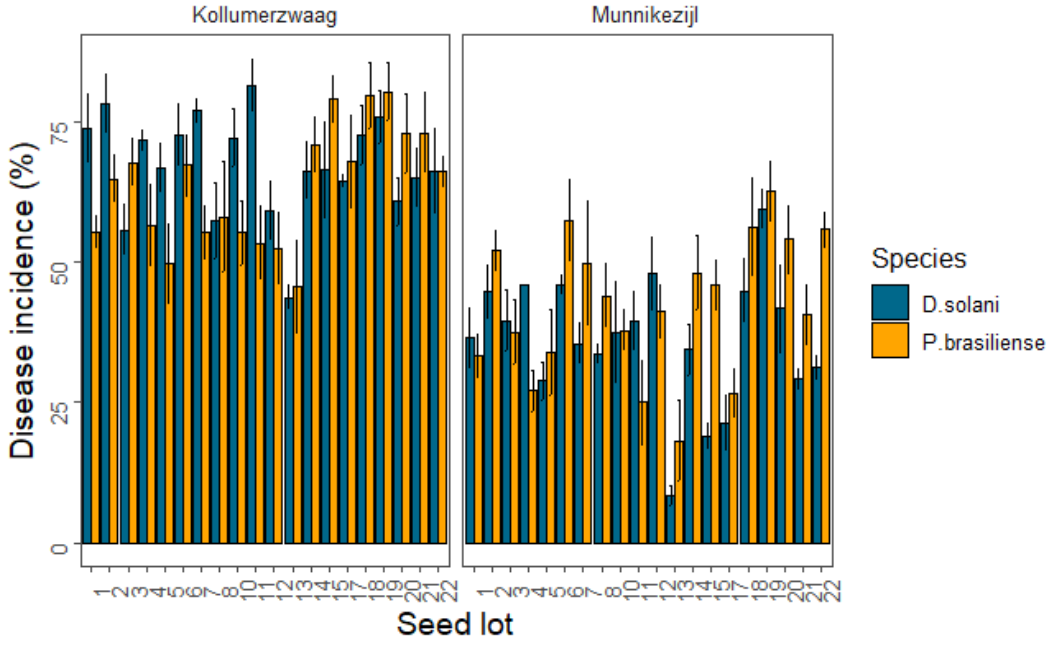


Fig. 15: This bar chart shows disease incidence (%) of the 20 lots of Kondor inoculated with *Dickeya solani and Pectobacterium brasiliense* at the location Kollumerzwaag and Munnekezijl; error bars represent the standard error.

3.3.2 Emergence in the field

Emergence was generally high in all treatments, however, it differed significantly between the species and locations (X2=189.7, p<0.01 and X2=189.7, p<0.01 respectively), but there was no interaction between species and location. Emergence was highest in treatments with *P. brasiliense*. There also were significant differences between lots (X^2=75.7, p<0.01) (Fig. 16). In addition, a slight interaction between location and lot could be detected, but the investigation of pairwise interactions did not show significant differences per lot and location.

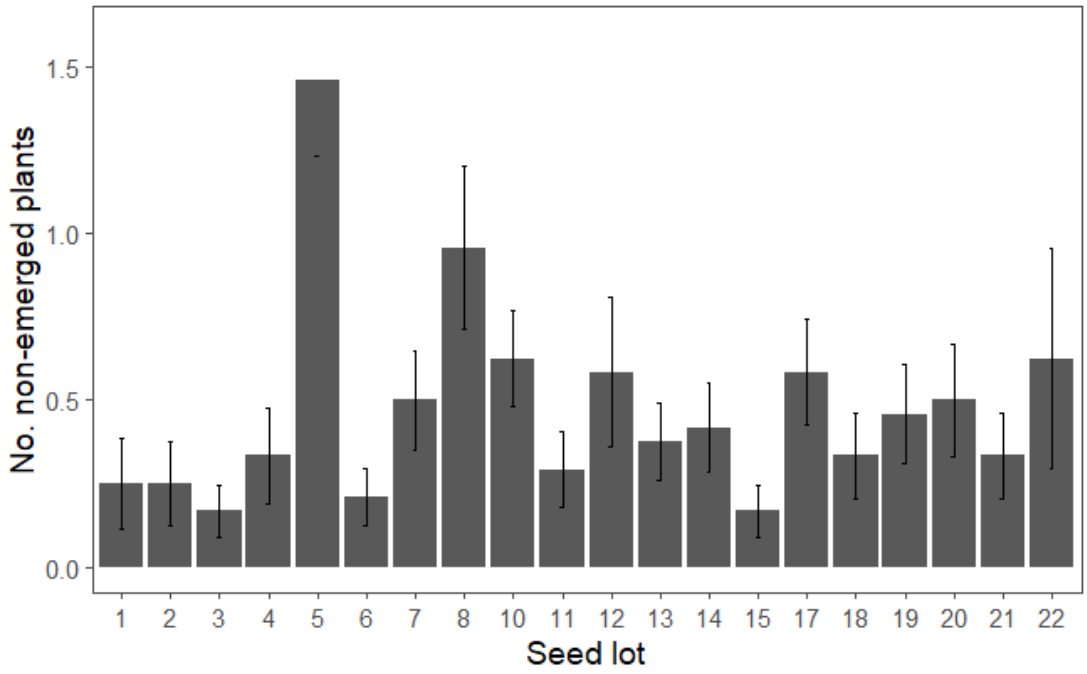


Fig.16: Average number of non-emerged plants for each seed lot; error bars represent the standard error.

3.3.3 Cut tubers

There were no differences between cut and uncut tubers with respect to disease incidence (X^2=0.1, p=0.7). However, cut tubers had on average a 7 times higher non-emergence than uncut tubers (X^2=25.0, p<0.01). Interactions between lots and cutting could not be assessed as only 24 tubers were cut per lot. Within the group of cut tubers there were significant differences between lots (X^2=51.2, p<0.01).

3.3.4 Fungal pathogens

In the year 2019 differences in disease incidence with. *C. coccodes* were no longer tested for, since no differences between lots had been found in the previous year with the fungal pathogens. For confirmation of this observation only *R. solani* was tested. There was a significant difference in disease index between *R. solani* treated tubers and the untreated control (X^2=169.3, p<0.01) with an average disease incidence of 36.7 for treated tubers and 2.6 for the control tubers. There was no effect of lot on disease incidence.

3.3.5 Microbiome analysis

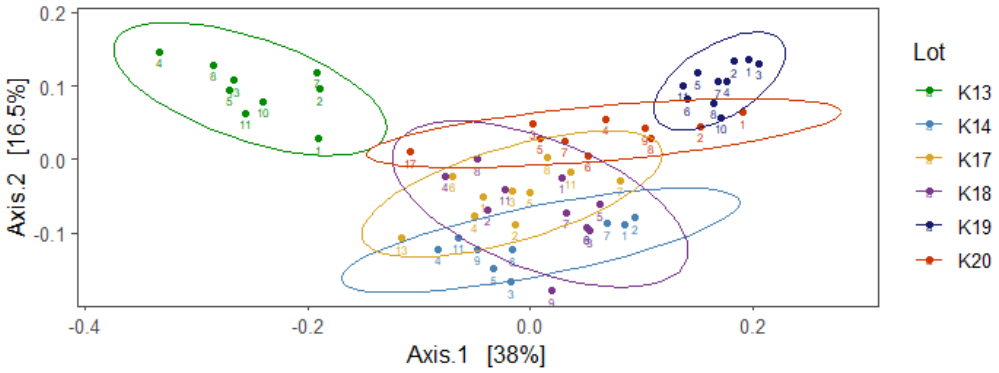
3.3.5.1 Bacterial 16S rDNA

3.3.5.1.1 NMDS for tubers

Non-metric dimensional scaling showed a clustering of samples belonging to the same lot (Fig. 17a). While most clusters showed some overlap, samples from lot 13 and 19 were most distinct from the others. Moreover, samples clustered according to disease incidence (Fig. 17b).

A permanova revealed significant differences between lots (F=32.4, p<0.01) as well as between high and low disease incidence tubers (F=10.7, p<0.01).

a



b

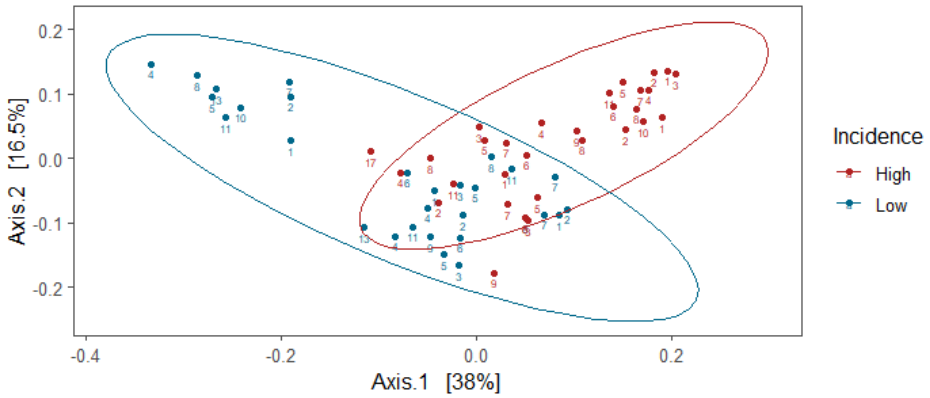
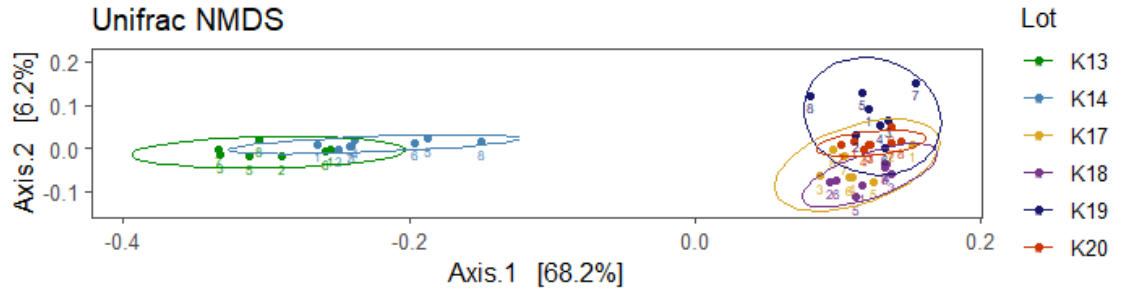


Fig. 17: Non-metric multidimensional scaling with weighted unifrac distances of tuber samples, colours and ellipses indicate a) lot, b) disease incidence of the lot (high, low).

3.3.5.1.2 NMDS for soil

Eight soil samples were analysed from each field that the seed tubers of the selected lots originated from. Non-metric dimensional scaling shows that lots and high and low disease incidence soils are mostly separated on the first axis (Fig.18), with lots 13 and 14 being clearly separated from the other lots. Both lots and high and low disease incidence tubers show significant differences according to a permanova ($F=38.1$, $p<0.01$ and $F=25.2$, $p<0.01$ respectively.

a



b

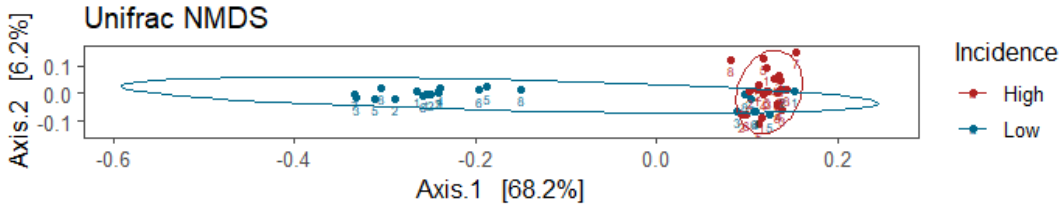


Fig. 18: Non-metric multidimensional scaling with weighted unifrac distances of soil samples, colours and ellipses indicate a) lot ,b) disease incidence of the lot (high, low).

3.3.5.1.3 NMDS for tuberparts

In order to assess if the microbiome differs between locations on the tuber, peel was taken from the stolon end, the rose-end and the middle of 20 tubers from the same lot. The non-metric multidimensional scaling showed no clustering according to location (Fig. 19) and also the permanova revealed no significant differences. Therefore, the microbiome of the different tuber parts was not analysed further.

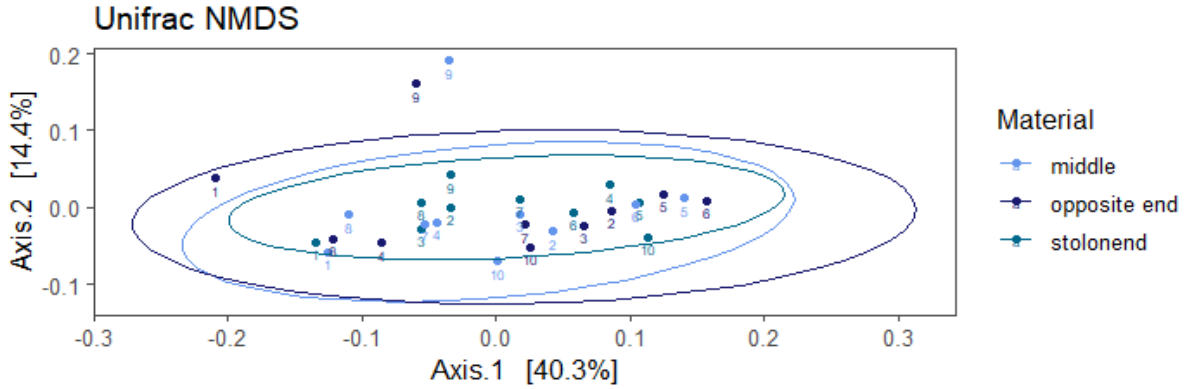


Fig 19: Non-metric dimensional scaling with weighted unifrac distances of tuber-part sample, colours and ellipses indicate the location.

3.3.5.1.4 SPLSDA for tubers

An splsda separated tubers with a high and a low disease incidence (Fig. 20). For the first component 90 OTUs were selected (Table S5). 29 taxa, belonging to 25 genera, were more abundant at a low disease incidence including the genera *Staphylococcus*, *Glutamicibacter*, *Pseudarthrobacter*, *Brevibacterium*, Candidatus Udaeobacter and members of the Bacillaceae (Fig. 21). Of the taxa, which were more abundant at low disease incidence, four were not detected at all at high disease incidence, i.e. *Chungangia*, *Sphingomonas, Oryzihumus* and *Marmoricola*. Taxa that were significantly more abundant in tubers producing plants with a high disease incidence included the genera *Pseudoxanthomonas*, *Lechevaliera*, *Sphingomonas* and *Brevundimonas.*

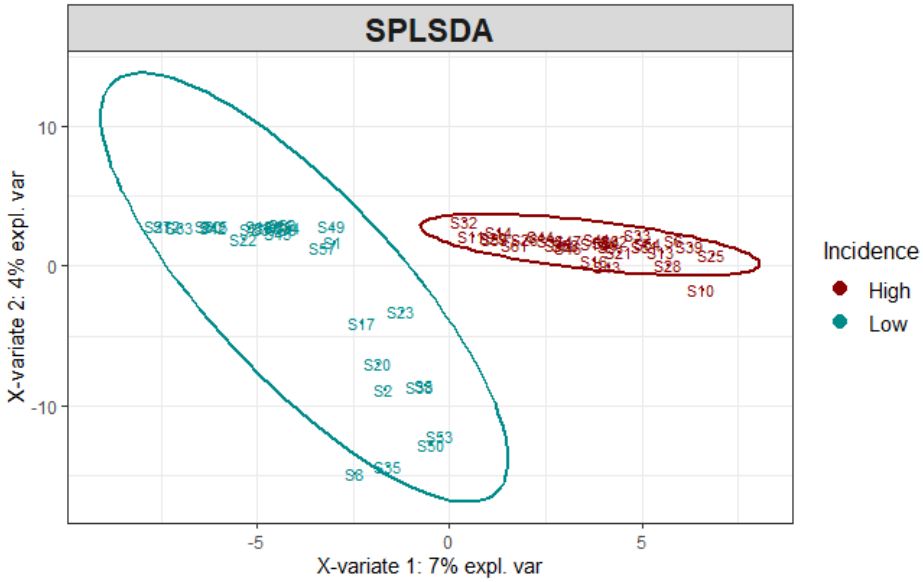


Fig. 20: SPLSDA of OTU abundances with Incidence as the separating factor. Two components and 160 variables were chosen for the final plot.

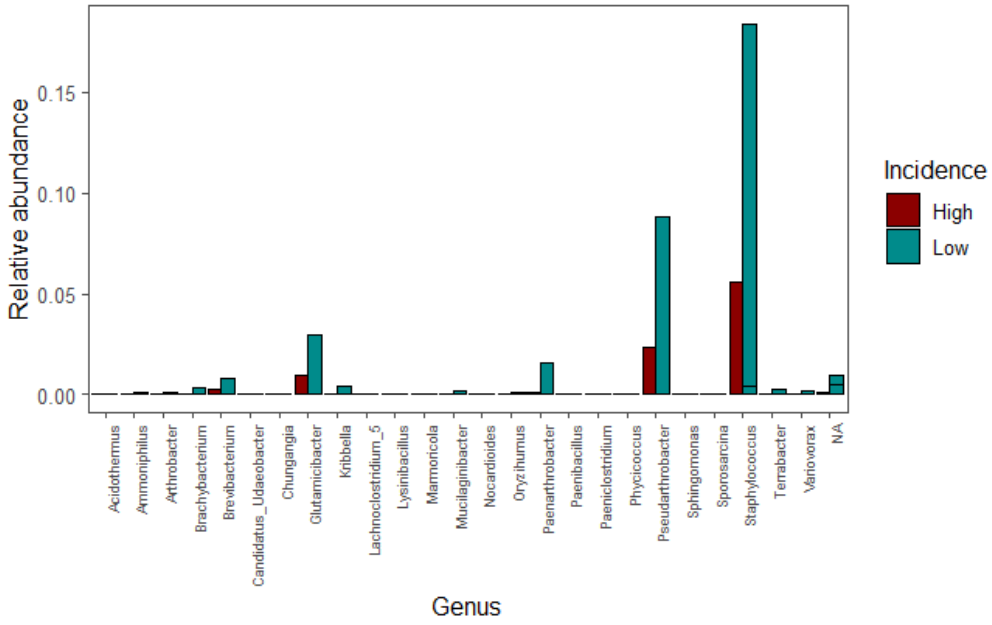


Fig. 21: Relative abundances of all 29 OTUs that were more abundant in samples with a low disease incidence and contributed to the separation in the SPLSDA; the column NA indicates taxa that could not be identified to genus level.

3.3.5.1.5 SPLSDA for soil

An splsda could clearly separate soils that produced lots with a high and low disease incidence (Fig. 22). For the first component, 55 OTUs were selected (Table S6). 34 taxa, belonging to 22 genera, were more abundant in the samples with a low disease incidence, including unidentified members of the families of Bacillaceae and Planoccocaceae, the genera Candidatus Udaeobacter and *Bradyrhizobium* (Fig. 23). 17 of the taxa that were more abundant at low disease incidences could not be detected at high disease incidence. Taxa that were significantly more abundant in soil producing tubers with a high disease incidence included a member of the family Burkholderiaceae and the genera RB41, *Adhaeribacter,* and *Agromyces*.

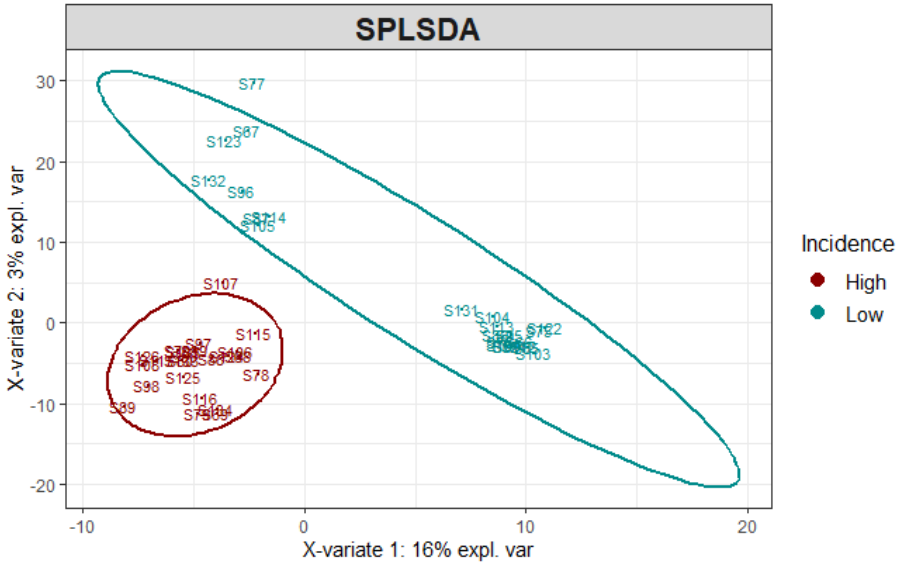


Fig. 22: SPLSDA of OTU abundances with Incidence as the separating factor. Two components and 105 variables were chosen for the final plot.

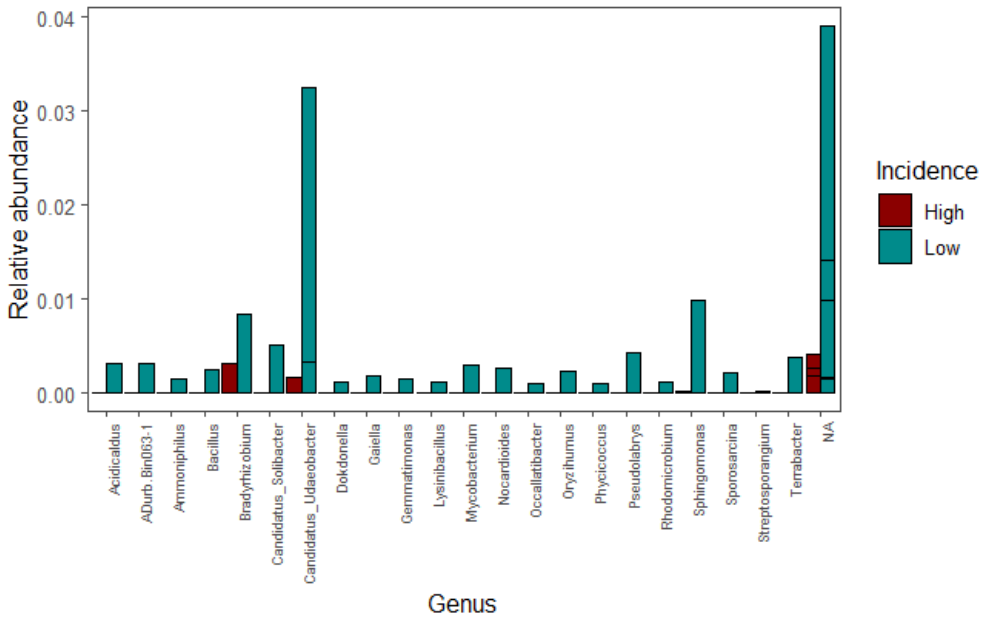


Fig. 23: Relative abundances of all 34 OTUs that were more abundant in samples with a low disease incidence and contributed to the separation in the SPLSDA; the column NA indicates taxa that could not be identified to genus level.

3.3.5.1.6 DESeq2 for tubers

Using the DESeq2 analysis 403 OTUs were detected as differing significantly in abundance between high and low disease incidence, 80 OTUs of which were more abundant in low disease incidence samples (Fig.24). Taxa with a high abundance again include *Staphylococcus, Glutamicibacter, Pseudarthrobacter, Brevibacterium*, Candidatus Udaeobacter and members of the Bacillaceae (Fig. 25).

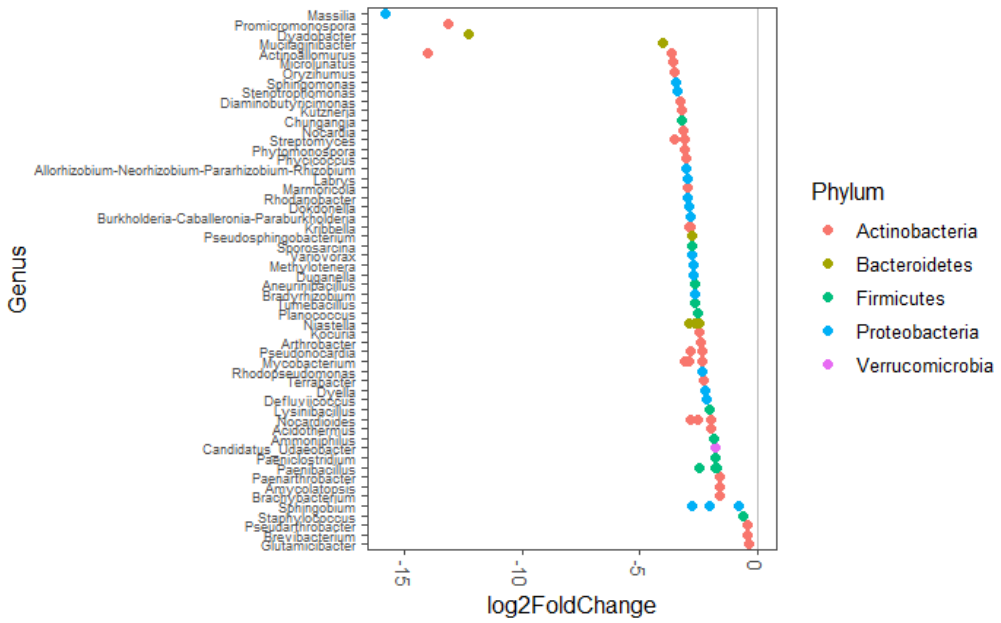


Fig. 24: log2-fold change in abundance in the low disease incidence samples compared to the high disease incidence samples of 80 taxa; each datapoint represents an OTU.

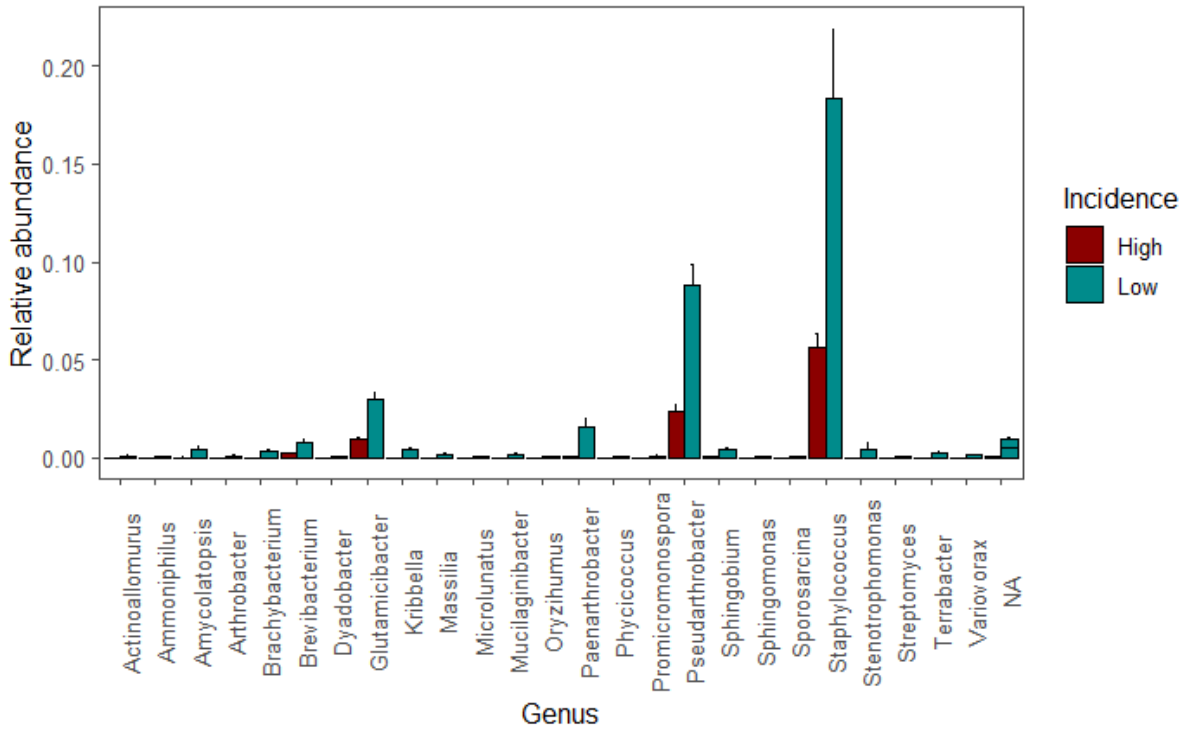


Fig. 25: Average relative abundance in lots with a high and low disease incidence, of the 30 most abundant OTUs, belonging to 25 genera, that showed a significant increase in lots with a low disease incidence according to the DESeq2 analysis; the column NA indicates taxa that could not be identified to genus level.

3.3.5.1.7 DESeq2 for soil

In soil, with DESeq2, 862 taxa were detected as significantly different between soils that were the origin of tubers with a low and a high disease incidence. Of these taxa, 336 were more abundant at low disease incidence and were identified to genus level (Fig. 26). Among the thirty most abundant taxa were Candidatus *Udaeobacter*, *Acidobacter*, *Sphingomonas* and *Bradyrhizobium*

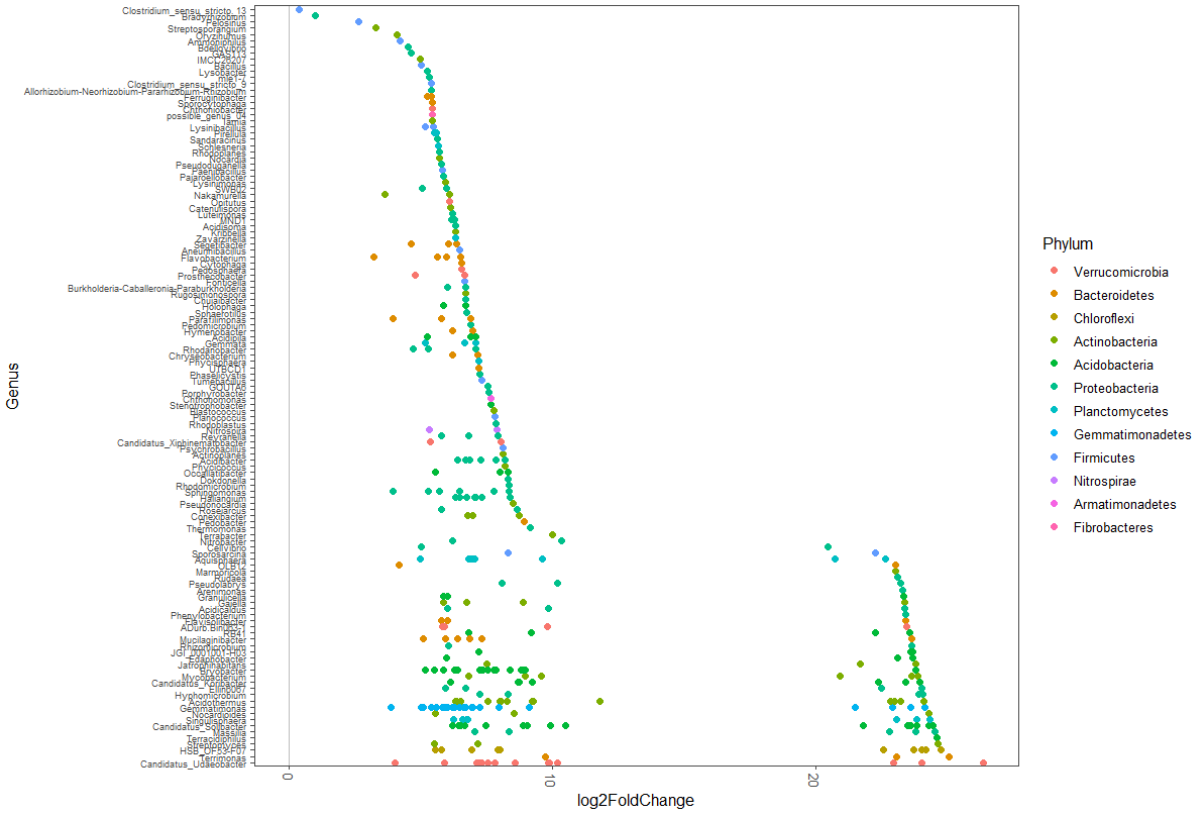


Fig. 26: log2-fold change in abundance in the low disease incidence samples compared to the high disease incidence samples of 62 taxa; each datapoint represents an OTU.

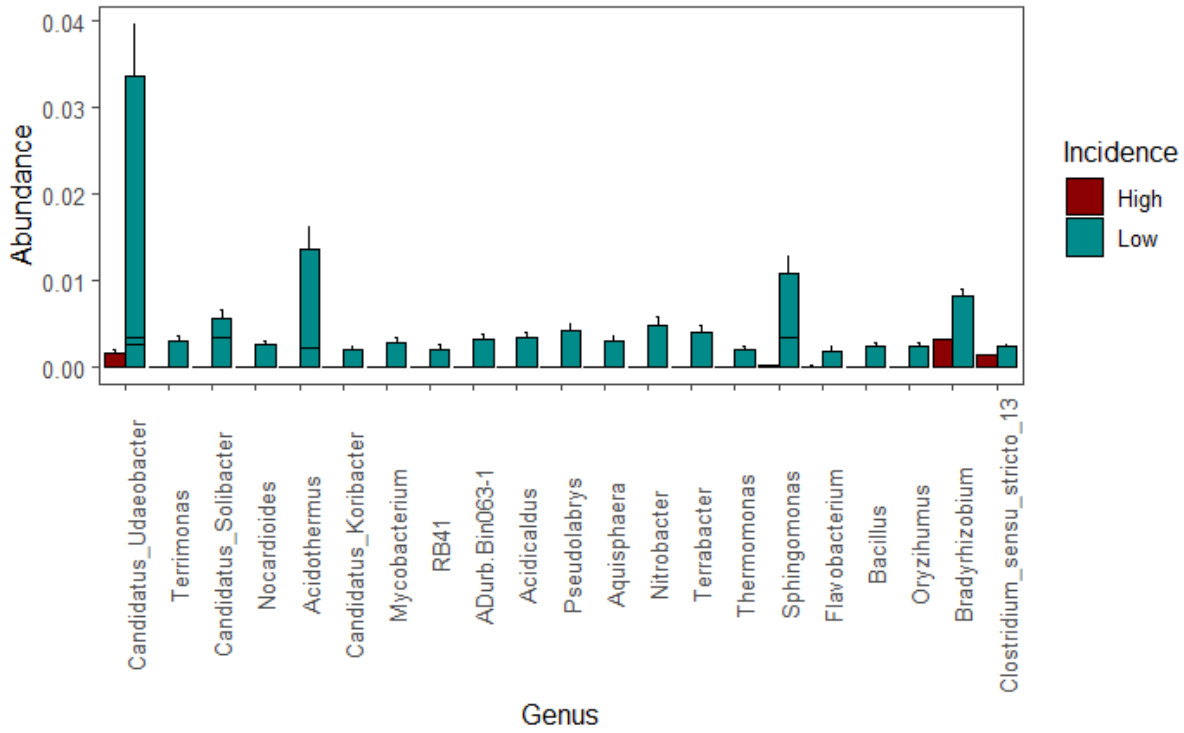


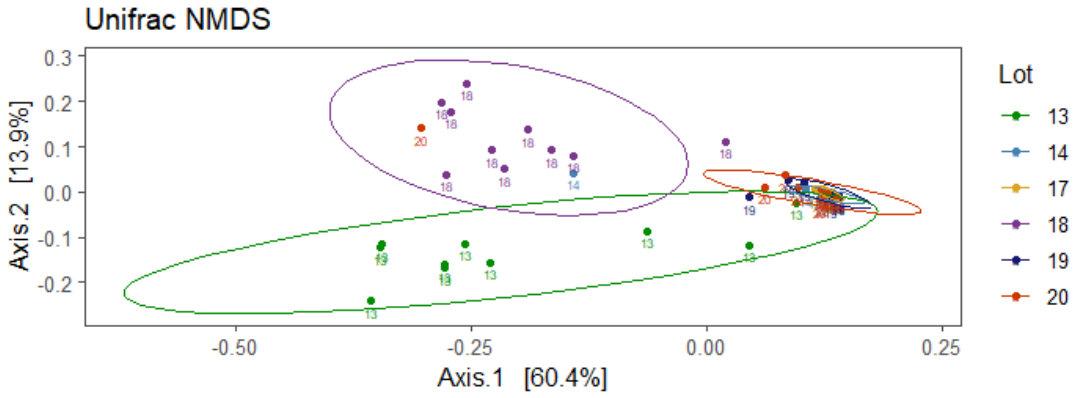
Fig. 27: Average relative abundance in lots with a high and low disease incidence, of the 30 most abundant OTUs, belonging 21 genera, that showed a significant increase in lots with a low disease incidence according to the DESeq2 analysis; the column NA indicates taxa that could not be identified to genus level.

3.3.5.2 Fungal ITS

3.3.5.2.1 NMDS for tubers

Non-metric multidimensional scaling showed that the lots 13 and 18 mostly group apart from the other lots (Fig. 28a). The permanova analysis detected a significant difference between lots ($F=10.8$, $p<0.01$). However, no clear separation can be observed between lots with a high disease incidence and a low disease incidence (Fig. 28b). Still, the permanova indicates a significant difference ($F=4.0$, $p=0.02$).

a



b

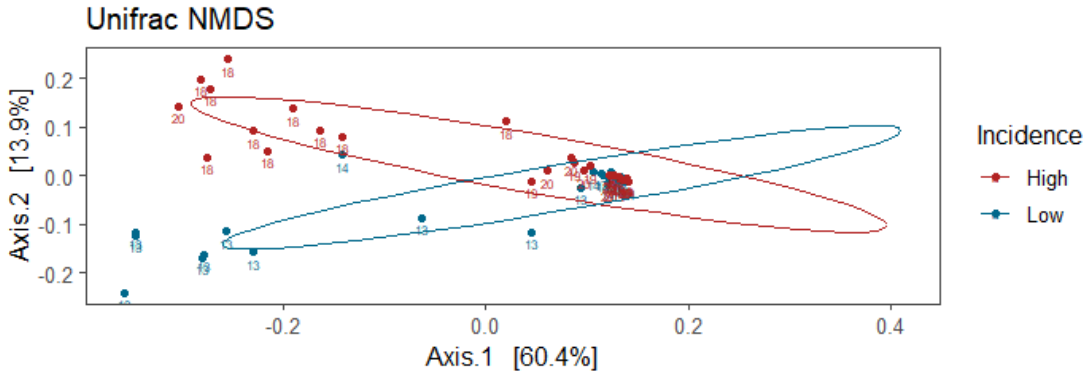
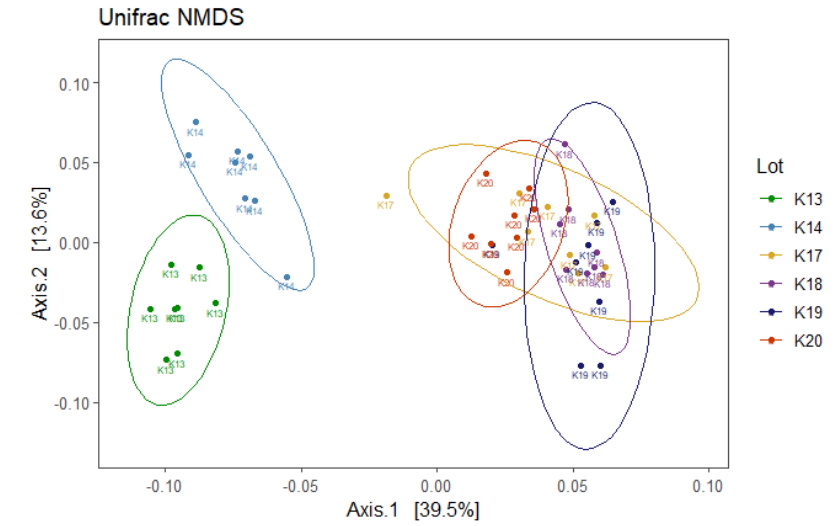


Fig. 28: Non-metric multidimensional scaling with weighted unifrac distances of tuber samples, colours and ellipses indicate a) lot ,b) disease incidence of the lot (high, low).

3.3.5.2.2 NMDS for soil

For the soil microbial community the lots 13 and 14 are clustered apart, while the other lots are not separated (Fig. 29a). Therefore, there is no clear separation between samples from high incidence and low incidence lots as well (Fig. 29b). However, the permanova analysis suggests significant differences ($F=16.5$, $p<0.01$ and $F=22.4$, $p<0.01$ respectively).

a



b

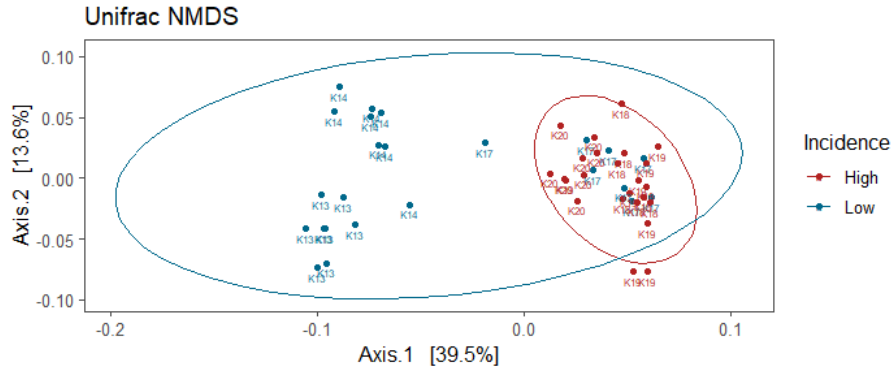


Fig. 29: Non-metric multidimensional scaling with weighted unifrac distances of soil samples, colours and ellipses indicate a) lot, b) disease incidence of the lot (high, low)

3.3.5.2.3 NMDS for tuberparts

Also for the ITS, data there were no differences in the fungal community composition between the different tuber parts (F=0.2, p=0.79) (Fig. 30).

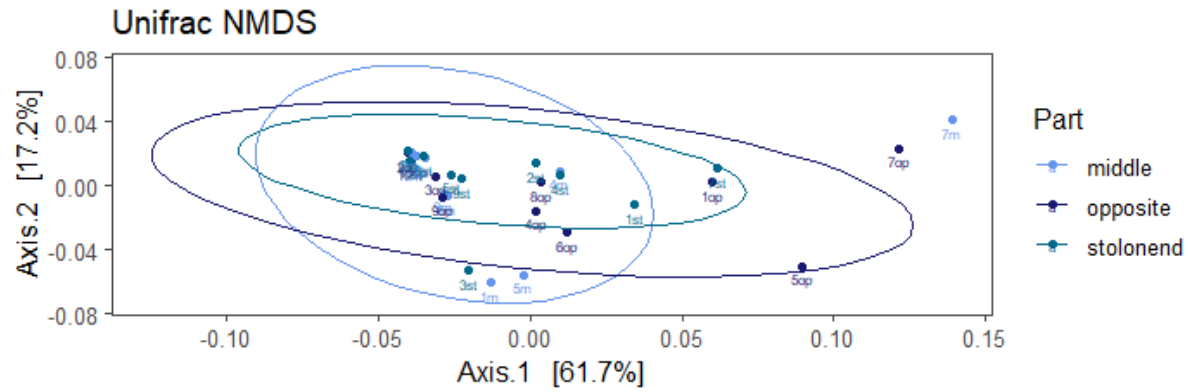


Fig 30: Non-metric dimensional scaling with weighted unifrac distances of tuber-part sample, colours and ellipses indicate the location.

3.3.5.2.4 SPLSDA for tubers

With the use of an SPLSDA, tubers from lots with a high and low disease incidence could more clearly be separated (Fig. 31). Two components were chosen for the final plot. The first component consisted of 90 OTUs, of which 45 (belonging to 24 genera) were more abundant at low disease incidence (Fig. 32). These included *Fusarium oxysporum*, *Debaryomyces hansenii*, *Plectosphaerella niemeijerarum*, *Vishniacozyma heimaeyensis, Mycosphaerella tassiana* and members of the genera *Alternaria* and *Cladiosporium* (Fig. 31). Of the species that were more abundant at a low disease incidence, 21 were not detected at all in samples from high disease incidence tubers. Among the 45 taxa that were more abundant in tubers of lots with a high disease incidence were *Mortierella minutissima*, *Plectosphaerella oligotrophica*, Pseudeurotium bakeri, *Fusarium oxysporum* and *Colletotrichum* sp..

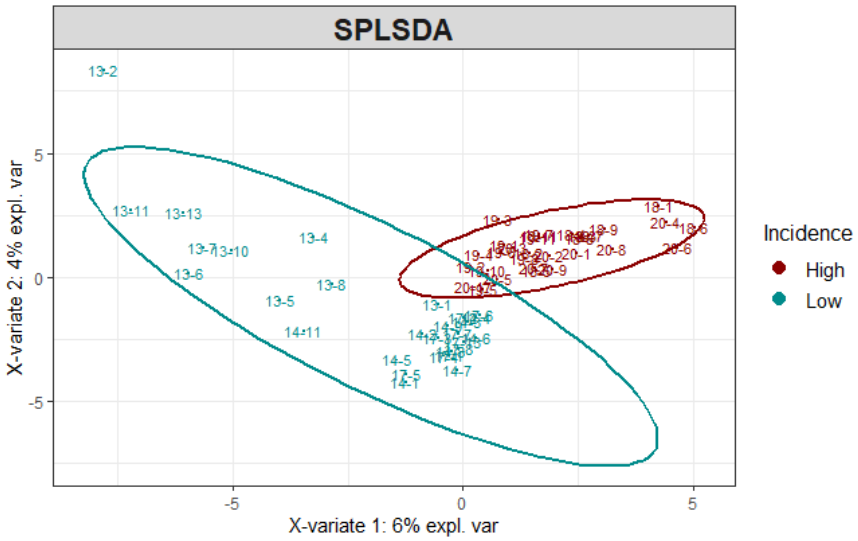


Fig. 31: SPLSDA of OTU abundances with Incidence as the separating factor. Two components and 190 variables were chosen for the final plot.

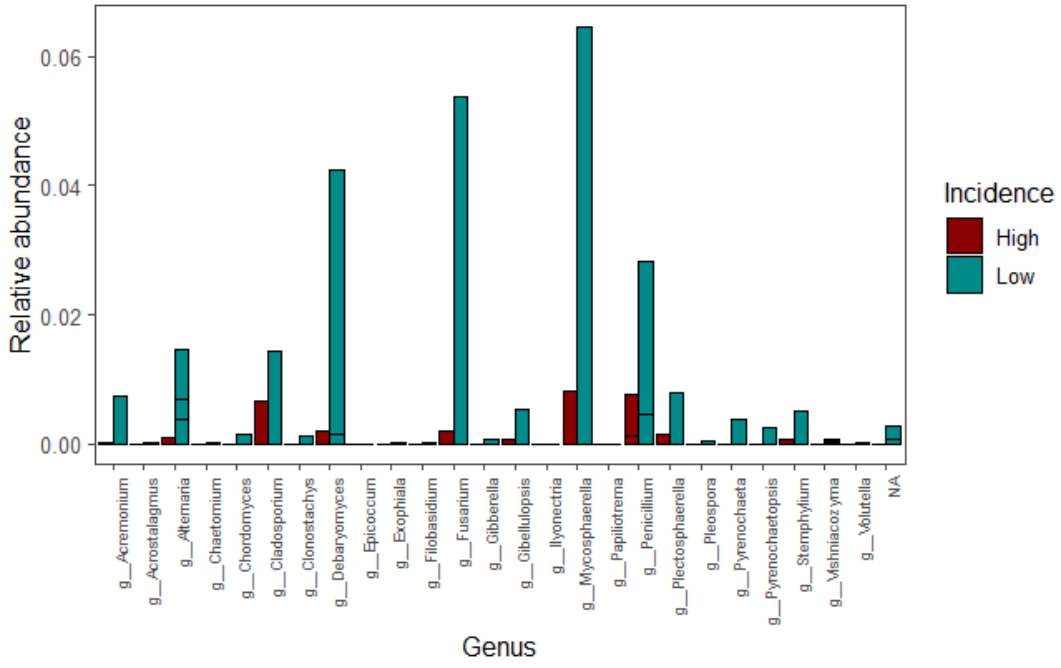


Fig. 32: Relative abundances of all 45 OTUs that were more abundant in samples with a low disease incidence and contributed to the separation in the SPLSDA; the column NA indicates taxa that could not be identified to genus level.

3.3.5.2.5 SPLSDA for soil

The splsda on soil communities shows a separation between samples with a high and a low disease incidence (Fig. 33). It is notable that for both tuber and soil the communities from tubers with a high disease incidence cluster closer together than those with a low disease incidence. Two components were

chosen for the final plot, including 40 and 6 OTUs respectively. For the first components, 31 of the 40 OTUs showed a higher abundance at lower disease incidence, among which *Cladosporium cladosporides, Saitozyma podzolica, Fusarium oxysporum* and two species of the genus *Solicoccozyma* (Fig.34). 17 OTUs were not present at all at samples with a high disease incidence. Species that were more abundant in soil that yielded tubers with high disease incidence include *Lophotrichus fimeti* and *Botryotrichum spirotrichum*.

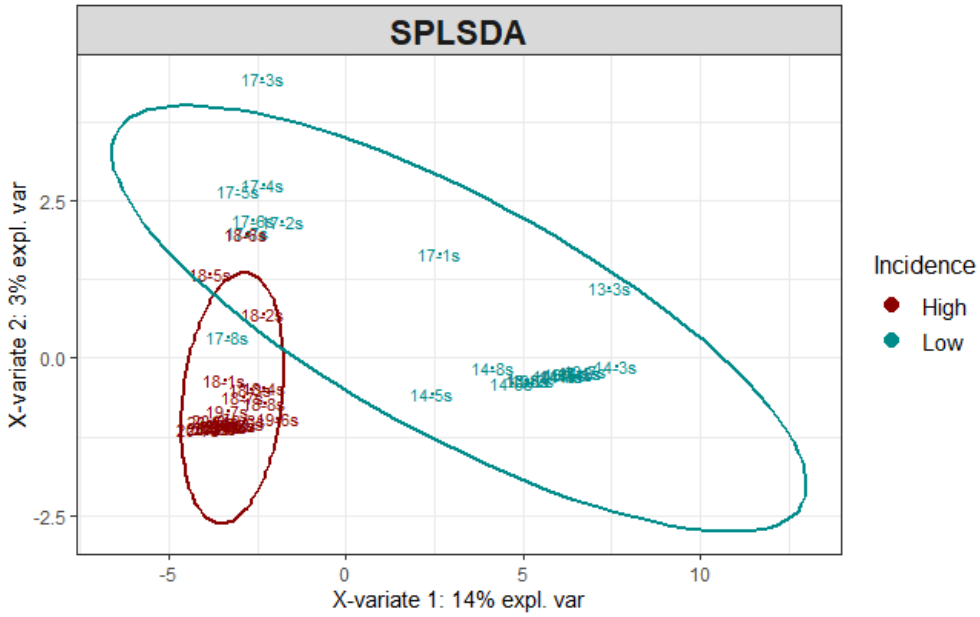


Fig. 33: SPLSDA of OTU abundances with Incidence as the separating factor. Two components and 46 variables were chosen for the final plot.

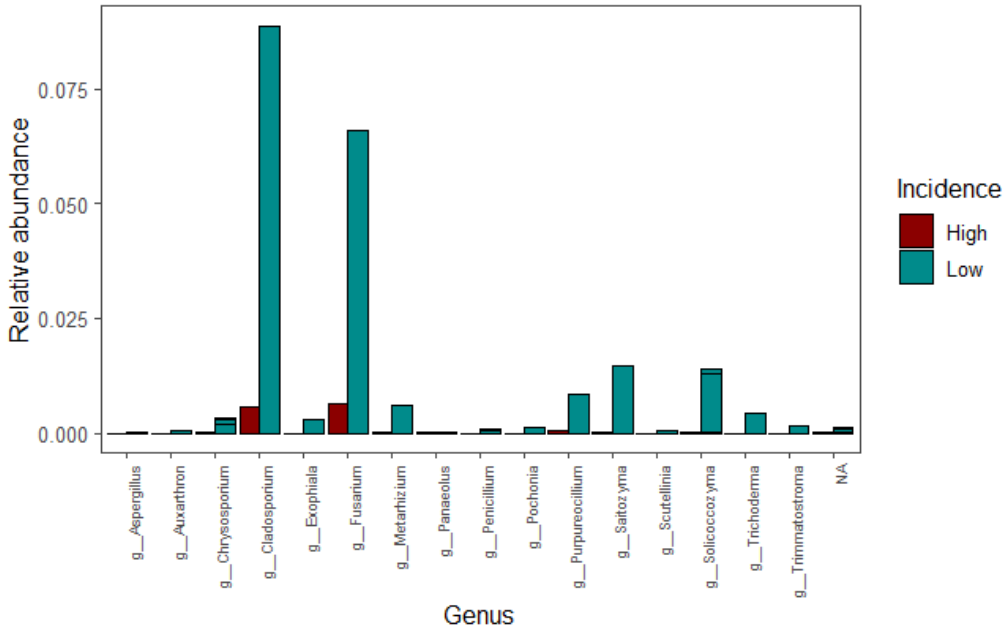


Fig. 34: Relative abundances of all 31 OTUs, belonging to 16 genera, that were more abundant in samples with a low disease incidence and contributed to the separation in the SPLSDA; the column NA indicates taxa that could not be identified to genus level.

3.3.5.2.6 DESeq2 for tubers

The DESeq analysis identified 27 OTUs as being significantly different between samples with a high and a low disease incidence. Among the 16 OTUs, belonging to 11 genera, that were more abundant in tubers that belonged to lots with a low disease incidence were the species *Fusarium oxysporium*, *Debaryomyces hansenii*, *Mycosphaerella tassiana* and *Plectosphaerella niemeijerarum* (Fig. 35, 36). All 16 OTUs were shared between the results of the DESeq and the SPLSDA analysis (Table 6).

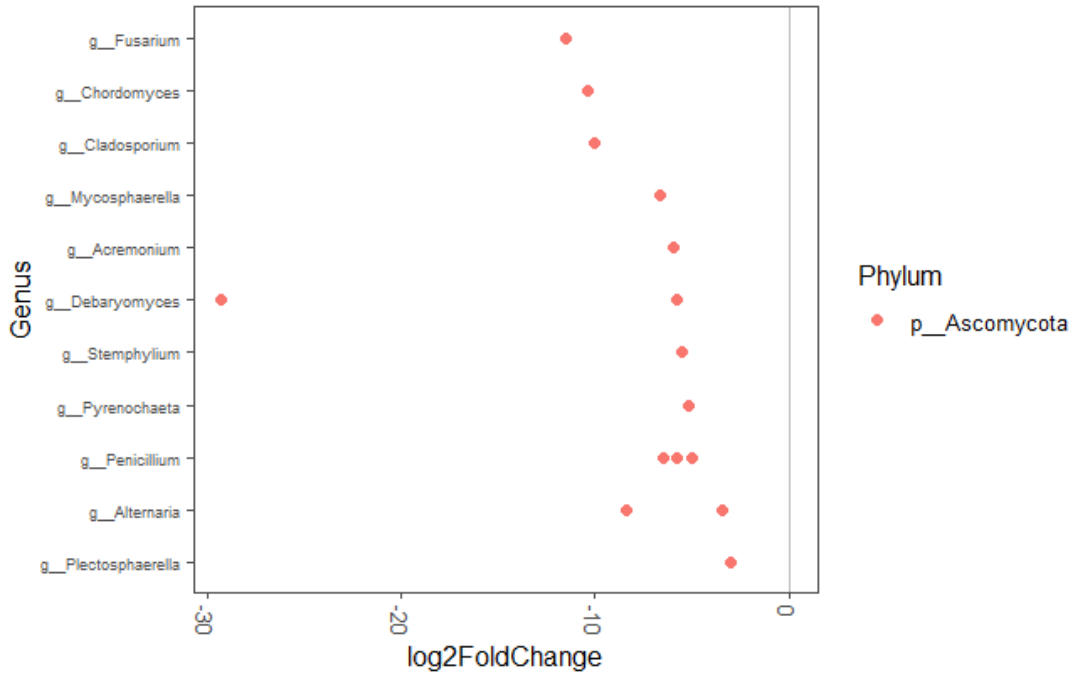


Fig. 35: log2-fold change in abundance in the low disease incidence samples compared to the high disease incidence samples of 16 taxa; each datapoint represents an OTU.

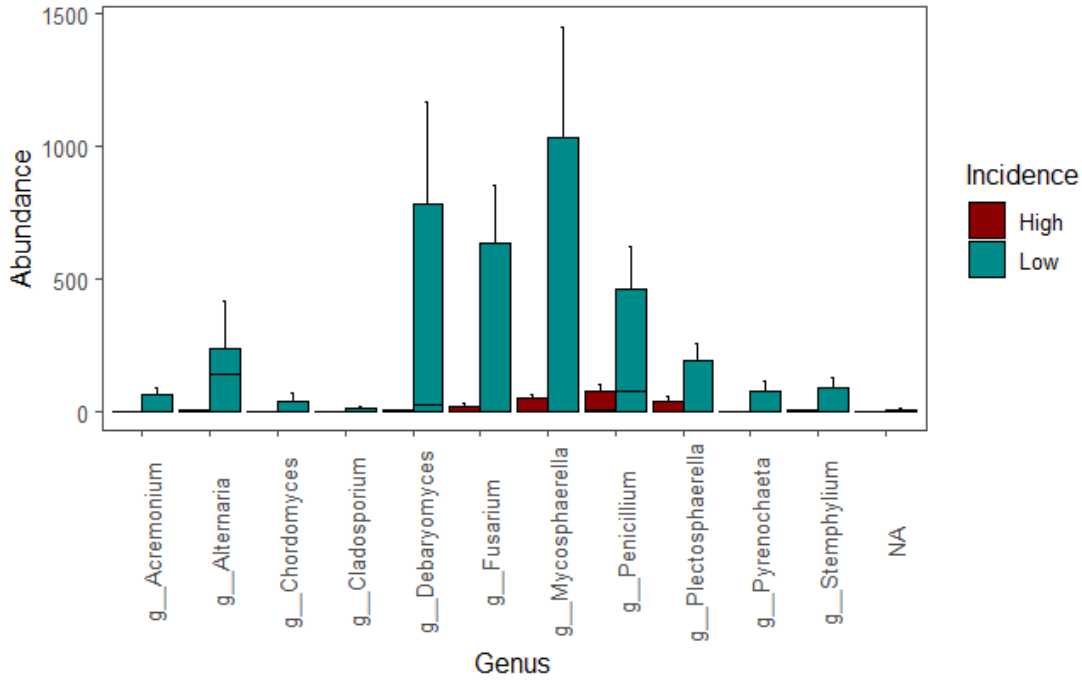


Fig. 36: Average relative abundance in lots with a high and low disease incidence, all OTUs that showed a significant increase in lots with a low disease incidence according to the DESeq2 analysis; the column NA indicates taxa that could not be identified to genus level.

Table 6: OTUs that have a significantly higher abundance in tubers with a low disease incidence compared to a high disease incidence in both the SPLSDA and DESeq anaysis; shown are the mean relative abundance at high and low disease incidence, the log-2-fold change as calculated by DESeq and the adjusted p-value.

OTU	Phylum	Genus	Species	Incidence	Relative abundance	log2FoldChange	padj
OTU9	Ascomycota	Fusarium	oxysporum	Low	0.053785	-11.4921	7.20E-18
OTU20	Ascomycota	Plectosphaerella	niemeijerarum	Low	0.007997	-3.0438	0.026366

OTU104	Ascomycota	Acremonium	furcatum	Low	0.007327	-5.93578	0.027615
OTU181	Ascomycota	Chordomyces	antarcticus	Low	0.001524	-10.3572	0.000223
OTU29	Ascomycota	Debaryomyces	hansenii	Low	0.042287	-5.79948	0.000447
OTU375	Ascomycota	Debaryomyces	hansenii	Low	0.001494	-29.2502	2.14E-22
OTU24	Ascomycota	Penicillium	brevicompactum	Low	0.028292	-5.79116	5.87E-09
OTU84	Ascomycota	Penicillium	bialowiezense	Low	0.004664	-4.99649	0.006587
OTU56	Ascomycota	Penicillium	jensenii	Low	0.00111	-6.45743	0.004347
OTU12	Ascomycota	Mycosphaerella	tassiana	Low	0.064517	-6.66685	8.50E-10
OTU16	Ascomycota	Alternaria	alternata	Low	0.014531	-3.46979	0.019512
OTU50	Ascomycota	Alternaria	NA	Low	0.00682	-8.43051	3.87E-06
OTU70	Ascomycota	Stemphylium	NA	Low	0.005001	-5.50661	0.005088
OTU150	Ascomycota	Pyrenochaeta	NA	Low	0.00378	-5.14412	0.019512
OTU256	Ascomycota	Cladosporium	NA	Low	0.001077	-10.0733	0.00218
OTU100	Ascomycota	NA	NA	Low	0.000479	-7.31017	0.000839
OTU181	Ascomycota	Chordomyces	antarcticus	High	1.38E-05	-10.3572	0.000223
OTU9	Ascomycota	Fusarium	oxysporum	High	0.001953	-11.4921	7.20E-18
OTU20	Ascomycota	Plectosphaerella	niemeijerarum	High	0.001479	-3.0438	0.026366
OTU104	Ascomycota	Acremonium	furcatum	High	0.00013	-5.93578	0.027615
OTU29	Ascomycota	Debaryomyces	hansenii	High	0.001964	-5.79948	0.000447
OTU375	Ascomycota	Debaryomyces	hansenii	High	0	-29.2502	2.14E-22
OTU24	Ascomycota	Penicillium	brevicompactum	High	0.007681	-5.79116	5.87E-09
OTU84	Ascomycota	Penicillium	bialowiezense	High	0.001199	-4.99649	0.006587
OTU56	Ascomycota	Penicillium	jensenii	High	0.000325	-6.45743	0.004347
OTU100	Ascomycota	NA	NA	High	2.98E-05	-7.31017	0.000839
OTU12	Ascomycota	Mycosphaerella	tassiana	High	0.008191	-6.66685	8.50E-10
OTU16	Ascomycota	Alternaria	alternata	High	0.001087	-3.46979	0.019512
OTU70	Ascomycota	Stemphylium	NA	High	0.000642	-5.50661	0.005088
OTU50	Ascomycota	Alternaria	NA	High	0.000532	-8.43051	3.87E-06
OTU150	Ascomycota	Pyrenochaeta	NA	High	0	-5.14412	0.019512
OTU256	Ascomycota	Cladosporium	NA	High	0	-10.0733	0.00218

3.3.5.2.7 DESeq2 for soil

For soil, the DESeq analysis identified 227 OTUs as significantly different in abundance between lots with high and low disease incidence, of which 171 OTUs were more abundant at a low disease incidence compared with a high disease incidence (Fig. 37). The most abundant OTUs at low disease incidence included *Cladiosporium cladosporioides*, *Fusarium oxysporium*, and *Paraphaeosphaeria sporulosa* and also *Saitozyma podzolica* and *Solicoccozyma* sp (Fig. 38). All 31 OTUs, belonging to 23 genera, that were identified as more abundant in soil samples that yielded tubers with a lower disease incidence by the SPLSDA were also significantly differing in abundance according to the DESeq analysis (Table 7).

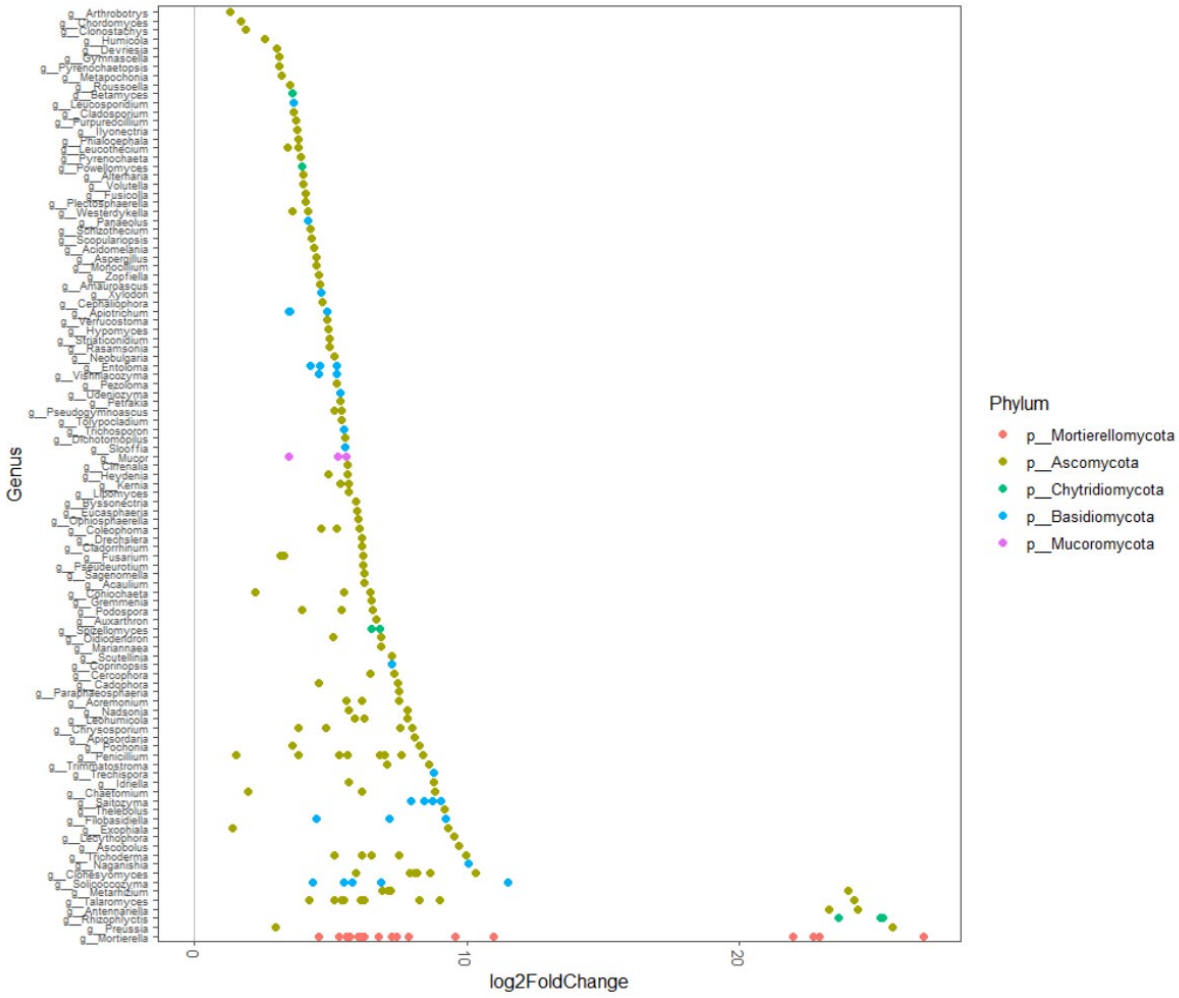


Fig. 37: log2-fold change in abundance in the low disease incidence samples compared to the high disease incidence samples of 171 taxa; each datapoint represents an OTU.

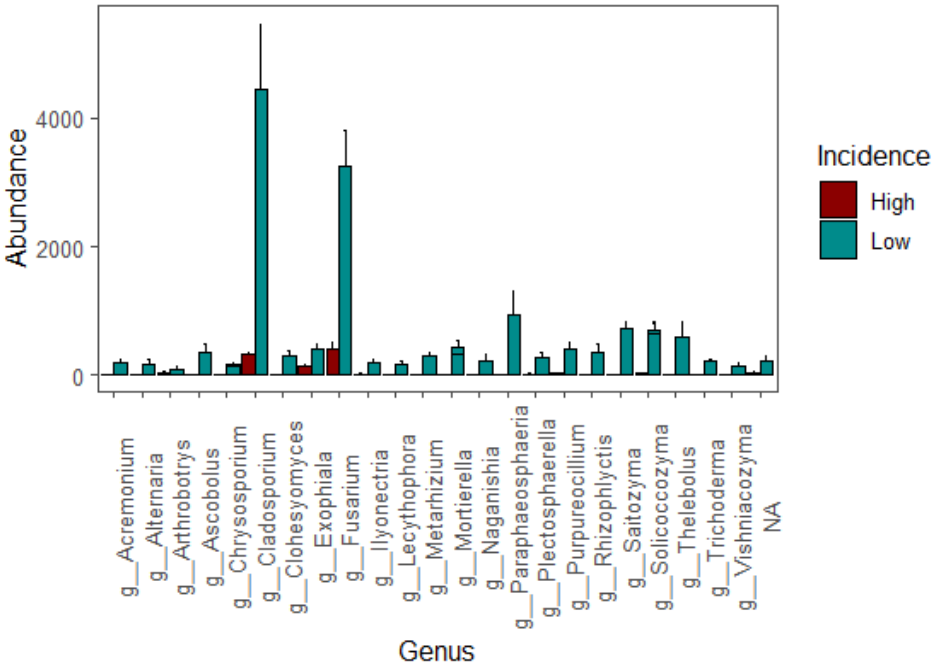


Fig. 38: Average relative abundance in lots with a high and low disease incidence, of the 30 most abundant OTUs that showed a significant increase in lots with a low disease incidence according to the DESeq2 analysis; the column NA indicates taxa that could not be identified to genus level.

Table 7: OTUs that have a significantly higher abundance in soil with a low disease incidence compared to a high disease incidence in both the SPLSDA and DESeq anaysis; shown are the mean relative abundance at high and low disease incidence, the log-2-fold change as calculated by DESeq and the adjusted p-value.

OTU	Phylum	Genus	Species	Incidence	Relative abundance	log2FoldChange	padj
OTU7	Ascomycota	Cladosporium	cladosporioides	Low	0.088657	-3.65968	1.38E-12
OTU9	Ascomycota	Fusarium	oxysporum	Low	0.066083	-3.30584	6.84E-09
OTU40	Basidiomycota	Saitozyma	podzolica	Low	0.014586	-8.4494	2.72E-12
OTU41	Basidiomycota	Solicoccozyma	terricola	Low	0.014104	-5.50378	2.12E-06
OTU46	Basidiomycota	Solicoccozyma	phenolica	Low	0.013057	-11.5152	3.27E-24
OTU66	Ascomycota	Purpureocillium	NA	Low	0.008353	-3.71763	2.55E-12
OTU98	Ascomycota	Metarhizium	carneum	Low	0.005977	-7.09943	5.90E-13
OTU130	Ascomycota	Trichoderma	hamatum	Low	0.004146	-9.98326	9.55E-19
OTU145	Ascomycota	Chrysosporium	merdarium	Low	0.003268	-3.83085	0.005195
OTU166	Ascomycota	Exophiala	equina	Low	0.002951	-9.32902	9.37E-19
OTU170	Ascomycota	Chrysosporium	pseudomerdarium	Low	0.002939	-7.98936	8.19E-12
OTU200	Basidiomycota	Saitozyma	podzolica	Low	0.00224	-9.02765	2.68E-16
OTU211	Ascomycota	Chrysosporium	pseudomerdarium	Low	0.00192	-4.8492	0.000164
OTU220	Basidiomycota	Saitozyma	podzolica	Low	0.001908	-8.74565	3.71E-12
OTU237	Ascomycota	Trimmatostroma	salicis	Low	0.001671	-8.63072	2.31E-15
OTU267	Ascomycota	Pochonia	cordycepisociata	Low	0.00134	-8.26617	2.73E-14
OTU273	Ascomycota	NA	NA	Low	0.001297	-6.14173	1.12E-09
OTU286	Ascomycota	NA	NA	Low	0.001208	-6.05834	8.37E-07
OTU291	Ascomycota	NA	NA	Low	0.001149	-7.99356	1.28E-13
OTU332	Ascomycota	NA	NA	Low	0.000901	-2.77555	0.007963
OTU351	Ascomycota	NA	NA	Low	0.000856	-7.60337	2.80E-13
OTU352	Ascomycota	Penicillium	sacculum	Low	0.000854	-7.59886	5.33E-10
OTU436	Ascomycota	Scutellinia	vitreola	Low	0.000584	-7.23402	1.08E-10
OTU501	Ascomycota	Penicillium	NA	Low	0.000468	-6.82012	1.68E-08
OTU531	Ascomycota	Auxarthron	umbrinum	Low	0.000453	-6.66972	5.56E-11
OTU697	Basidiomycota	Panaeolus	papilionaceus	Low	0.000247	-4.18931	0.000413
OTU738	Basidiomycota	Solicoccozyma	aeria	Low	0.000236	-5.79	5.28E-08
OTU749	Basidiomycota	NA	NA	Low	0.000225	-5.75206	1.70E-07
OTU789	Ascomycota	NA	NA	Low	0.000201	-4.41501	4.98E-05
OTU942	Ascomycota	NA	NA	Low	0.000156	-5.04735	1.27E-06
OTU1229	Ascomycota	Aspergillus	chlamydosporus	Low	9.63E-05	-4.47649	0.00025
OTU9	Ascomycota	Fusarium	oxysporum	High	0.006498	-3.30584	6.84E-09
OTU7	Ascomycota	Cladosporium	cladosporioides	High	0.005659	-3.65968	1.38E-12
OTU66	Ascomycota	Purpureocillium	NA	High	0.000471	-3.71763	2.55E-12
OTU41	Basidiomycota	Solicoccozyma	terricola	High	0.000275	-5.50378	2.12E-06
OTU145	Ascomycota	Chrysosporium	merdarium	High	0.00015	-3.83085	0.005195
OTU211	Ascomycota	Chrysosporium	pseudomerdarium	High	8.64E-05	-4.8492	0.000164
OTU332	Ascomycota	NA	NA	High	7.75E-05	-2.77555	0.007963
OTU40	Basidiomycota	Saitozyma	podzolica	High	4.73E-05	-8.4494	2.72E-12
OTU98	Ascomycota	Metarhizium	carneum	High	3.67E-05	-7.09943	5.90E-13
OTU286	Ascomycota	NA	NA	High	1.98E-05	-6.05834	8.37E-07
OTU273	Ascomycota	NA	NA	High	1.70E-05	-6.14173	1.12E-09
OTU697	Basidiomycota	Panaeolus	papilionaceus	High	1.27E-05	-4.18931	0.000413

OTU789	Ascomycota	NA	NA	High	7.27E-06	-4.41501	4.98E-05
OTU170	Ascomycota	Chrysosporium	pseudomerdarium	High	6.41E-06	-7.98936	8.19E-12
OTU1229	Ascomycota	Aspergillus	chlamydosporus	High	0	-4.47649	0.00025
OTU130	Ascomycota	Trichoderma	hamatum	High	0	-9.98326	9.55E-19
OTU166	Ascomycota	Exophiala	equina	High	0	-9.32902	9.37E-19
OTU200	Basidiomycota	Saitozyma	podzolica	High	0	-9.02765	2.68E-16
OTU220	Basidiomycota	Saitozyma	podzolica	High	0	-8.74565	3.71E-12
OTU237	Ascomycota	Trimmatostroma	salicis	High	0	-8.63072	2.31E-15
OTU267	Ascomycota	Pochonia	cordycepisociata	High	0	-8.26617	2.73E-14
OTU291	Ascomycota	NA	NA	High	0	-7.99356	1.28E-13
OTU351	Ascomycota	NA	NA	High	0	-7.60337	2.80E-13
OTU352	Ascomycota	Penicillium	sacculum	High	0	-7.59886	5.33E-10
OTU436	Ascomycota	Scutellinia	vitreola	High	0	-7.23402	1.08E-10
OTU46	Basidiomycota	Solicoccozyma	phenolica	High	0	-11.5152	3.27E-24
OTU501	Ascomycota	Penicillium	NA	High	0	-6.82012	1.68E-08
OTU531	Ascomycota	Auxarthron	umbrinum	High	0	-6.66972	5.56E-11
OTU738	Basidiomycota	Solicoccozyma	aeria	High	0	-5.79	5.28E-08
OTU749	Basidiomycota	NA	NA	High	0	-5.75206	1.70E-07
OTU942	Ascomycota	NA	NA	High	0	-5.04735	1.27E-06

3.3.5.3 Comparison between soil and tuber microbiome

3.3.5.3.1 Bacterial 16S rDNA

Next we determined how many OTUs were shared between soil and tubers. This could indicate that the shared OTUs originate from soil. Of all OTUs identified in soil and tuber, 1068 occurred exclusively in soil, 316 were found only in the tuber and 1661 were shared between the two. Of the OTUs that the previous analysis has identified as being most significantly different between lots with a high and a low disease incidence, the genera *Pseudarthrobacter, Rhodococcus,* Candidatus Udaeobacter, *Brevundimonas,* and *Lechevalieria* were detected in both soil and tuber. *Pseudarthrobacter* was found to be present in all lots, but was less abundant in soil than in tubers (Fig. 39). Also *Rhodococcus* was low abundant in soil compared to tubers. The same was found for the genus *Lechevaliera*, which has been correlated with a high disease incidence. In contrast, Candidatus Udaeobacter had high abundances in soil, but was only present in tubers at very low abundances (Fig. 40). The genus *Glutamicibacter* was only present in tubers and not found in soil (Fig. 41). Also *Brevibacterium* and *Staphylococcus* show almost exclusive presence in tubers.

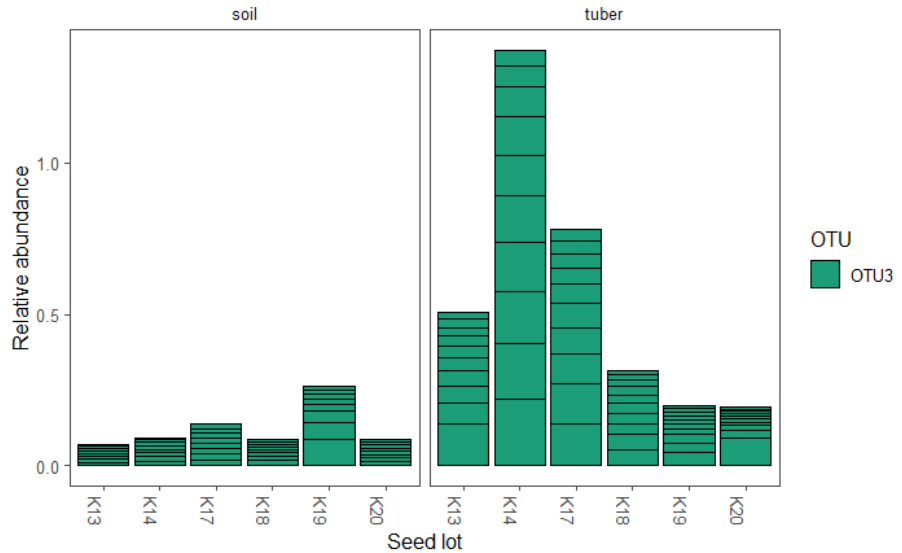


Fig. 39: Relative abundance of the genus *Pseudarthrobacter (*OTU3) in all six lots in soil and tuber.

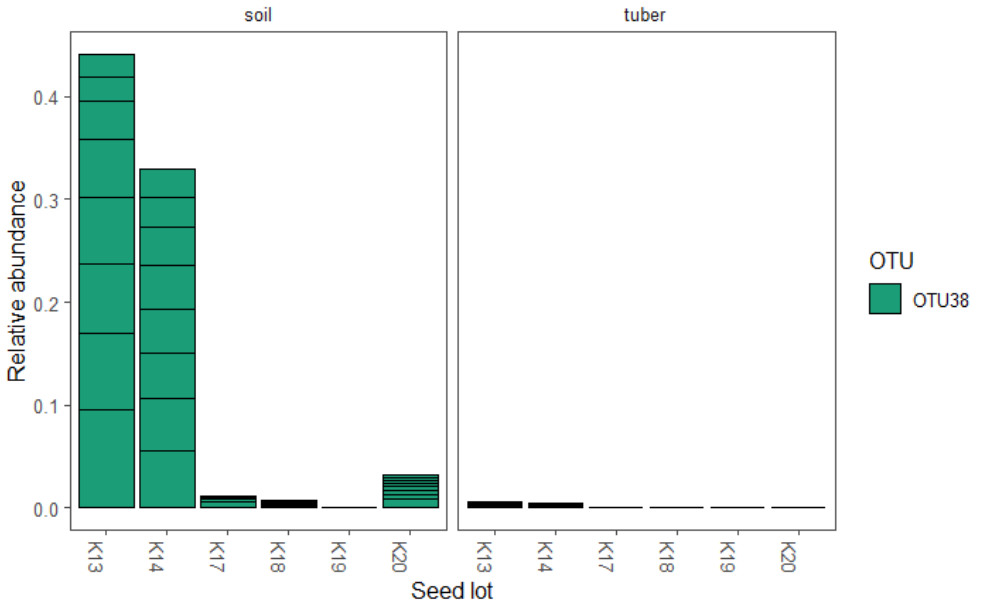


Fig. 40: Relative abundance of the genus Candidatus Udaeobacter (OTU38) in all six lots in soil and tuber.

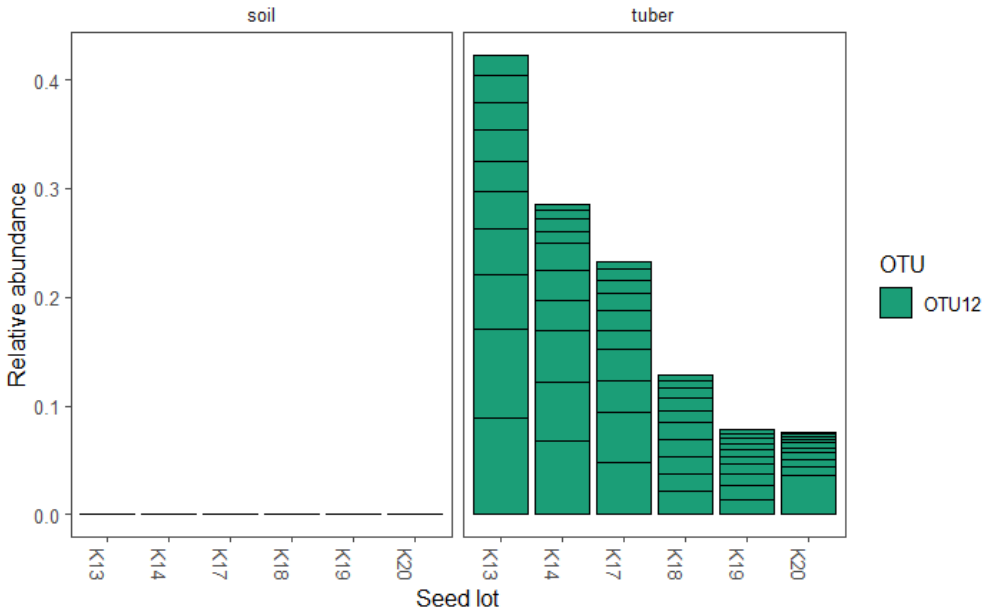


Fig. 41: Relative abundance of the genus *Glutamicibacter* (OTU12) in all six lots in soil and tuber.

3.3.5.3.2 Fungal ITS

For fungal taxa, it could be observed that most were present in soil, with 1400 OTUs. Only 15 were exclusively detected in tubers and 211 were shared between the two habitats. Of the taxa that were found to differ most between lots with a low and a high disease incidence, most occurred in soil as well as in tubers and showed differences in abundance only between lots. However, *Saitozyma podzolica* and several OTUs of the genus *Solicoccozyma* were mostly found in soil and only in low abundances in tubers (Fig. 42, 43), while *Debaroymyces hansenii* was found only in tubers with high abundances in lot 17 (Fig. 44).

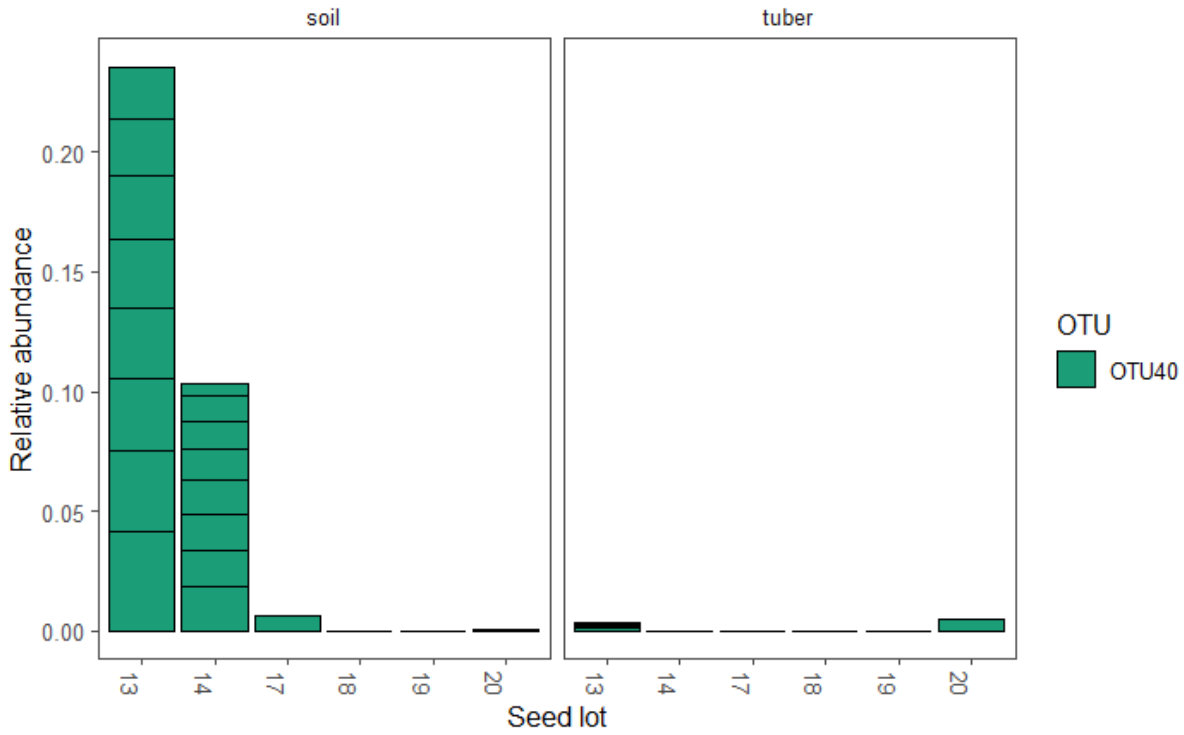


Fig. 42: Relative abundance of the genus *Saitozyma podzolica* (OTU40) in all six lots in soil and tuber.

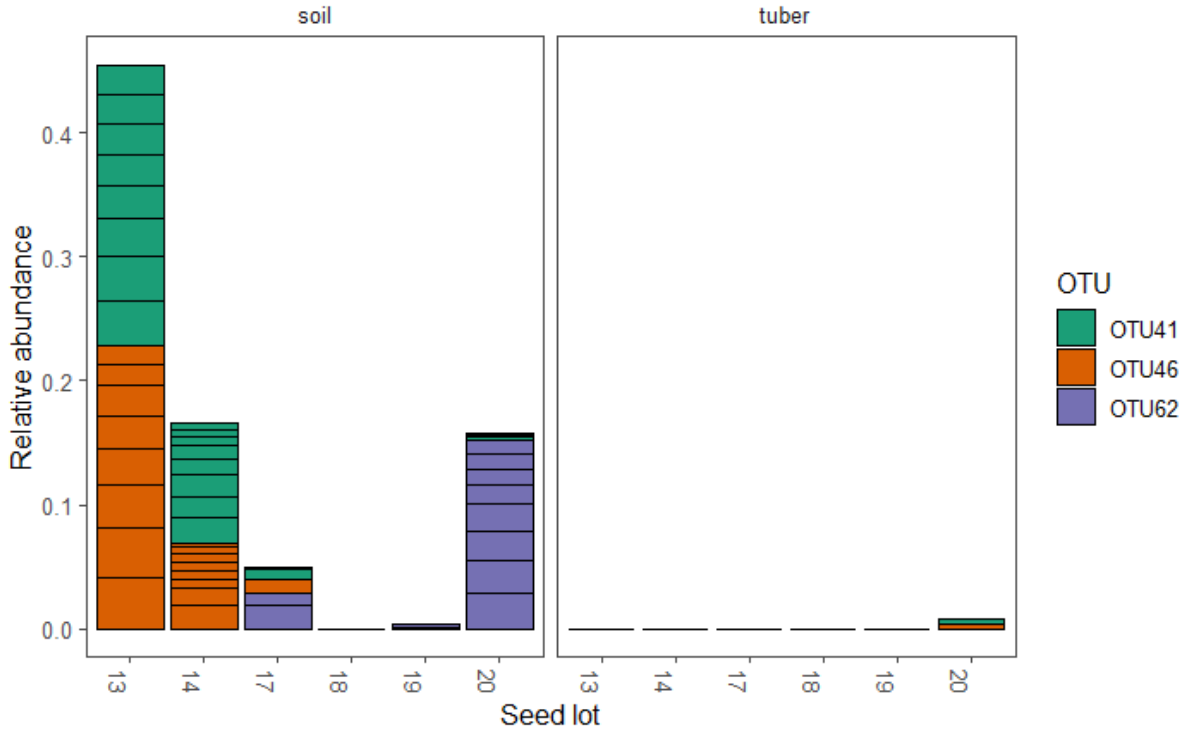


Fig. 43: Relative abundance of the genus *Solicoccozyma* (OTU41, OTU 46 and OTU 62) in all six lots in soil and tuber.

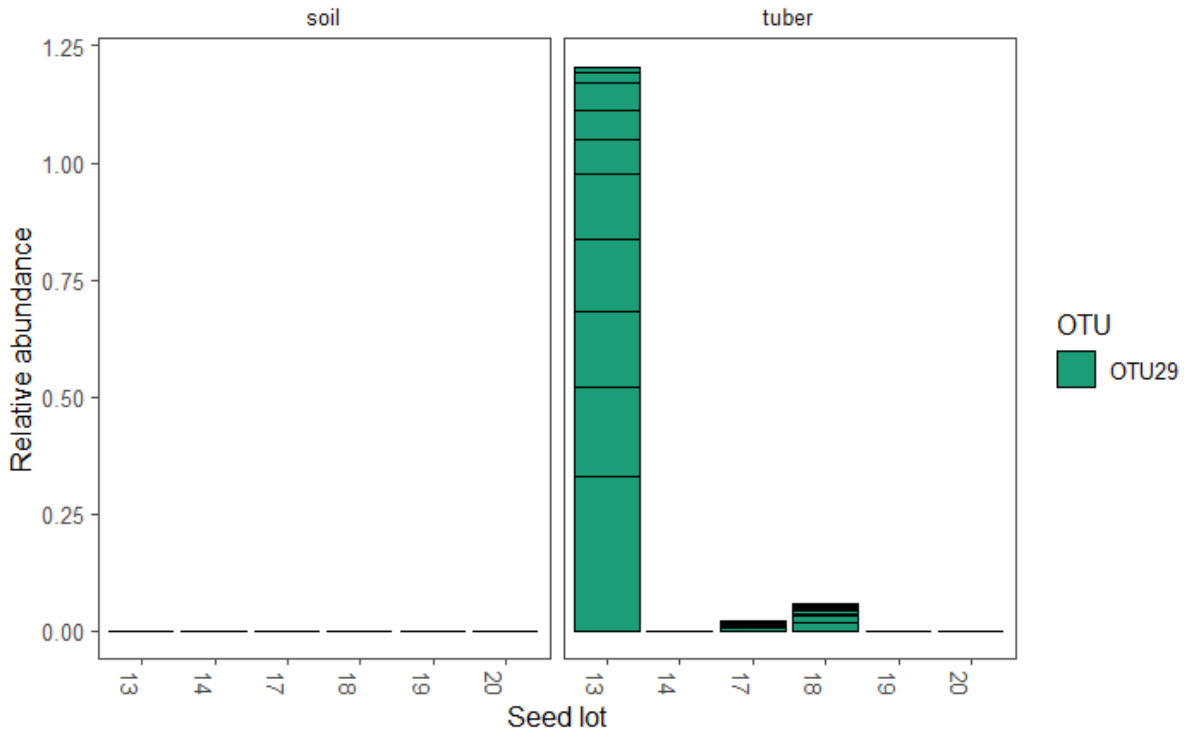


Fig. 44: Relative abundance of *Debaryomyces hansenii* (OTU29) in all six lots in soil and tuber.

3.3.6 Summary of tuber resistance

Table 8 shows a summary of the disease incidence in the selected lots in 2018 and 2019. The results are averaged over location and pathogen. Bacterial and fungal taxa with the highest abundances in tubers with low disease incidence are included as well.

Table 8: Summary of disease incidence and differentially abundant species in 2018 and 2019

Year	Lot	Disease incidence %	Group	Bacterial species with a higher abundance at low disease incidence	Fungal species with a higher abundance at low disease incidence
2018	K6	24.68	Low	*Pseudomonas sp.,* *Curtobacterium sp.,* *Pantoea sp.,* *Rhodococcus sp.*	*Vishniacozyma heimaeyensis,* *Penicillium brevicompactum,* *Plectosphaerella cucumerina* *Debaryomyces hansenii,* *Rhodotorula babjevae*
2018	K8	17.85	Low		
2018	K10	27.26	Low		
2018	K9	54.36	High		
2018	K13	46.51	High		
2018	K14	51.55	High		
2019	K13	26.06	*Low*	*Staphylococcus spp.,* *Pseudarthrobacter sp.,* *Glutamicibacter sp.,* *Paenarthrobacter sp.,* *Brevibacterium sp.,* *Canidatus Udaeobacter,* Bacillacae	*Fusarium oxysporum,* *Debaryomyces hansenii,* *Plectosphaerella niemejerarum,* *Vishniacozyma heimaeyensis,* *Mycosphaerella tassiana*
2019	K14	50.29	*Low*		
2019	K17	42.90	*Low*		
2019	K18	58.70	*High*		
2019	K19	67.59	*High*		
2019	K20	51.20	*High*		

3.4 Metabolome

PCA analysis showed a weak separation according to disease incidence if used as a continuous variable in the data from 2018 (Fig. S1). However, there was a high variation between lots. In both years the presence/absence of metabolites was not significantly associated with disease incidence. When metabolite quantities were considered, no metabolites were associated with low or high disease

incidence in 2018. In 2019, seven metabolites were associated with disease incidence. After imputation of values under the Limit of Detection, five metabolites were significantly associated with disease incidence (Table 8, Fig. 15). Three of these metabolites could be tentatively identified. Metabolite 26000 might be a jasmonate conjugate, possibly rhodojaponin IV; metabolite 16506 has been putatively identified as an (yet unknown) alkaloid; 13588 has also identified as an alkaloid, possibly strictosidinic acid.

Table 8: Significant metabolites associated with disease incidence in 2019.

Centrotype (Metabolite)	Small value imputation	p-value	Adj. p-value
26000	no	0.000	0.001
28106	no	0.000	0.001
16506	no	0.000	0.008
17386	no	0.000	0.004
13588	no	0.000	0.002
13692	no	0.000	0.001
15216	no	0.000	0.009
28106	yes	0.000	0.004
17386	yes	0.000	0.016
13692	yes	0.000	0.004
15216	yes	0.000	0.032
29999	yes	0.000	0.040

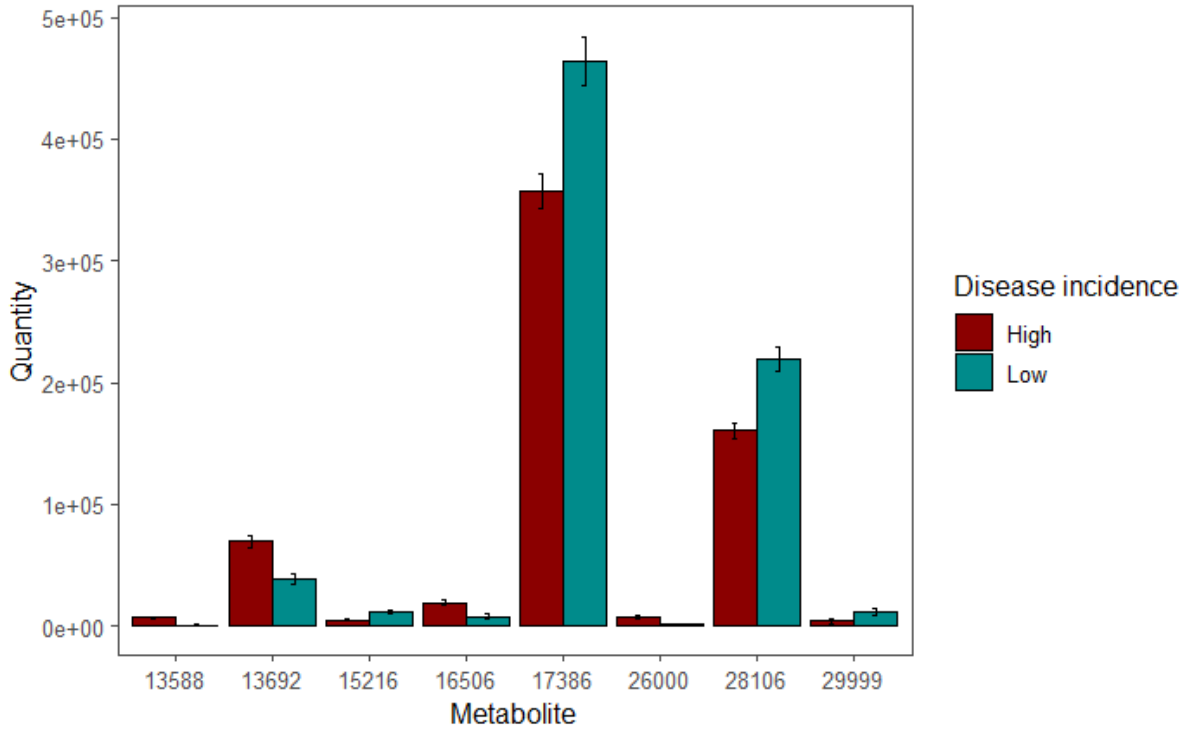


Fig. 15: Relative quantity (MS response) of those metabolites (codes indicated on X axis) that differed significantly between lots with a low and a high disease incidence in 2019; error bars represent the standard error.

3.5 Soil abiotic parameters

An overview of all measured variables can be found in Table S1.
A stepwise general linear model indicated that disease incidence is associated with the parameters pH, C:N ratio, available sulphur, organic C, available calcium, available phosphor, C:organic matter ratio, available K, soil Na, clay, organic matter and sand. However, no parameter alone showed a strong correlation with disease incidence (rho> 0.5 or rho <-0.5).

Most of these parameters, except for C:N ratio and organic C had a significant association with bacterial community composition in soil (Table 10). An NMDS was performed as described in 3.3.5.1.2., showing differences between bacterial communities in two dimensions. By indicating the respective soil parameter in the respective lot on a colour scale, it can be seen that mostly lot 13 and 14, which have a low disease incidence, share similar values, while lot 17 is often distinct (Fig. 45). Fungal community composition was significantly associated with variables except organic C (Table 11). Also for the fungal community it can be observed that the measured parameters do not correlate with disease incidence (Fig. 46).

Table 10: Results of a Permanova on the weighted unifrac distances for the bacterial communities.

Parameter	F-model	p-value
pH	91.64	<0.01
C:N ratio	1.80	0.14
Plant available S	12.92	<0.01
Plant available P	14.62	<0.01
Plant available Ca	20.83	<0.01
Organic C	0.52	0.62
C:OM ratio	7.54	<0.01
Soil Na	8.79	<0.01
Soil K	13.80	<0.01
Clay	25.51	<0.01
OM	6.24	0.01
Sand	41.36	<0.01

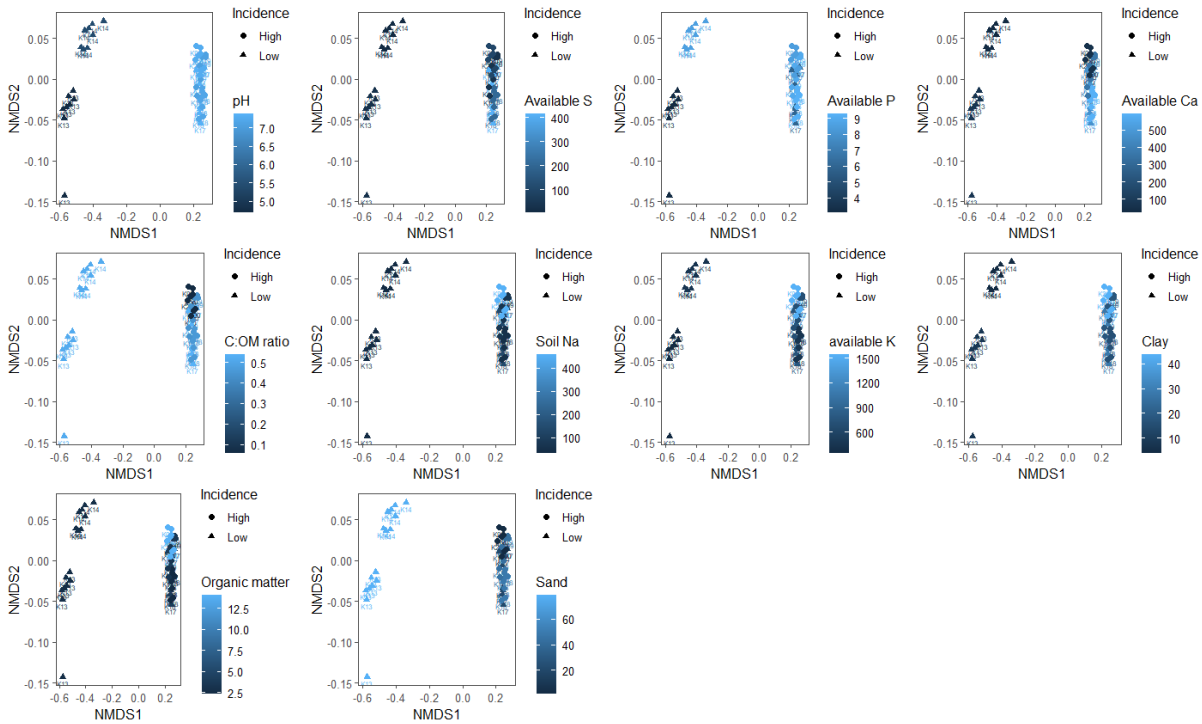


Fig. 45: NMDS plot of bacterial communities in the different samples; colour indicates pH, plant available S (kg S/ha), plant available P (kg P/ha), plant available Ca (kg Ca/ha), C:OM ratio, soil Na (kg Na/ha), available K (kg K/ha), Clay (%), Organic matter (%) and Sand (%).

Table 11: Results of a Permanova on the weighted unifrac distances for the fungal communities.

Parameter	F-model	p-value
pH	26.06	<0.01
C:N ratio	4.05	<0.01
Plant available S	6.86	<0.01
Plant available P	9.33	<0.01
Plant available Ca	14.31	<0.01
Organic C	0.99	0.38
C:OM ratio	4.62	<0.01

Soil Na	5.16	<0.01
Soil K	6.69	<0.01
Clay	10.23	<0.01
OM	4.37	0.01
Sand	14.19	<0.01

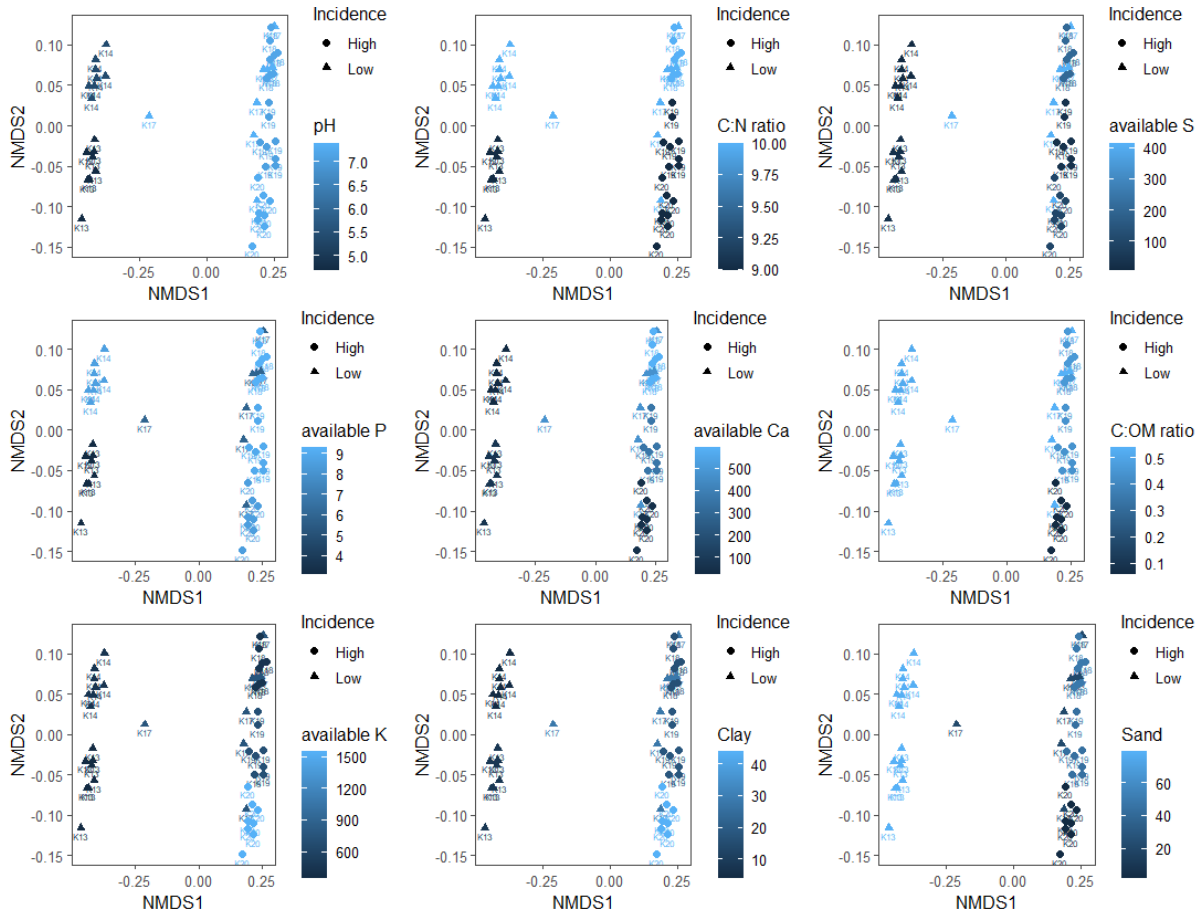


Fig. 46: NMDS plot of fungal communities in the different samples; colour indicates pH, C:N ratio, plant available S (kg S/ha), plant available P (kg P/ha), plant available Ca (kg Ca/ha), C:OM ratio, soil Na (kg Na/ha), available K (kg K/ha), Clay (%), Organic matter (%) and Sand (%).

3.6 Analysis of merged microbiome data from 2018 and 2019

For the merged data only the tuber microbiome was analyzed as no soil samples were taken in 2018.

3.6.1 Bacterial 16S rDNA

3.6.1.1 Taxonomic analysis

Visually inspecting the relative abundance of bacterial phyla in 2018 and 2019 showed that at low disease incidence a higher abundance of Actinobacteria and Firmicutes could often be observed compared to tubers with a high disease incidence (Fig. 47). In the latter, Proteobacteria dominated. Still, there was a high variation between lots.

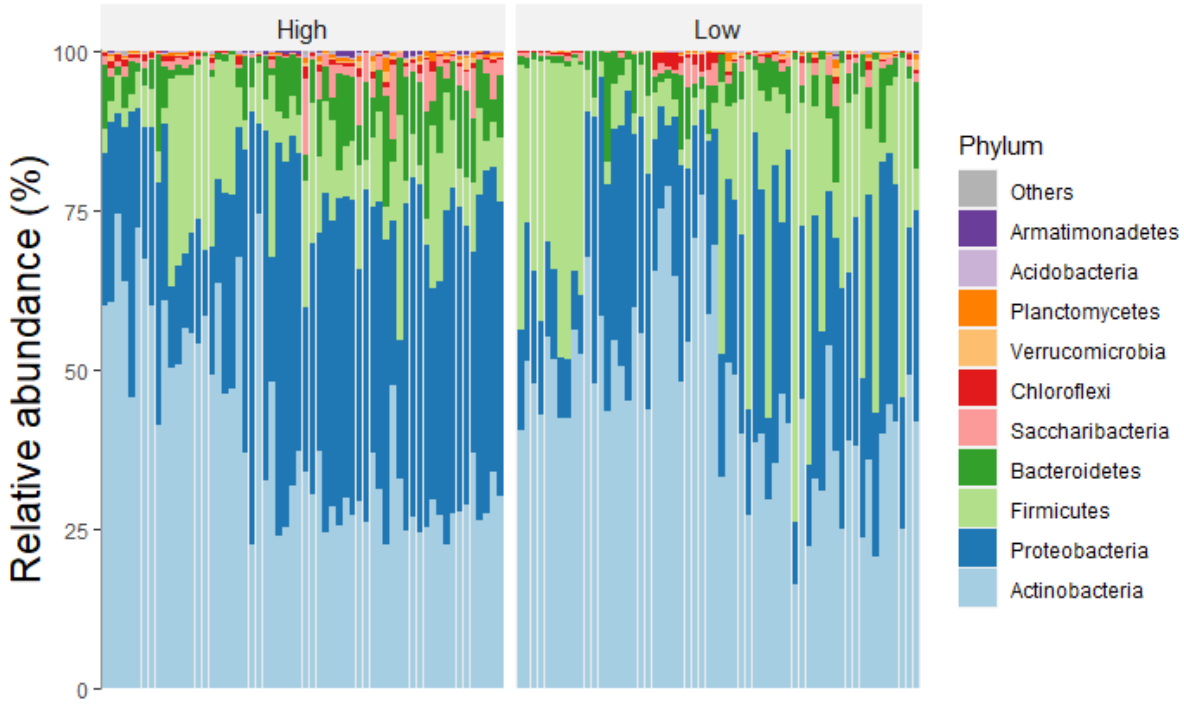


Figure 47: Relative abundance of the present bacterial phyla in all lots analyzed in 2018 and 2019, separated according to disease incidence.

3.6.1.2 NMDS

Similar to the analysis of the separate years, there is a large overlap between communities originating from lots with a high or low disease incidence respectively (Fig. 48). Nevertheless, there was a significant difference between those two groups ($F=7.88$, $p<0.01$) and between lots ($F=27.31$, $p<0.01$).

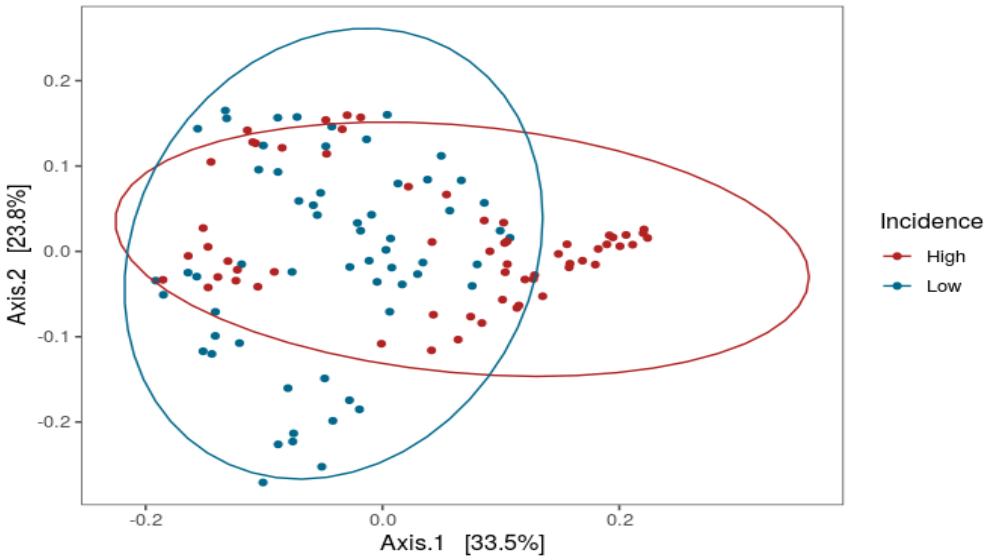


Figure 48: Non-metric multidimensional scaling with weighted unifrac distances of tuber samples in 2018 and 2019, colours and ellipses indicate disease incidence of the lot (high, low).

3.6.1.3 SPLSDA

An SPLDSA could more clearly separate bacterial communities from lots with a high and low disease incidence (Fig. 49). The taxa that contributed to this separation and showed a higher relative abundance in tubers with a low disease incidence include *Arthrobacter, Curtobacterium, Pantoea, Pseudomonas, Rhodococcus* and *Staphylococcus* (Fig. 50). Results obtained by DESeq2 were similar (data not shown).

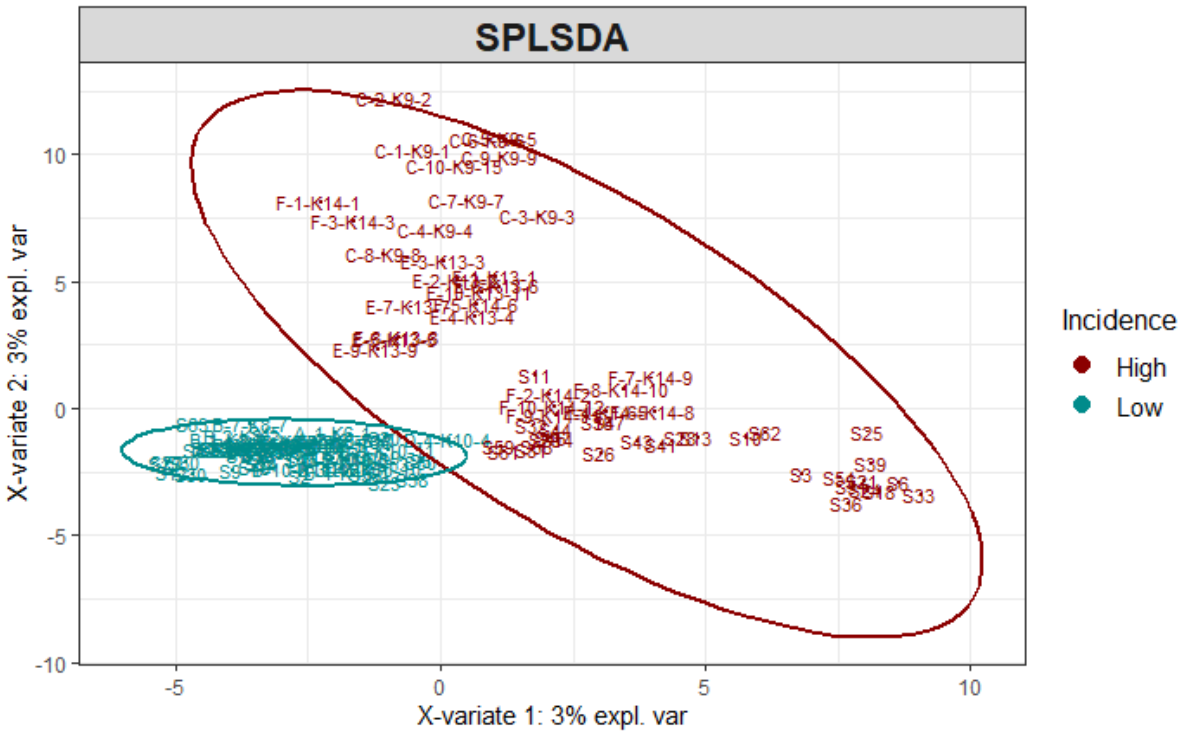


Figure 49: SPLSDA of OTU abundances with Incidence as the separating factor. Three components and 260 variables were chosen for the final plot.

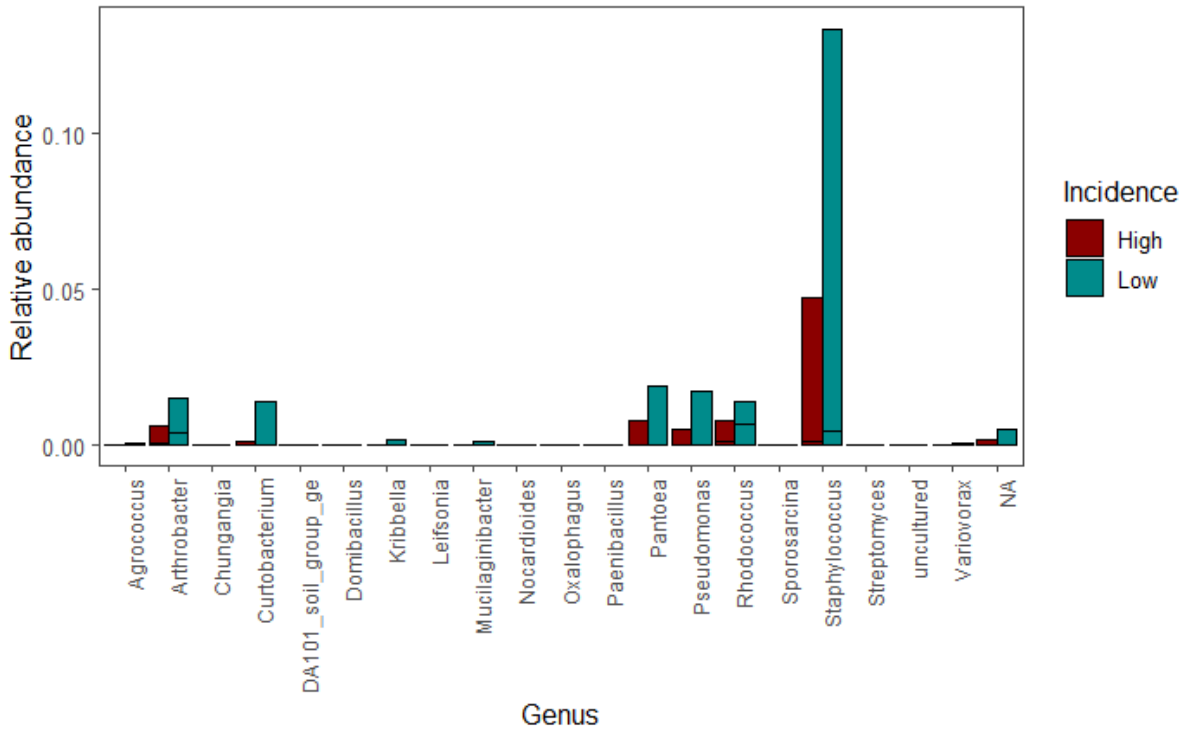


Figure 50: Relative abundances of all 26 OTUs, belonging to19 genera, that were more abundant in samples with a low disease incidence and contributed to the separation in the SPLSDA in the first component.

3.6.1.4 Comparison between 2018 and 2019

66.8% of all OTUs were shared between 2018 and 2019 (Fig. 51). Plotting the abundance of differentially abundant species in each lot for each year showed that abundance was highly variable between lots. Nevertheless, the genera *Rhodococcus, Arthrobacter* and *Curtobacterium* showed a higher abundance at low disease incidence in almost all lots (Fig. 52, 53, 54).

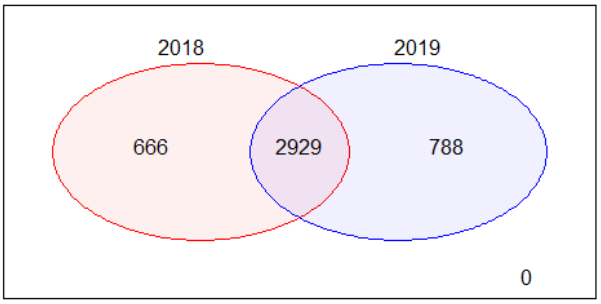


Fig. 51: Venn diagram for all OTUs in 2018 and 2019.

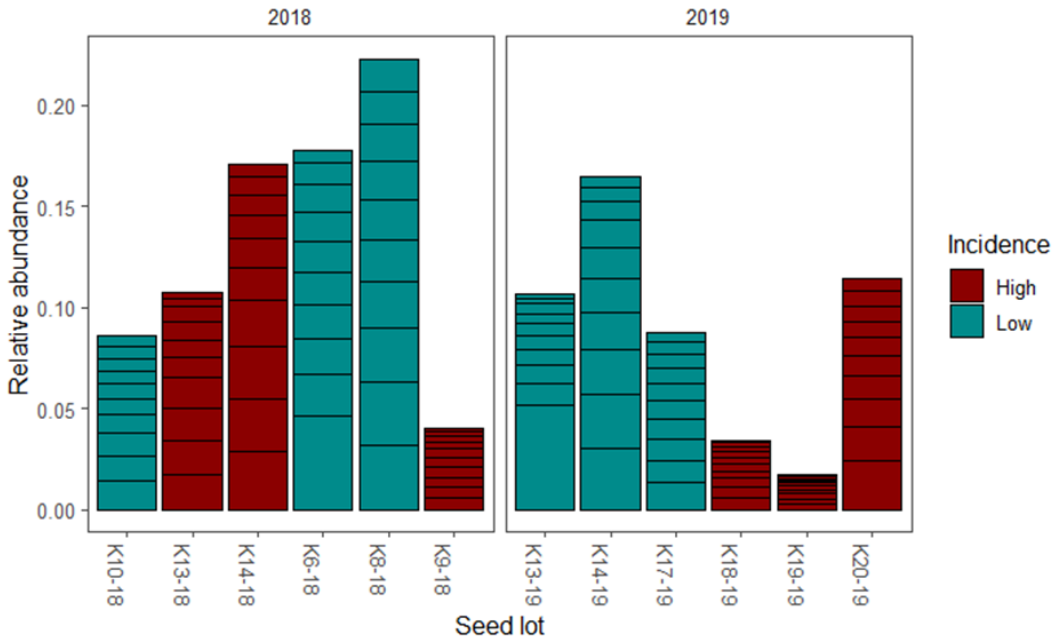


Fig. 52: Relative abundance of the genus *Rhodococcus* in lots with a high and low disease incidence in the years 2018 and 2019.

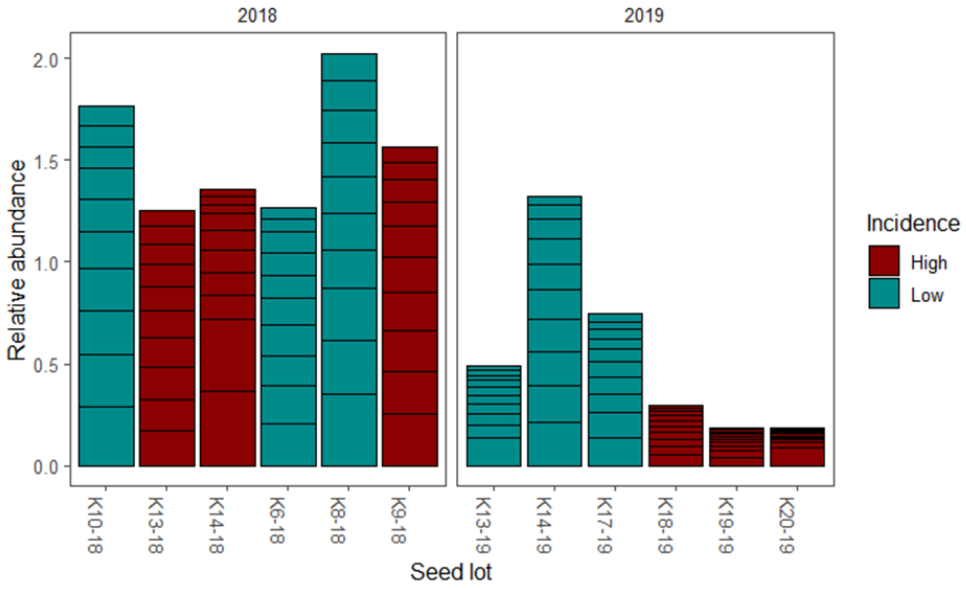


Fig. 53: Relative abundance of the genus *Arthrobacter* in lots with a high and low disease incidence in the years 2018 and 2019.

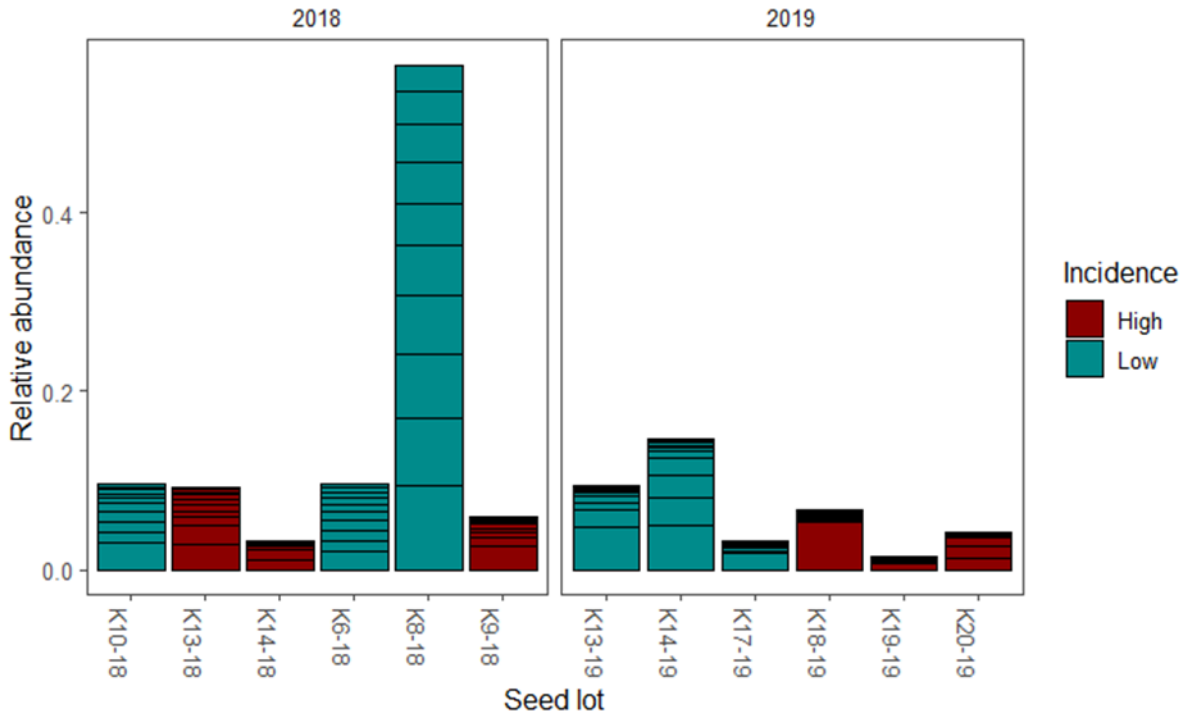


Fig. 54: Relative abundance of the genus *Curtobacterium* in lots with a high and low disease incidence in the years 2018 and 2019.

3.6.2 Fungal ITS

3.6.2.1 Taxonomic analysis

Visual inspection of the abundance of all fungal classes in the different lots showed a high variation between lots (Fig. 55). No classes were observed with a generally higher abundance at a low disease incidence.

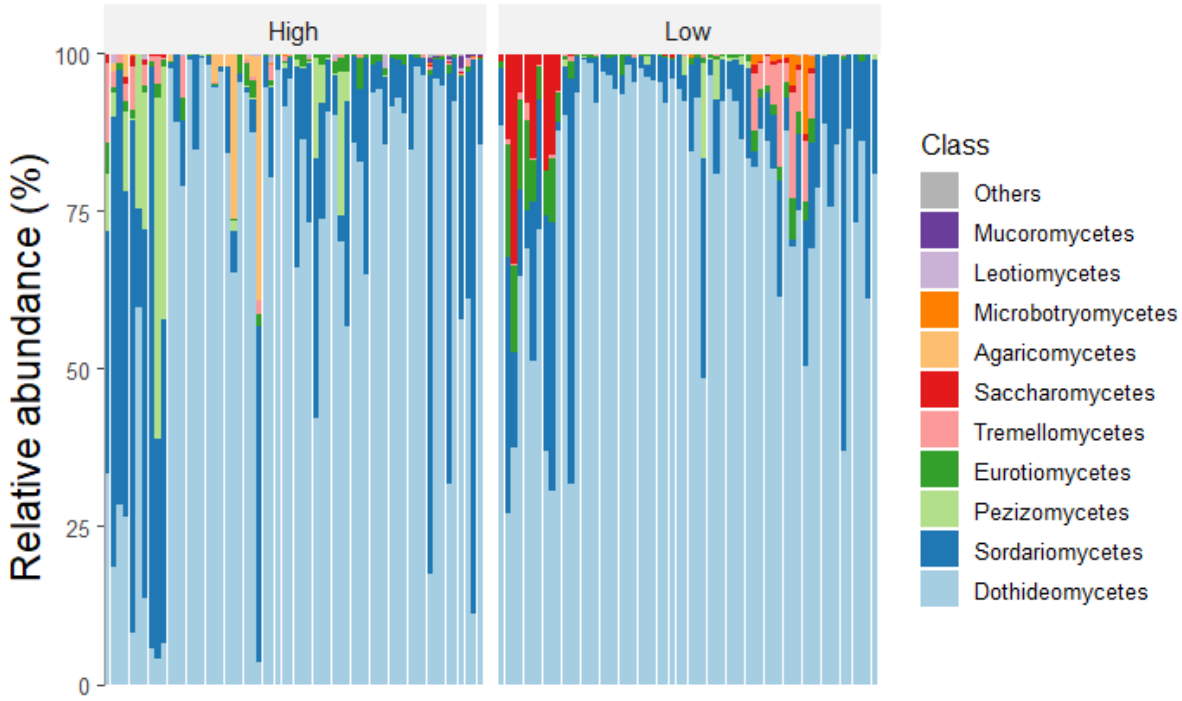


Fig. 55: Relative abundance of the present fungal classes in all lots analyzed in 2018 and 2019, separated according to disease incidence.

3.6.2.2 NMDS

An NMDS shows no clear separation between communities from lots with a high and low disease incidence in two dimensions(Fig. 56). Nevertheless, there was a significant difference between disease incidences ($F=8.10$, $p<0.01$) and between lots ($F=9.95$, $p<0.01$).

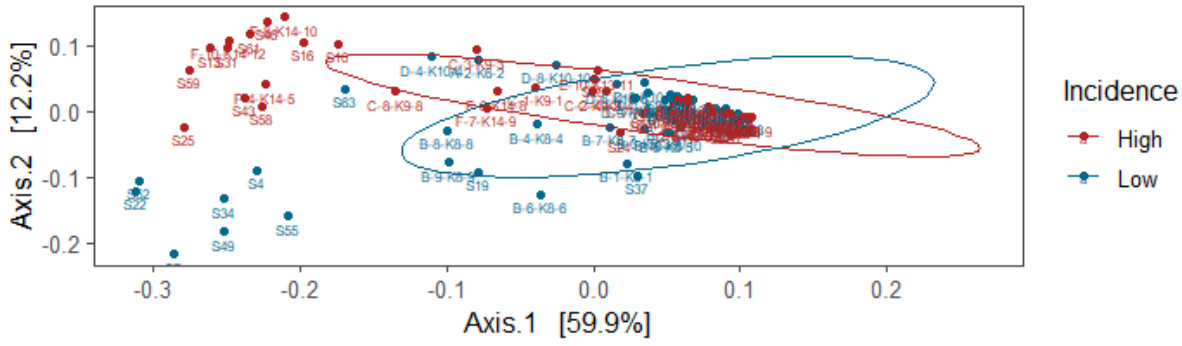


Fig. 56: Non-metric multidimensional scaling with weighted unifrac distances of tuber samples in 2018 and 2019, colors and ellipses indicate disease incidence of the lot (high, low).

3.6.2.3 SPLSDA

A SPLSDA did show some separation, indicating small differences between a high and a low disease incidence (Fig. 57). Among the taxa that were significantly more abundant at low disease incidence were *Vishniacozyma heimayensis, Plectosphaerella niemeijerarum, Mycosphaerella tassiana*, *Debaryomyces, Saitozyma podzolica* and *Penicillium brevicompactum*. A subset of those taxa was also identified by DESeq2 as significantly different (Fig. 58).

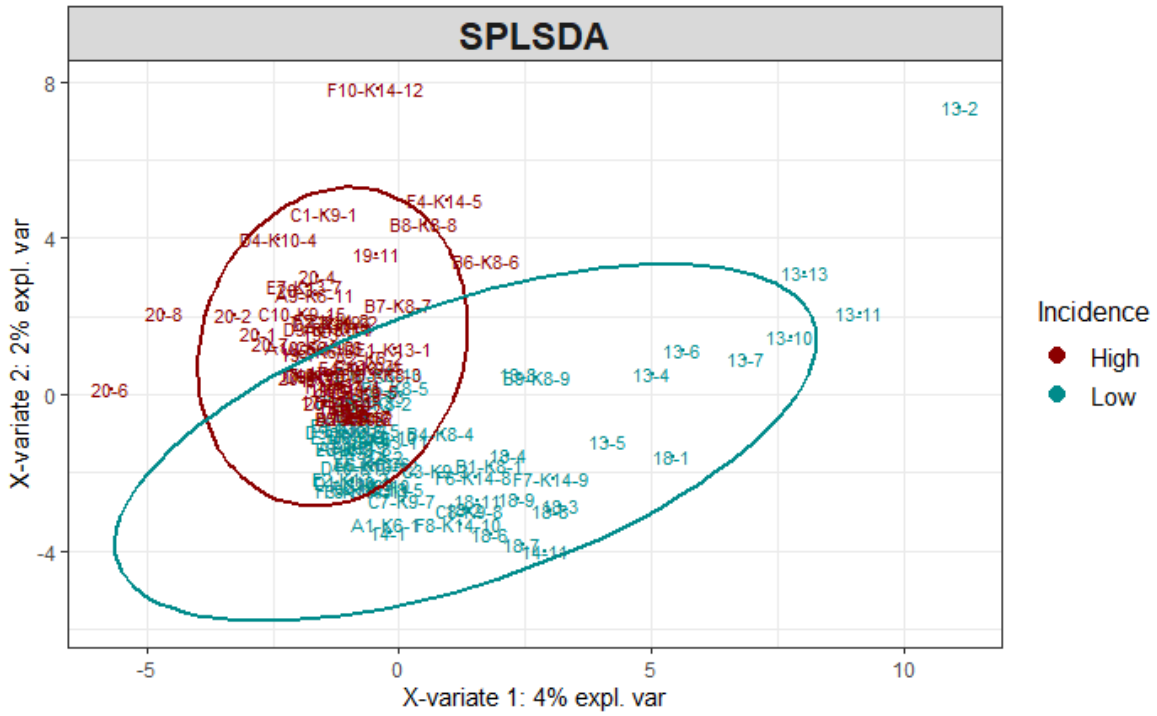


Figure 57: SPLSDA of OTU abundances with Incidence as the separating factor. Two components and 170 variables were chosen for the final plot.

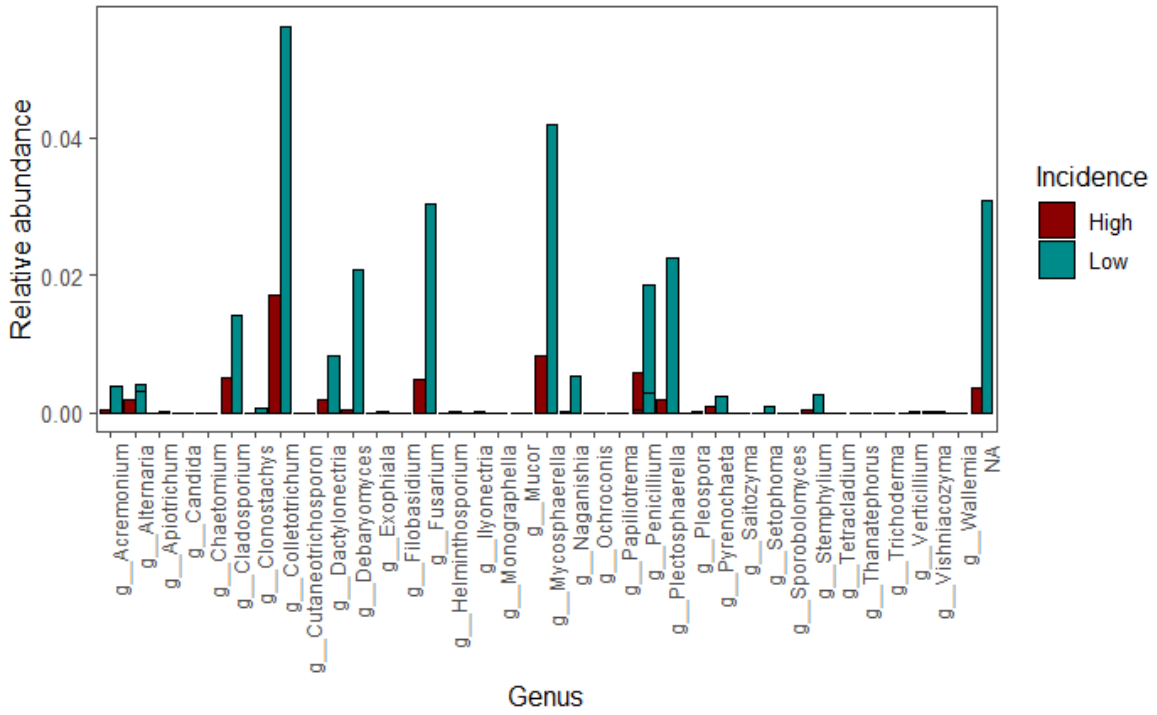


Figure 58: Relative abundances of all 52 OTUs, belonging to 37 genera, that were more abundant in samples with a low disease incidence and contributed to the separation in the SPLSDA in the first component.

3.6.2.4 Comparison between 2018 and 2019

Comparing both years, 50% of all OTUs were shared, while 38% were unique to year 2018 and 12% were unique to year 2019 (Fig. 59). Differentially abundant taxa showed a high variation between lots

and years. For example, the genera *Vishniacozyma* was highly abundant in lot 8 in 2018 but showed a much lower abundance in the other lots (data not shown). *Mycosphaerella* and *Penicillium* were highly abundant in both lot 8 in year 2018 and lot 13 in year 2ß19. In contrast, the lower abundant taxa *Acremonium* sp,. and *Pyrenochaeta* sp. showed a higher abundance at low disease incidence compared to high disease incidence in both years (Fig. 60, 61).

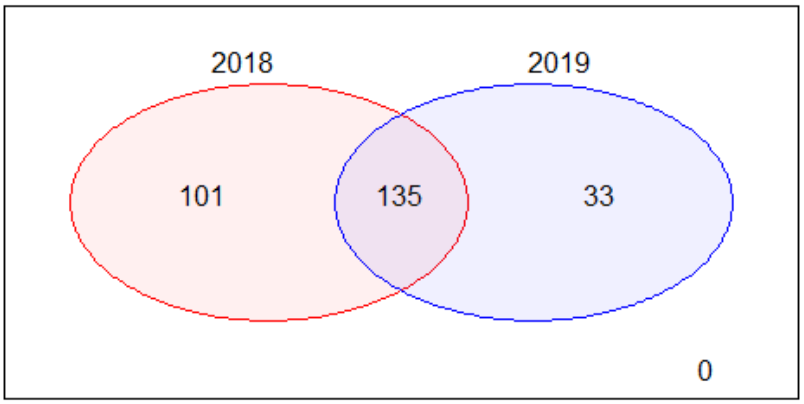


Fig. 59: Venn diagram for all OTUs in 2018 and 2019.

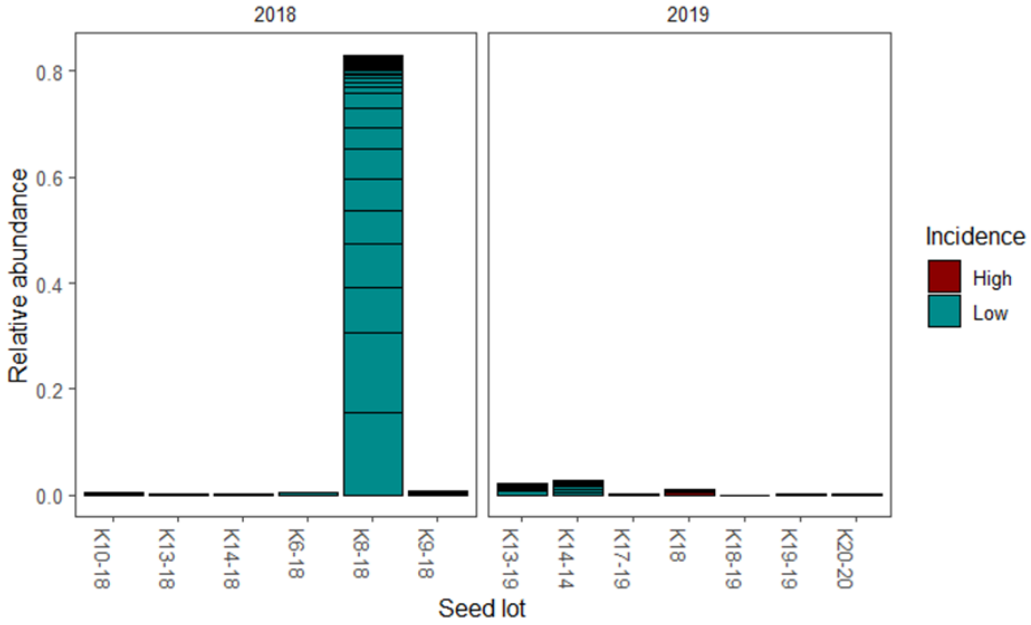


Fig. 60: Relative abundance of the genus *Acremonium* in lots with a high and low disease incidence in the years 2018 and 2019.

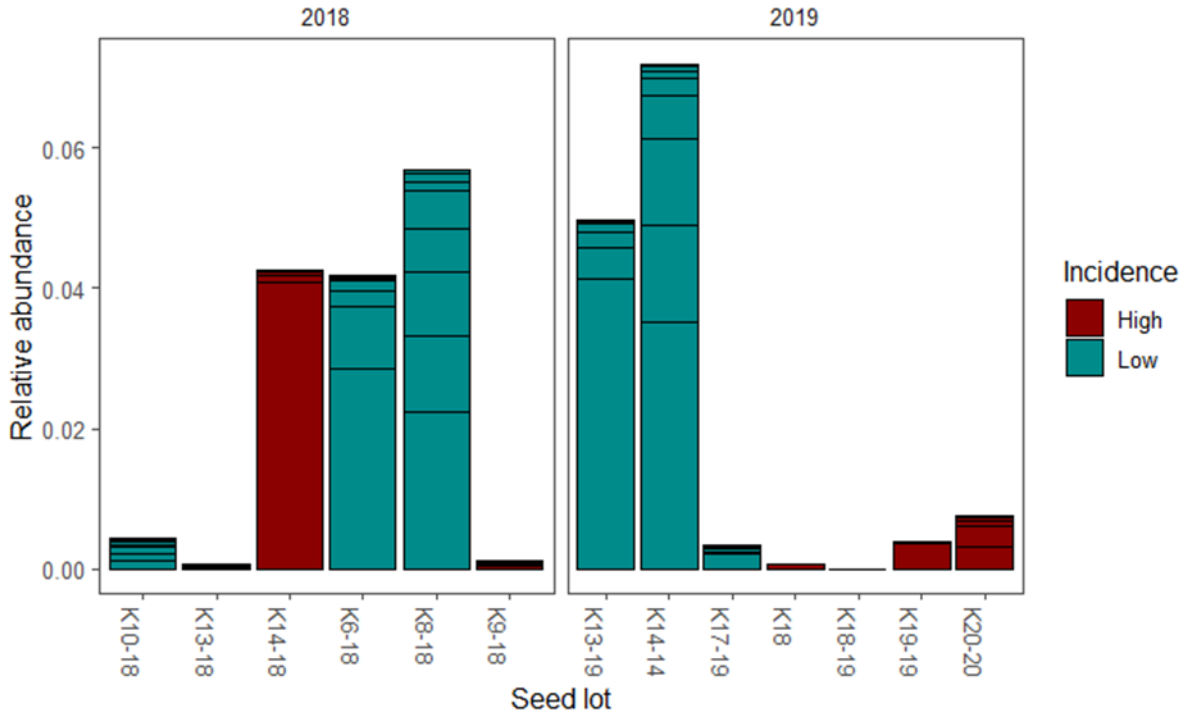


Fig. 61: Relative abundance of the genus *Pyrenochaeta* in lots with a high and low disease incidence in the years 2018 and 2019.

4 Discussion

This study shows that lots of the same variety of potato, originating from different locations, differ in their susceptibility to *P. brasiliense* and *D. solani*. This is supported by earlier findings in 2015 and 2016 using only *D. solani*. These differences indicate the influence of the origin of the mother tuber on the resistance of the next field generation. General findings include pronounced differences between lots of the cultivar Kondor, but less between lots of the cultivar Spunta, as disease incidence in Spunta was generally low. It was also observed that disease incidence was generally higher in sandy soils than in clay soils. This was expected as sandy soils are generally warmer and therefore more conducive to the growth and as a consequence the spread of the species *D. solani* and *P. brasiliense* (Du Raan et al., 2016). *D. solani* was on average more aggressive than *P. brasiliense*, but the susceptibility of seed lots against the two pathogens did not always coincide. In other words, lots that were less susceptible to *P. brasiliense* were not necessarily more resistant to *D. solani* and vice versa.

While the origin of the lot had an effect on the susceptibility against SRPs, no such effect could be seen with respect the fungal pathogens *Rhizoctonia solani* and *Colletotrichum coccodes*. On the one hand, this could have been due to a high disease pressure that masked potential effects of lot. On the other hand, these fungi are soil-borne and thus suppressiveness in the soil of the present growth location itself might play a dominant role in risks for disease expression. Specific suppressiveness conveyed by the presence of certain bacterial taxa has been found to reduce disease incidence caused by *R. solani* (Postma et al., 2008). It is possible that also suppressiveness against *C. coccodes* cannot be transferred from the mother tuber, but is a trait of the soil. Several soil amendments have been found to reduce *C. coccodes* disease incidence, indicating that suppressiveness might be based on the general activity of microorganisms, competing for resources (Avilés et al., 2011).

In both years, there were differences in the microbiome between lots with a high and a low disease incidence and differences in the tuber metabolome. Still, differences between the individual lots were in most cases larger than differences between high and low disease incidence in general. Also taxa associated with suppressiveness differed between years. This indicates that a range of different microorganisms and metabolites can be effective against SRPs and that there is not one species or community composition that conveys resistance. It is likely that different combinations of taxa and the interactions between those taxa affect suppressiveness (Latz et al., 2016). The differences between lots also confirm that the soil of origin influences the endophytic and epiphytic microbiome. Our results are supported by the finding that the soil has a larger effect on the tuber microbiome than the cultivar (Buchholz et al., 2019).

As mentioned previously, there was no clear separation in the microbiome composition between lots with a high and a low disease incidence. Nevertheless, several bacterial and fungal taxa emerged as significantly different in abundance between these two categories. For bacteria, these taxa also differed between the two years of the study, reflecting the use of different lots originating from different soils. In 2018, the genera *Pantoea, Pseudomonas, Curtobacterium* and *Rhodococcus* were highly abundant in lots with a low disease incidence. Especially, fluorescent *Pseudomonas* are widely accepted as biocontrol agents, possessing antibiotic producing machinery, as for example for the production of 2,4-diacetylphloroglucinol or pyrrolnitrin (Weller, 2007). They are also successful competitors for iron with the production of siderophores. Several species of *Pantoea*, like *P. vagans* and *P. agglormerans* have likewise been described as biocontrol agents, for example against *Erwinia amylovora*, the causal agent of fire blight (Pusey et al., 2011). Different *Curtobacterium* strains are supposed to be able to against pathogens like *Xylella fastidiosa* (Garrido et al., 2016) and *Rhodoccus* has even been described as a biocontrol agent against *P. atrosepticum* (Barbey et al., 2013). Thus, all these taxa are have the potential to act as biocontrol agents according to literature. However, here we can only show a correlation between abundance and decline in disease incidence. A causal relationship needs yet to be established.

In 2019, the taxa that were most abundant in lots with a low disease incidence belonged to the genera *Staphylococcus, Glutamicibacter, Pseudarthrobacter, Brevibacterium* and Candidatus Udaeobacter. The genera *Glutamicibacter* and *Pseudarthrobacter* have only recently been reclassified from being subspecies of *Arthrobacter* sp. (Busse, 2016). Therefore, their biocontrol ability is not well characterized. However, *Glutamicibacter* has been found as an endophyte in potato previously (Liu et al., 2020b).

Arthrobacter sp. on the other hand has already been described as a growth promoter and a biocontrol agent against phytopathogenic fungi (Velázquez-Becerra et al., 2013). Also species from the genus *Staphylococcus* and *Brevibacterium* are mainly known as effective agents against fungi (Labuschagne et al., 2010). Candidatus Udaeobacter is ubiquitous in soil, but as many members of the Verrucomicrobiaceae, resists cultivation (Hofer, 2016). Therefore, biocontrol abilities could not yet be assessed.

In contrast to bacterial taxa, several fungal taxa were in both years associated with low disease incidence, like the yeast species *Debaryomyces hansenii* and *Vishniacozyma heimayensis*. There is little to no information about *V. heimayensis* and it is likely that this species was misidentified or is closely related to better known species such as *V. victoriae* as their taxonomy is complex and was subject to recent changes (Liu et al., 2015). *D. hansenii* and *V. victoriae* have been described in many studies as a biocontrol agent against molds on fruit, such as *Penicillium expansum* and *Botrytis cinerea* (Gramisci et al., 2018; Ming et al., 2020), and dairy molds (Liu and Tsao, 2009), but have not yet been found to be effective against bacterial diseases in general or SRPs specifically. Also other species found in either year, such as *Penicillium brevicompactum* (Nicoletti et al., 2004) and *Mucor hiemalis* (Ziedan et al., 2013), were demonstrated to be antagonistic to other fungi. The genus *Plectosphaerella* was associated with decreased root tot in sugar beet (Kusstatscher et al., 2019). In 2019, several fungal species were also found to be more abundant in the soil of the origin of the seed lots with a low disease incidence. Strains of *Cladosporium cladosporioides* and *Fusarium oxysporum* are known for the production of antimicrobial compounds and the yeast genera *Solicoccozyma* and *Saitozyma* have been found to produce phytohormones (Son et al., 2008; Streletskii et al., 2016; Sarabia et al., 2018; Yehia et al., 2020). However, there are only few reports of fungal antagonists effective against bacterial pathogens (see e.g. Tagawa et al. (2010)) and the potential of fungal antagonists remains largely unexplored.

In addition, microbiome data from both years was analyzed together in order to discover common patterns that might indicate taxa that are more generally involved in suppressiveness. For bacteria, a higher abundance of Firmicutes and Actinobacteria was apparent in both years at a low disease incidence. Both phyla are frequently linked to increased suppressiveness against a variety of pathogens (Mendes et al., 2011). Members of both phyla are known to be producers of antimicrobial compounds and to have plant growth promoting properties (Sánchez et al., 2017; Lee et al., 2021). In spite of variation between years and lots the genera *Arthrobacter* (i.e. *Pseudarthrobacter*), *Curtobacterium*, and *Rhodococcus* were constantly higher abundant at low disease incidence, indicating a role in disease suppressiveness against SRPs. In contrast to bacteria, merging data from fungal communities showed a high difference between years and as a consequence little common differences between disease incidences. This indicates that variation between the years of e.g. abiotic parameters might have affected fungal communities more than bacterial communities. Nevertheless, also for fungi only few taxa could be identified that were differentially different in both years, which were *Plectosphaerella niemeijerarum*, *Debaryomyces sp., Vishniacozyma heimayensis* and *Pyrenochaeta* sp. Interestingly, *Pyrenochaeta* has often been described as a pathogen on other plant species than potato and it is poorly understood if certain strains can have antagonistic activity against bacterial pathogens.

As lots from different locations and different years show differences in their microbiome, it seems likely that the soil community at these locations should be the major source of differences in the tuber microbiome (Buchholz et al., 2019). In support of this assumption, most of the taxa that were found to be associated with a low disease incidence could also be found in the soil in the year 2019. However, in most cases, these taxa were present in soils from all lots and did not show a higher abundance in soils that were the origin of lots with lower disease incidence. This indicates that the mere presence of potentially beneficial bacterial taxa does not generally lead to a more resistant tuber. Rather, these taxa have to be recruited in high abundances as epiphytes or endophytes in the tuber. Recruitment can be affected by a number of factors, as for example the field location, soil type and cultivation practices (Edwards et al., 2015; Liu et al., 2017). Interestingly, a number of strains in the tubers were not found in soil, indicating that they originated either from the mother tuber or entered the above-ground part of the plant (Frank et al., 2017).

In addition to taxa that occurred both in tuber and soil and in tubers only, several taxa, such as Candidatus Udaeobacter and *Solicoccozyma* sp. were almost exclusively retrieved from soil. Still, they were correlated significantly with a low disease incidence in tubers. This finding indicates that those taxa might have had an indirect influence on tuber resistance, e.g. by priming systemic plant defenses. It has been shown previously induction of the systemic acquired resistance (SAR) pathway can induce resistance in the next generation of plants (Luna et al., 2012). Kuźnicki et al. (2019) could demonstrate the same principle for potato tubers and resistance against *Phytophtora infestans*. Still, the increased

abundance of taxa in soil of tubers with a low disease incidence could also be explained by factors unrelated to disease suppressiveness, such as abiotic parameters and co-occurrence with other taxa.

Potato plants are able to produce a wide array of secondary metabolites, many of which are present in the tubers. These comprise polyphenols and glycoalkaloids, some of which have been found to be toxic to a number of pathogenic species (Nogawa et al., 2019). However, the relationship between disease incidence and tuber metabolome could not be assessed comprehensively due to the high variation between and within lots that hampered statistical analysis. A number of maximally seven metabolites, out of several hundred compounds including series of (poly)phenolic compounds and glycoalkaloids, were significantly different in their relative quantity between tubers from the high and low disease incidence lots. Identification of these metabolites based on their mass spectrometry data yielded only tentative results, due to a lack of both standards and mass spectral data from metabolite databases to verify their elemental formula and putative annotations. The three metabolites that could be annotated have not been reported in potato before, and therefore their annotations should be viewed with caution. There were no compounds specifically present or absent in low or high incident lots. Therefore, it cannot be concluded that resistance of tuber lots from different locations is based on significant differences in the composition or content of the hundreds secondary metabolites detectable by the applied untargeted LCMS approach (De Vos et al., 2007). Worth to note here that the complete metabolome cannot be determined by a single analytical platform; for instance, highly polar compounds as well as lipid-soluble and volatile compounds are not detectable by the specific LCMS approach used here. Other, complementary metabolomics platforms, e.g. lipidomics (LCMS of lipid-soluble compounds), GCMS-volatiles (natural volatile organic compounds) and GCMS-derivatized polar extracts (highly polar compounds) should be used in order to determine which platform(s) are most promising in screening low and high incidence tuber lots for metabolome differences.

A combination of soil parameters was associated with a low disease incidence. However, no single parameter showed a clear correlation with disease incidence, indicating that disease incidence of blackleg is not dependent on one particular value, such as an increased calcium and magnesium concentration as opposed to a low nitrogen concentration, as has been suggested previously (Charkowski, 2015). This was supported by the finding that there was no association of soil parameters with microbial community composition at low and high disease incidence. Still, several soil parameters were related to microbial community composition, indicating their role in shaping microbial communities. Especially for bacterial, communities, several parameters differed between the lots 13 and 14 as compared to the other lots, which could be due to their high sand content and originating from the same region (Texel, North-Holland). In order to account for the effect of soil type and overall variability between the lots, a higher amount of lots from different soil types would have to be investigated. Overall, this comparison shows that soil parameters were not suitable to predict microbial community composition or disease incidence in this study.

Although the present study could successfully identify a number of taxa that were differentially abundant between tubers and soils with a high and a low disease incidence, there are limits to the implications of these results. First, it is obvious that the correlations that we observed here do not necessarily imply a causative influence of these taxa on resistance. This would have to be tested comprehensively by isolating the respective taxa and inoculating them on tubers, either as single strains or in artificial communities and determine disease incidence after challenge with the pathogen. Isolation is constrained due to many taxa still resisting cultivation and due to the fact that the sequencing of marker genes as 16S rRNA and ITS only allows identification to the genus level or even to the species level. However, it is known that microbial species differ tremendously with regard to their traits even at strain level. Therefore, isolation of the same strains as discovered by sequencing is challenging. Moreover, it is possible that no single strains or even a limited set of strains is responsible for resistance, but the combined activity of and the interactions between the community members. In addition, this study was focused on the dominant community members, which are most likely to have a significant effect on resistance. However, it has been repeatedly shown that also rare taxa can play a role. Due to their low abundances these taxa are difficult to cultivate (but see Kurm et al. (2019)).

5 Conclusions and outlook

This two year study shows that disease incidence of blackleg disease differs between lots of a potato cultivar, originating from different locations, and that a low disease incidence is correlated with the increased abundance of several microbial taxa with potential biocontrol ability. A majority of these taxa might originate from the soil of the previous generation. However, it is yet unclear which factors determine the colonization of the tubers with these taxa.
Moreover, our results indicate that testing the resistance of different cultivars against pathogens should not rely on only one seed lot due to the differences in resistance between seed lots.
In order to confirm the biocontrol ability of the microbial taxa found in this study, isolation of the respective species followed by *in-vitro* and field studies are needed. For identification of potential secondary metabolites involved in disease resistance it should be determined if inhomogeneous distribution among tubers could be responsible for failure of detection. In addition, other methods should be used to enable the detection of metabolites not covered by LC-MS.

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Supplementary information

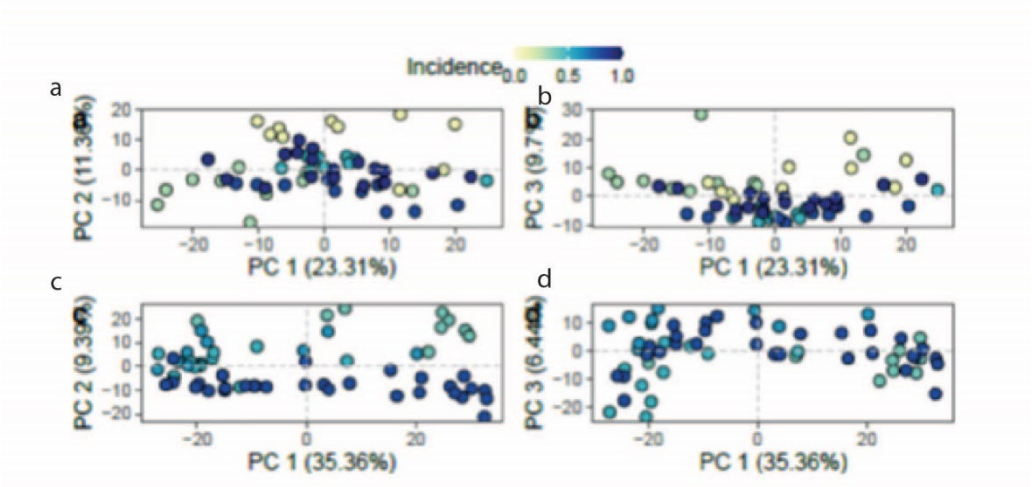

Figure S1: PCA score of the detected metabolites in the a, b) 2018 data set and the c, d) 2019 data set. The data points represent samples are coloured according to disease incidence at lot level.

Table S1: Seed lots used in 2018 and 2019.

Lot no.	**Cultivar**	**Area**	**Year**
1	Spunta	Groningen	2018
2	Spunta	Groningen	2018
3	Spunta	Groningen	2018
4	Spunta	Groningen	2018
5	Spunta	Groningen	2018
6	Spunta	Groningen	2018
7	Spunta	Groningen	2018
8	Spunta	Friesland	2018
9	Spunta	Friesland	2018
10	Spunta	Friesland	2018
11	Spunta	N-Holland	2018
12	Spunta	Friesland	2018
13	Spunta	N-Holland	2018
14	Spunta	Flevoland	2018
15	Kondor	N-Brabant	2018
16	Kondor	N-Holland (Texel)	2018

17	Kondor	N-Holland (Texel)	2018
18	Kondor	N-Holland	2018
19	Kondor	Friesland	2018
20	Kondor	Zeeland	2018
21	Kondor	N-Holland	2018
22	Kondor	N-Holland (Texel)	2018
23	Kondor	N-Holland	2018
24	Kondor	N-Holland	2018
25	Kondor	N-Holland	2018
26	Kondor	N-Holland	2018
27	Kondor	Groningen	2018
28	Kondor	Drenthe	2018
1	Kondor	Friesland	2019
2	Kondor	Groningen	2019
3	Kondor	Groningen	2019
4	Kondor	Groningen	2019
5	Kondor	Drenthe	2019
6	Kondor	Drenthe	2019
7	Kondor	Drenthe	2019
8	Kondor	Flevoland	2019
10	Kondor	Flevoland	2019
11	Kondor	Noord-Holland	2019
12	Kondor	Noord-Holland	2019
13	Kondor	Noord-Holland	2019
14	Kondor	Noord-Holland	2019
15	Kondor	Noord-Holland	2019
17	Kondor	Noord-Holland	2019
18	Kondor	Noord-Holland	2019

19	Kondor	Noord-Holland	2019
20	Kondor	Zeeland	2019
21	Kondor	Noordbrabant	2019
22	Kondor	Zeeland	2019

Table S2: Soil parameters at the locations of the seed lot origins; background infections with *Pectobacterium atrosepticum* (Pa), generic *Dickeya*, *P. parmentieri* (Pp), and *P. brasilience* (Pcb) were assessed in 4 subsamples of 50 tubers.

Partij	Partijnummer	Plaats	Jaar	Pa	Dickeya	Pp	Pcb	Provincie
K1	1	Lioessens	3	0	0	0	1	Friesland
K2	2	Bierum	5	0	0	0	0	Groningen
K3	3	Oudeschip	3	0	0	0	0	Groningen
K4	4	Rottum	4	0	0	0	0	Groningen
K5	5	Zuidvelde	4					Drenthe
K6	6	Nooitgedacht	4					Drenthe
K7	7	Wapse	4					Drenthe
K8	8	Rutten	3	0	0	0	2	Flevoland
K10	10	Dronten	2	0	0	0	0	Flevoland
K11	11	Anna Paulowna	3	0	0	0	0	Noord-Holland
K12	12	Middenmeer	3	0	0	1	4	Noord-Holland
K13	13	Den Burg	3	0	0	0	0	Noord-Holland
K14	14	Den Hoorn	3	0	0	0	0	Noord-Holland
K15	15	De Cocksdorp	2	0	0	0	0	Noord-Holland
K17	17	Zuidschermer	3	0	0	0	4	Noord-Holland
K18	18	Wieringerwerf	2	0	0	0	0	Noord-Holland
K19	19	Anna Paulowna	3	0	0	0	0	Noord-Holland
K20	20	Kerkwerve	3	0	0	0	0	Zeeland
K21	21	Heerle	4					Noordbrabant
K22	22	Axel	3	0	0	0	0	Zeeland

Table S2: Continued

Partij	N-totaal (kg N/ha)	C/N ratio	N-leverend vermogen (kg N/ha)	S plant beschikaar (kg S/ha)	S-totaal (kg S/ha)	C/S ratio	S-leverend vermogen (kg S/ha)	P-beschikbaar (kg P/ha)	P-bodemvoraad (kg P/ha)	K-beschikbaar (kg K/ha)	K-bodemvoraad (kg K/ha)
K1	3180	8	65	14	515	49	9	16.4	895	300	450
K2	4650	9	90	84	740	58	12	18.5	580	200	410
K3	3040	9	60	37	475	59	8	9.5	665	425	695
K4	3930	9	75	35	750	48	14	16.8	860	460	600
K5	5560	18	60	28	885	116	5	4.8	640	485	385
K6	7590	16	95	41	1305	92	14	5.1	540	650	275
K7	6030	14	90	28	685	126	3	5.1	870	260	210
K8	5150	18	55	108	910	101	8	6.5	325	205	290
K10	2950	11	55	29	1350	25	30	9	1015	220	365
K11	2720	10	50	51	475	60	8	5.4	720	375	460
K12	6200	10	115	364	7225	9	45	7.1	840	575	650
K13	4920	9	90	28	770	55	13	3.1	470	205	350
K14	4330	10	80	8	705	61	11	8.6	740	210	445
K15	2280	9	45	47	515	41	10	35	755	455	400
K17	6820	10	120	417	1045	63	16	5.9	620	235	760
K18	3290	10	60	209	915	37	18	9.3	905	215	450
K19	4970	9	95	35	1700	27	37	9	760	380	705
K20	2430	9	45	100	540	40	11	8.5	545	315	1550
K21	3950	14	55	8	665	81	8	14.8	1015	175	305
K22	3180	7	70	12	600	39	12	5.7	730	325	395

Partij	Ca-beschikbaar (kg Ca/ha)	Ca-bodem (kg Ca/ha)	Mg-beschikbaar (mg Mg/kg)	Mg-bodemvooraad (kg Mg/ha)	Na-beschikbaar (mg Na/kg)	Na-bodemvooraad (kg Na/ha)	Si-beschikbaar	Fe-beschikbaar	Zn-beschikbaar	Mn beschibaar
K1	155	6425	265	220	85	50	106030	7280	< 320	1190
K2	175	8155	315	500	90	55	128470	< 6230	< 310	< 770
K3	150	3745	480	545	65	65	59870	7670	1010	13590
K4	75	6940	335	535	95	65	110680	< 6300	310	< 780
K5	115	2760	435	405	65	50	18880	< 5670	7160	22190
K6	65	3345	490	435	100	50	19180	< 5430	2830	9170
K7	205	1710	235	360	50	35	15340	< 5750	3390	3640
K8	45	2395	310	275	45	35	13780	< 5720	6630	30170
K10	150	6525	165	215	95	45	93430	< 6260	310	780
K11	430	4505	175	190	60	60	64240	< 6370	< 320	< 790
K12	380	13200	295	445	90	95	237820	< 6050	< 300	< 740
K13	75	1665	355	410	75	40	33000	6300	4060	22460
K14	25	1910	330	435	60	35	39490	< 6180	2860	9590
K15	75	1990	240	255	65	45	45890	10550	3210	21300
K17	475	12060	230	395	110	140	220040	< 5990	< 300	< 740
K18	595	8515	195	190	80	55	222880	< 6260	310	1430
K19	360	8565	305	405	80	70	116620	< 6080	< 300	< 750
K20	55	14550	335	1410	90	460	100420	< 4900	< 240	< 610
K21	145	2785	225	210	20	20	18480	< 6070	3950	5680
K22	25	6685	225	245	30	50	118470	9540	< 310	< 790

Partij	Cu-beschikbaar	Co-beschikbaar	B-beschikbaar	Mo-beschikbaar	Se-beschikbaar	pH	C-organisch (%)	Organische stof (%)	C/OS ratio	Koolzure kalk (%)
K1	155	< 10	1160	< 10	12	7.2	0.8	1.3	0.62	1.5
K2	70	< 10	625	20	18	7.2	1.4	2.6	0.54	0.2
K3	210	25	1075	< 10	7.6	5.8	0.9	1.7	0.53	< 0,2
K4	110	< 10	1420	20	13	7	1.1	2.2	0.5	0.3
K5	95	45	595	< 10	6.7	4.7	3.6	5.9	0.61	< 0.2
K6	90	15	410	< 10	10	5.2	4.5	7.6	0.59	< 0.2
K7	110	15	225	< 10	8.8	5.2	3	5.4	0.56	< 0.2
K8	< 60	50	405	< 10	6.8	5	3.2	5.4	0.59	< 0.2
K10	85	< 10	520	20	11	7.4	1.1	2.4	0.46	6.9
K11	75	< 10	1415	110	11	6.9	0.9	1.7	0.53	2.3
K12	115	< 10	1420	70	16	7.2	2.1	3.9	0.54	7.4
K13	100	65	335	< 10	7.7	4.7	1.4	2.7	0.52	<0,2
K14	110	20	400	< 10	9.8	5.4	1.4	2.8	0.52	< 0,2
K15	85	25	550	< 10	< 6,8	5.7	0.7	1.3	0.54	< 0,2
K17	90	< 10	1450	40	26	7.4	2.2	4.1	0.54	13.5
K18	125	10	770	70	12	7.2	1.1	2.4	0.46	5.3
K19	160	< 10	1515	200	18	7.1	1.5	3.4	0.44	4.4
K20	65	< 5	890	20	8	7.3	0.9	14.1	0.06	0.8
K21	120	20	355	< 10	6.6	5.3	1.8	3.3	0.55	< 0,2
K22	100	10	775	20	9.8	7.3	0.7	1.9	0.37	0.9

Partij	Klei	Silt	Zand	Slib	Klei-humus (CEC) (mmol+/kg)	CEC bezetting	Ca bezetting	Mg bezetting	K-bezetting	Na bezetting
K1	14	35	47	25	110	100	91	5.2	3.3	0.6
K2	14	39	44	26	149	100	89	8.9	2.3	0.5
K3	11	28	59	19	80	100	74	18	7	1.1
K4	16	37	45	27	131	100	85	11	3.7	0.7
K5	3	18	73	8	77	85	64	15	4.5	1
K6	2	11	79	5	89	89	70	15	2.9	0.9
K7	2	7	86	4	55	78	55	19	3.5	0.9
K8	1	8	86	3	67	80	63	12	3.9	0.7
K10	9	28	54	17	114	100	92	5	2.6	0.5
K11	8	13	75	12	80	100	89	6.3	4.6	1
K12	29	39	21	41	247	98	90	5	2.3	0.6
K13	8	10	79	11	47	88	57	23	6.2	1.3
K14	4	15	78	9	52	91	60	23	7.1	1
K15	4	12	83	8	45	91	69	14	7.1	1.3
K17	28	33	21	38	224	100	91	4.9	2.9	0.9
K18	16	27	49	24	147	100	93	3.5	2.5	0.5
K19	18	30	44	27	160	100	89	6.9	3.8	0.6
K20	44	39	2	56	457	84	67	11	3.7	1.9
K21	2	17	78	7	60	91	77	9.5	4.3	0.5
K22	10	25	62	18	116	100	91	5.5	2.8	0.6

Partij	H bezetting	Al bezetting	Verkruimelbaarheid	Verslemping	Stuifgevoeligheid	Microbiele biomassa	Microbiele activiteit	Schimmel/bacterie ratio	K getal
K1	< 0.1	< 0.1	8.1	3.4	8.5	203	16	0.8	23
K2	< 0,1	< 0,1	8.2	3.9	8.6	157	38	1.5	17
K3	< 0,1	< 0,1	8.5	3.4	8.2	156	20	0.3	32
K4	< 0,1	< 0,1	7.8	4.1	8.6	180	28	0.7	32
K5	0.3	1.6	10	8.2	7.4	414	40	0.7	46
K6	< 0.1	< 0.1	10	8.5	5.3	605	54	0.6	65
K7	0.2	< 0.1	10	8.1	3.4	763	91	0.9	26
K8	< 0.1	< 0.1	10	8.1	3.4	382	40	0.7	19
K10	< 0.1	< 0.1	8.9	4.3	8.2	331	23	1.2	18
K11	< 0,1	< 0,1	9	4.7	7.4	24	20	0.6	28
K12	< 0,1	< 0,1	5.7	6.1	9	267	36	0.8	38
K13	0.4	1.6	9.1	5.1	7.3	548	49	0.9	17
K14	< 0,1	< 0,1	10	7.5	7.3	480	62	0.7	19
K15	< 0,1	< 0,1	10	7.3	5.6	217	29	0.7	39
K17	< 0,1	< 0,1	5.9	5.8	8.8	493	49	0.8	21
K18	< 0,1	< 0,1	7.8	4.2	8.4	109	21	0.6	19
K19	< 0,1	< 0,1	7.5	4.8	8.5	200	29	0.8	28
K20	< 0,1	< 0,1	7.7	8.1	9.2	815	66	0.7	32
K21	0.2	< 0,1	10	7.7	5.8	277	32	0.9	15
K22	< 0,1	< 0,1	8.7	3.7	8.1	24	10	0.6	25

Table S3: Detection *of Dickeya, P. atrosepticum, P. brasiliense* and *P. parmentieri* in progeny tubers in 2018.

Location	Lot	Cultivar	Pathogen	Concentration	*Dickeya*	*P. atrosepticum*	*P. brasiliense*	*P. parmentieri*
Driezum	20	K	water	0	0	0	4	0

Driezum	22	K	water	0	0	0	1	0
Driezum	24	K	water	0	0	0	6	0
Driezum	23	K	water	0	0	0	7	0
Driezum	27	K	water	0	0	0	2	1
Driezum	28	K	water	0	0	0	3	3
Driezum	8	S	water	0	0	0	3	0
Driezum	9	S	water	0	0	0	0	0
Driezum	12	S	water	0	0	0	4	0
Driezum	4	S	water	0	0	0	4	0
Driezum	5	S	water	0	0	0	6	0
Driezum	7	S	water	0	0	0	4	0
Driezum	20	K	*D. solani*	10^6	10	0	5	0
Driezum	22	K	*D. solani*	10^6	6	0	0	0
Driezum	24	K	*D. solani*	10^6	10	0	6	0
Driezum	23	K	*D. solani*	10^6	7	0	6	0
Driezum	27	K	*D. solani*	10^6	10	0	7	1
Driezum	28	K	*D. solani*	10^6	8	0	0	6
Driezum	8	S	*D. solani*	10^6	8	0	2	0
Driezum	9	S	*D. solani*	10^6	6	0	6	0

Driezum	12	S	*D. solani*	10^6	10	0	8	0
Driezum	4	S	*D. solani*	10^6	9	0	1	0
Driezum	5	S	*D. solani*	10^6	7	0	3	0
Driezum	7	S	*D. solani*	10^6	6	0	1	0
Driezum	20	K	*P. brasiliense*	10^6	0	0	9	1
Driezum	22	K	*P. brasiliense*	10^6	2	0	10	0
Driezum	24	K	*P. brasiliense*	10^6	0	0	10	0
Driezum	23	K	*P. brasiliense*	10^6	0	0	9	0
Driezum	27	K	*P. brasiliense*	10^6	1	0	10	0
Driezum	28	K	*P. brasiliense*	10^6	0	0	9	7
Driezum	8	S	*P. brasiliense*	10^6	0	0	10	0
Driezum	9	S	*P. brasiliense*	10^6	0	0	10	0
Driezum	12	S	*P. brasiliense*	10^6	0	0	10	0
Driezum	4	S	*P. brasiliense*	10^6	0	0	8	0
Driezum	5	S	*P. brasiliense*	10^6	4	0	10	0
Driezum	7	S	*P. brasiliense*	10^6	0	0	10	0

Table S4: SPLSDA components of bacterial taxa in 2018

OTU	High	Low	Contrib.High	Contrib.Low	Contrib	GroupContrib	importance	Kingdom	Phylum	Class	Order	Family	Genus
OTU100	-0.38752	0.387523	FALSE	TRUE	FALSE	Low	-0.0487	Bacteria	Actinobacteria	Actinobacteria	Propionibacteriales	Nocardioidaceae	Marmoricola
OTU1020	-0.36706	0.36706	FALSE	TRUE	FALSE	Low	-0.0118	Bacteria	Actinobacteria	Thermoleophilia	Solirubrobacterales	Elev-16S-1332	NA
OTU1027	0.371761	-0.37176	TRUE	FALSE	FALSE	High	0.020276	Bacteria	Actinobacteria	Actinobacteria	Micromonosporales	Micromonosporaceae	NA
OTU1050	0.366027	-0.3663	TRUE	FALSE	FALSE	High	0.009936	Bacteria	Actinobacteria	Thermoleophilia	Solirubrobacterales	Elev-16S-1332	NA
OTU1057	-0.37688	0.376876	FALSE	TRUE	FALSE	Low	-0.0295	Bacteria	Cyanobacteria	Cyanobacteria	SubsectionIV	FamilyI	Nostoc
OTU116	-0.40243	0.402433	FALSE	TRUE	FALSE	Low	-0.07559	Bacteria	Actinobacteria	Actinobacteria	Micrococcales	Microbacteriaceae	Plantibacter
OTU12	0.386996	-0.387	TRUE	FALSE	FALSE	High	0.047749	Bacteria	Actinobacteria	Actinobacteria	Micrococcales	Dermabacteraceae	Brachybacterium
OTU127	-0.36484	0.364841	FALSE	TRUE	FALSE	Low	-0.0078	Bacteria	Proteobacteria	Alphaproteobacteria	Rhodospirillales	Rhodospirillaceae	Skermanella
OTU128	0.498744	-0.49874	TRUE	FALSE	FALSE	High	0.249261	Bacteria	Actinobacteria	Actinobacteria	Micrococcales	Microbacteriaceae	Agromyces
OTU1333	0.377409	-0.37741	TRUE	FALSE	FALSE	High	0.030461	Bacteria	Actinobacteria	Actinobacteria	Frankiales	Geodermatophilaceae	Geodermatophilus
OTU142	0.375737	-0.37574	TRUE	FALSE	FALSE	High	0.027446	Bacteria	Actinobacteria	Actinobacteria	Micrococcales	Sanguibacteraceae	Sanguibacter
OTU149	-0.3986	0.398596	FALSE	TRUE	FALSE	Low	-0.06867	Bacteria	Proteobacteria	Betaproteobacteria	Methylophilales	Methylophilaceae	Methylophilus
OTU16	-0.45407	0.454066	FALSE	TRUE	FALSE	Low	-0.16869	Bacteria	Proteobacteria	Gammaproteobacteria	Pseudomonadales	Pseudomonadaceae	Pseudomonas

OTU162	0.388009	-0.38801	TRUE	FALSE	FALSE	High	0.049575	Bacteria	Actinobacteria	Actinobacteria	Micrococcales	Sanguibacteraceae	Sanguibacter
OTU168	-0.36507	0.365068	FALSE	TRUE	FALSE	Low	-0.00821	Bacteria	Proteobacteria	Alphaproteobacteria	Rhodospirillales	Rhodospirillaceae	Skermanella
OTU171	-0.39409	0.394093	FALSE	TRUE	FALSE	Low	-0.06055	Bacteria	Firmicutes	Bacilli	Bacillales	Bacillaceae	Bacillus
OTU172	0.398588	-0.39859	TRUE	FALSE	FALSE	High	0.068652	Bacteria	Actinobacteria	Actinobacteria	Corynebacteriales	Nocardiaceae	Rhodococcus
OTU174	0.370595	-0.3706	TRUE	FALSE	FALSE	High	0.018173	Bacteria	Firmicutes	Bacilli	Bacillales	Paenibacillaceae	Paenibacillus
OTU177	-0.41065	0.410655	FALSE	TRUE	FALSE	Low	-0.09041	Bacteria	Actinobacteria	Actinobacteria	Propionibacteriales	Nocardioidaceae	Nocardioides
OTU18	-0.42065	0.42065	FALSE	TRUE	FALSE	Low	-0.10844	Bacteria	Proteobacteria	Gammaproteobacteria	Enterobacteriales	Enterobacteriaceae	Pantoea
OTU181	-0.38303	0.383033	FALSE	TRUE	FALSE	Low	-0.0406	Bacteria	Actinobacteria	Actinobacteria	Propionibacteriales	Nocardioidaceae	Marmoricola
OTU194	-0.39957	0.399572	FALSE	TRUE	FALSE	Low	-0.07043	Bacteria	Actinobacteria	Actinobacteria	Micrococcales	Microbacteriaceae	Agromyces
OTU203	-0.39026	0.390263	FALSE	TRUE	FALSE	Low	-0.05364	Bacteria	Actinobacteria	Actinobacteria	Pseudonocardiales	Pseudonocardiaceae	Actinomycetospora
OTU210	-0.51806	0.518061	FALSE	TRUE	FALSE	Low	-0.2841	Bacteria	Actinobacteria	Actinobacteria	Micrococcales	Microbacteriaceae	Agrococcus
OTU22	-0.42769	0.427688	FALSE	TRUE	FALSE	Low	-0.12113	Bacteria	Actinobacteria	Actinobacteria	Micrococcales	Microbacteriaceae	Curtobacterium
OTU241	0.366366	-0.36637	TRUE	FALSE	FALSE	High	0.010546	Bacteria	Proteobacteria	Gammaproteobacteria	Xanthomonadales	Xanthomonadales_Incertae_Sedis	Acidibacter
OTU271	-0.37033	0.370334	FALSE	TRUE	FALSE	Low	-0.0177	Bacteria	Actinobacteria	Actinobacteria	Propionibacteriales	Nocardioidaceae	Nocardioides
OTU285	-0.3907	0.390698	FALSE	TRUE	FALSE	Low	-0.05442	Bacteria	Actinobacteria	Actinobacteria	Micrococcales	Intrasporangiaceae	Intrasporangium

OTU301	0.440201	-0.4402	TRUE	FALSE	FALSE	High	0.143692	Bacteria	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	Lachnoclostridium_5
OTU309	0.380071	-0.38007	TRUE	FALSE	FALSE	High	0.035261	Bacteria	Proteobacteria	Alphaproteobacteria	Rhizobiales	Brucellaceae	Ochrobactrum
OTU312	0.498489	-0.49849	TRUE	FALSE	FALSE	High	0.248801	Bacteria	Firmicutes	Bacilli	Bacillales	Planococcaceae	NA
OTU313	0.41872	-0.41872	TRUE	FALSE	FALSE	High	0.104957	Bacteria	Actinobacteria	Actinobacteria	Micrococcales	Microbacteriaceae	Leucobacter
OTU320	0.463479	-0.46348	TRUE	FALSE	FALSE	High	0.185668	Bacteria	Actinobacteria	Actinobacteria	Streptomycetales	Streptomycetaceae	Streptomyces
OTU321	-0.38361	0.383614	FALSE	TRUE	FALSE	Low	-0.04165	Bacteria	Actinobacteria	Actinobacteria	Propionibacteriales	Nocardioidaceae	Marmoricola
OTU323	0.48324	-0.48324	TRUE	FALSE	FALSE	High	0.221303	Bacteria	Firmicutes	Negativicutes	Selenomonadales	Veillonellaceae	Anaerosinus
OTU324	0.365833	-0.36583	TRUE	FALSE	FALSE	High	0.009586	Bacteria	Proteobacteria	Gammaproteobacteria	Pseudomonadales	Pseudomonadaceae	Pseudomonas
OTU343	0.362233	-0.36223	TRUE	FALSE	FALSE	High	0.003093	Bacteria	Actinobacteria	Actinobacteria	Micrococcales	Microbacteriaceae	Leucobacter
OTU348	0.46691	-0.46691	TRUE	FALSE	FALSE	High	0.191855	Bacteria	Actinobacteria	Thermoleophilia	Gaiellales	Gaiellaceae	Gaiella
OTU357	-0.39934	0.399339	FALSE	TRUE	FALSE	Low	-0.07001	Bacteria	Actinobacteria	Actinobacteria	Micrococcales	Micrococcaceae	Arthrobacter
OTU36	-0.44554	0.445541	FALSE	TRUE	FALSE	Low	-0.15332	Bacteria	Actinobacteria	Actinobacteria	Corynebacteriales	Nocardiaceae	Rhodococcus
OTU363	-0.40814	0.408135	FALSE	TRUE	FALSE	Low	-0.08587	Bacteria	Proteobacteria	Alphaproteobacteria	Rhizobiales	Methylobacteriaceae	Microvirga
OTU367	0.363065	-0.36306	TRUE	FALSE	FALSE	High	0.004594	Bacteria	Actinobacteria	Actinobacteria	Propionibacteriales	Nocardioidaceae	Aeromicrobium
OTU400	-0.37375	0.373754	FALSE	TRUE	FALSE	Low	-0.02387	Bacteria	Actinobacteria	Actinobacteria	Micrococcales	Ruaniaceae	Haloactinobacterium

OTU405	0.411775	-0.41177	TRUE	FALSE	FALSE	High	0.092432	Bacteria	Actinobacteria	Actinobacteria	Micrococcales	Microbacteriaceae	Leucobacter
OTU410	0.481134	-0.48113	TRUE	FALSE	FALSE	High	0.217505	Bacteria	Firmicutes	Bacilli	Lactobacillales	Leuconostocaceae	Leuconostoc
OTU441	0.41546	-0.41546	TRUE	FALSE	FALSE	High	0.099077	Bacteria	Proteobacteria	Alphaproteobacteria	Sphingomonadales	Sphingomonadaceae	Sphingobium
OTU443	0.390039	-0.39004	TRUE	FALSE	FALSE	High	0.053235	Bacteria	Actinobacteria	Actinobacteria	Propionibacteriales	Nocardioidaceae	Marmoricola
OTU45	-0.36995	0.369947	FALSE	TRUE	FALSE	Low	-0.017	Bacteria	Proteobacteria	Gammaproteobacteria	Pseudomonadales	Pseudomonadaceae	Pseudomonas
OTU450	-0.40978	0.409784	FALSE	TRUE	FALSE	Low	-0.08884	Bacteria	Actinobacteria	Actinobacteria	Micrococcales	Microbacteriaceae	Leifsonia
OTU452	0.369695	-0.36969	TRUE	FALSE	FALSE	High	0.01655	Bacteria	Actinobacteria	Thermoleophilia	Solirubrobacterales	Elev-16S-1332	NA
OTU456	0.366273	-0.36627	TRUE	FALSE	FALSE	High	0.010379	Bacteria	Firmicutes	Bacilli	Bacillales	Family_XII	Exiguobacterium
OTU458	-0.36145	0.361446	FALSE	TRUE	FALSE	Low	-0.00167	Bacteria	Actinobacteria	Actinobacteria	Micrococcales	Promicromonosporaceae	Promicromonospora
OTU46	0.428697	-0.4287	TRUE	FALSE	FALSE	High	0.122948	Bacteria	Actinobacteria	Actinobacteria	Propionibacteriales	Nocardioidaceae	Nocardioides
OTU463	-0.39288	0.39288	FALSE	TRUE	FALSE	Low	-0.05836	Bacteria	Firmicutes	Bacilli	Bacillales	Planococcaceae	NA
OTU466	0.414699	-0.4147	TRUE	FALSE	FALSE	High	0.097706	Bacteria	Actinobacteria	Thermoleophilia	Gaiellales	Gaiellaceae	Gaiella
OTU47	-0.5422	0.542197	FALSE	TRUE	FALSE	Low	-0.32762	Bacteria	Actinobacteria	Actinobacteria	Micrococcales	Micrococcaceae	Arthrobacter
OTU479	0.441941	-0.44194	TRUE	FALSE	FALSE	High	0.146829	Bacteria	Proteobacteria	Alphaproteobacteria	Caulobacterales	Caulobacteraceae	NA
OTU483	-0.39532	0.395323	FALSE	TRUE	FALSE	Low	-0.06276	Bacteria	Actinobacteria	Actinobacteria	Propionibacteriales	Nocardioidaceae	Nocardioides
OTU486	0.374298	-0.3743	TRUE	FALSE	FALSE	High	0.02485	Bacteria	Proteobacteria	Alphaproteobacteria	Rhodospirillales	Rhodospirillaceae	Azospirillum

OTU489	0.401468	-0.40147	TRUE	FALSE	FALSE	High	0.073845	Bacteria	Proteobacteria	Alphaproteobacteria	Rhizobiales	Xanthobacteraceae	Xanthobacter
OTU501	-0.40621	0.406212	FALSE	TRUE	FALSE	Low	-0.0824	Bacteria	Actinobacteria	Actinobacteria	Kineosporiales	Kineosporiaceae	Kineococcus
OTU502	-0.36344	0.363436	FALSE	TRUE	FALSE	Low	-0.00526	Bacteria	Actinobacteria	Actinobacteria	Micrococcales	Intrasporangiaceae	Ornithinibacter
OTU512	0.364391	-0.3643	TRUE	FALSE	FALSE	High	0.006985	Bacteria	Actinobacteria	Actinobacteria	Micrococcales	Intrasporangiaceae	Lapillicoccus
OTU518	0.376061	-0.37606	TRUE	FALSE	FALSE	High	0.02803	Bacteria	Proteobacteria	Alphaproteobacteria	Rhizobiales	Hyphomicrobiaceae	Devosia
OTU524	-0.37021	0.370211	FALSE	TRUE	FALSE	Low	-0.01748	Bacteria	Firmicutes	Bacilli	Bacillales	Bacillaceae	NA
OTU53	-0.37861	0.378611	FALSE	TRUE	FALSE	Low	-0.03263	Bacteria	Proteobacteria	Gammaproteobacteria	Enterobacteriales	Enterobacteriaceae	NA
OTU535	-0.39442	0.394419	FALSE	TRUE	FALSE	Low	-0.06113	Bacteria	Proteobacteria	Alphaproteobacteria	Rhodospirillales	Rhodospirillaceae	Skermanella
OTU538	-0.41198	0.411982	FALSE	TRUE	FALSE	Low	-0.09281	Bacteria	Actinobacteria	Actinobacteria	Frankiales	Geodermatophilaceae	Blastococcus
OTU548	0.447834	-0.44783	TRUE	FALSE	FALSE	High	0.157456	Bacteria	Firmicutes	Negativicutes	Selenomonadales	Veillonellaceae	Pelosinus
OTU551	-0.47487	0.47487	FALSE	TRUE	FALSE	Low	-0.20621	Bacteria	Actinobacteria	Actinobacteria	Propionibacteriales	Nocardioidaceae	Nocardioides
OTU574	0.405352	-0.40535	TRUE	FALSE	FALSE	High	0.080849	Bacteria	Proteobacteria	Alphaproteobacteria	Rhizobiales	Brucellaceae	Pseudochrobactrum
OTU604	0.378654	-0.37865	TRUE	FALSE	FALSE	High	0.032707	Bacteria	Planctomycetes	Planctomycetacia	Planctomycetales	Planctomycetaceae	Pirellula
OTU615	0.376203	-0.3762	TRUE	FALSE	FALSE	High	0.028285	Bacteria	Bacteroidetes	Sphingobacteriia	Sphingobacteriales	Chitinophagaceae	Niastella
OTU622	-0.3907	0.390702	FALSE	TRUE	FALSE	Low	-0.05443	Bacteria	Actinobacteria	Actinobacteria	Micrococcales	Micrococcaceae	NA

OTU654	0.430565	-0.43056	TRUE	FALSE	FALSE	High	0.126315	Bacteria	Actinobacteria	Thermoleophilia	Solirubrobacterales	Elev-16S-1332	NA
OTU663	-0.37018	0.370177	FALSE	TRUE	FALSE	Low	-0.01742	Bacteria	Proteobacteria	Alphaproteobacteria	Rhizobiales	Hyphomicrobiaceae	Hyphomicrobium
OTU666	-0.36486	0.36486	FALSE	TRUE	FALSE	Low	-0.00783	Bacteria	Actinobacteria	Actinobacteria	Frankiales	Geodermatophilaceae	NA
OTU677	0.372153	-0.37215	TRUE	FALSE	FALSE	High	0.020983	Bacteria	Actinobacteria	Actinobacteria	Micrococcales	Micrococcaceae	Glutamicibacter
OTU683	0.374806	-0.37481	TRUE	FALSE	FALSE	High	0.025766	Bacteria	Proteobacteria	Alphaproteobacteria	Rhizobiales	Hyphomicrobiaceae	Devosia
OTU697	-0.41543	0.415427	FALSE	TRUE	FALSE	Low	-0.09902	Bacteria	Firmicutes	Bacilli	Bacillales	Alicyclobacillaceae	Tumebacillus
OTU743	-0.39844	0.398445	FALSE	TRUE	FALSE	Low	-0.06839	Bacteria	Actinobacteria	Thermoleophilia	Solirubrobacterales	Solirubrobacteraceae	Solirubrobacter
OTU750	0.410527	-0.41053	TRUE	FALSE	FALSE	High	0.090181	Bacteria	Actinobacteria	Actinobacteria	Micromonosporales	Micromonosporaceae	Micromonospora
OTU757	0.362846	-0.36285	TRUE	FALSE	FALSE	High	0.004199	Bacteria	Bacteroidetes	Sphingobacteriia	Sphingobacteriales	Chitinophagaceae	Ferruginibacter
OTU768	-0.37578	0.375783	FALSE	TRUE	FALSE	Low	-0.02753	Bacteria	Actinobacteria	Actinobacteria	Micrococcales	Intrasporangiaceae	Oryzihumus
OTU774	0.415324	-0.41532	TRUE	FALSE	FALSE	High	0.098833	Bacteria	Firmicutes	Bacilli	Bacillales	Thermoactinomycetaceae	Thermoactinomyces
OTU785	0.390573	-0.39057	TRUE	FALSE	FALSE	High	0.054199	Bacteria	Firmicutes	Clostridia	Clostridiales	Ruminococcaceae	Ruminococcus_1
OTU804	0.383185	-0.38319	TRUE	FALSE	FALSE	High	0.040877	Bacteria	Firmicutes	Bacilli	Bacillales	Planococcaceae	Lysinibacillus
OTU871	0.42571	-0.42571	TRUE	FALSE	FALSE	High	0.117561	Bacteria	Actinobacteria	Thermoleophilia	Solirubrobacterales	Elev-16S-1332	NA

OTU875	0.367894	-0.36789	TRUE	FALSE	FALSE	High	0.013303	Bacteria	Proteobacteria	Gammaproteobacteria	Pseudomonadales	Moraxellaceae	Acinetobacter
OTU893	-0.40068	0.400679	FALSE	TRUE	FALSE	Low	-0.07242	Bacteria	Firmicutes	Bacilli	Bacillales	Paenibacillaceae	Paenibacillus
OTU898	-0.40511	0.405113	FALSE	TRUE	FALSE	Low	-0.08042	Bacteria	Proteobacteria	Alphaproteobacteria	Rhodobacterales	Rhodobacteraceae	NA
OTU914	0.379602	-0.3796	TRUE	FALSE	FALSE	High	0.034414	Bacteria	Proteobacteria	Gammaproteobacteria	Xanthomonadales	Xanthomonadaceae	Tahibacter
OTU92	-0.40792	0.407917	FALSE	TRUE	FALSE	Low	-0.08547	Bacteria	Actinobacteria	Actinobacteria	Micrococcales	Microbacteriaceae	Clavibacter
OTU928	0.400131	-0.40013	TRUE	FALSE	FALSE	High	0.071436	Bacteria	Firmicutes	Bacilli	Bacillales	Staphylococcaceae	Jeotgalicoccus
OTU930	0.403296	-0.4033	TRUE	FALSE	FALSE	High	0.077143	Bacteria	Firmicutes	Bacilli	Lactobacillales	Streptococcaceae	Lactococcus
OTU949	0.363955	-0.36395	TRUE	FALSE	FALSE	High	0.006199	Bacteria	Acidobacteria	Solibacteres	Solibacterales	Solibacteraceae_(Subgroup_3)	Bryobacter
OTU95	0.415392	-0.41539	TRUE	FALSE	FALSE	High	0.098955	Bacteria	Firmicutes	Bacilli	Bacillales	Planococcaceae	NA
OTU965	0.360668	-0.36067	TRUE	FALSE	FALSE	High	0.000272	Bacteria	Proteobacteria	Deltaproteobacteria	Myxococcales	Haliangiaceae	Haliangium
OTU979	0.453714	-0.45371	TRUE	FALSE	FALSE	High	0.168059	Bacteria	Proteobacteria	Alphaproteobacteria	Rhizobiales	Hyphomicrobiaceae	Pedomicrobium
OTU986	-0.43014	0.430137	FALSE	TRUE	FALSE	Low	-0.12554	Bacteria	Proteobacteria	Alphaproteobacteria	Rhizobiales	Rhodobiaceae	NA

Table S5: SPLSDA components of bacterial taxa in 2019 tubers.

OTU	High	Low	Contrib.High	Contrib.Low	Contrib	GroupContrib	importance	Kingdom	Phylum	Class	Order	Family	Genus
OTU1	-0.41666	0.431027	FALSE	TRUE	FALSE	Low	-0.0032	Bacteria	Firmicutes	Bacilli	Bacillales	Staphylococcaceae	Staphylococcus

OTU100	0.480666	-0.49724	TRUE	FALSE	FALSE	High	0.083019	Bacteria	Actinobacteria	Actinobacteria	Micrococcales	Microbacteriaceae	Galbitalea
OTU1004	0.416476	-0.43084	TRUE	FALSE	FALSE	High	0.002969	Bacteria	Proteobacteria	Alphaproteobacteria	Rhizobiales	Devosiaceae	Devosia
OTU1009	0.444924	-0.46027	TRUE	FALSE	FALSE	High	0.038446	Bacteria	Firmicutes	Bacilli	Lactobacillales	Enterococcaceae	Enterococcus
OTU103	0.546747	-0.5656	TRUE	FALSE	FALSE	High	0.165427	Bacteria	Proteobacteria	Alphaproteobacteria	Rhizobiales	Rhizobiaceae	Neorhizobium
OTU104	0.484279	-0.50098	TRUE	FALSE	FALSE	High	0.087525	Bacteria	Actinobacteria	Actinobacteria	Micrococcales	Sanguibacteraceae	Sanguibacter
OTU105	0.550185	-0.56916	TRUE	FALSE	FALSE	High	0.169714	Bacteria	Proteobacteria	Alphaproteobacteria	Sphingomonadales	Sphingomonadaceae	Sphingomonas
OTU107	0.471456	-0.48771	TRUE	FALSE	FALSE	High	0.071534	Bacteria	Bacteroidetes	Bacteroidia	Sphingobacteriales	Sphingobacteriaceae	Pedobacter
OTU1082	0.417807	-0.43221	TRUE	FALSE	FALSE	High	0.004629	Bacteria	Proteobacteria	Alphaproteobacteria	Rhizobiales	Rhizobiaceae	Aurantimonas
OTU1087	0.442857	-0.45813	TRUE	FALSE	FALSE	High	0.035869	Bacteria	Bacteroidetes	Bacteroidia	Flavobacteriales	Flavobacteriaceae	Flavobacterium
OTU1116	-0.42506	0.439715	FALSE	TRUE	FALSE	Low	-0.01367	Bacteria	Firmicutes	Bacilli	Bacillales	Planococcaceae	Chungangia
OTU112	0.485218	-0.50195	TRUE	FALSE	FALSE	High	0.088696	Bacteria	Proteobacteria	Alphaproteobacteria	Rhizobiales	Rhizobiaceae	Ensifer
OTU113	0.423705	-0.43832	TRUE	FALSE	FALSE	High	0.011985	Bacteria	Proteobacteria	Alphaproteobacteria	Caulobacterales	Caulobacteraceae	Caulobacter
OTU114	-0.42562	0.440295	FALSE	TRUE	FALSE	Low	-0.01437	Bacteria	Actinobacteria	Actinobacteria	Frankiales	Acidothermaceae	Acidothermus
OTU118	-0.43838	0.453501	FALSE	TRUE	FALSE	Low	-0.03029	Bacteria	Proteobacteria	Alphaproteobacteria	Sphingomonadales	Sphingomonadaceae	Sphingomonas
OTU12	-0.57019	0.589856	FALSE	TRUE	FALSE	Low	-0.19467	Bacteria	Actinobacteria	Actinobacteria	Micrococcales	Micrococcaceae	Glutamicibacter

OTU133	0.580505	-0.60052	TRUE	FALSE	FALSE	High	0.207526	Bacteria	Actinobacteria	Actinobacteria	Micrococcales	Microbacteriaceae	Agromyces
OTU136	0.440976	-0.45618	TRUE	FALSE	FALSE	High	0.033522	Bacteria	Bacteroidetes	Bacteroidia	Cytophagales	Microscillaceae	NA
OTU138	0.544694	-0.56348	TRUE	FALSE	FALSE	High	0.162866	Bacteria	Proteobacteria	Alphaproteobacteria	Rhizobiales	Devosiaceae	Devosia
OTU143	0.416349	-0.43071	TRUE	FALSE	FALSE	High	0.002812	Bacteria	Proteobacteria	Alphaproteobacteria	Rhizobiales	Devosiaceae	Devosia
OTU1464	0.437787	-0.45288	TRUE	FALSE	FALSE	High	0.029546	Bacteria	Actinobacteria	Actinobacteria	Corynebacteriales	Nocardiaceae	Nocardia
OTU151	-0.42126	0.435784	FALSE	TRUE	FALSE	Low	-0.00893	Bacteria	Firmicutes	Bacilli	Bacillales	Staphylococcaceae	Staphylococcus
OTU1554	0.427098	-0.44183	TRUE	FALSE	FALSE	High	0.016215	Bacteria	Proteobacteria	Alphaproteobacteria	Rhizobiales	Rhizobiaceae	Aureimonas
OTU157	0.535722	-0.5542	TRUE	FALSE	FALSE	High	0.151678	Bacteria	Actinobacteria	Actinobacteria	Micromonosporales	Micromonosporaceae	NA
OTU160	-0.57037	0.590037	FALSE	TRUE	FALSE	Low	-0.19489	Bacteria	Actinobacteria	Actinobacteria	Micrococcales	Intrasporangiaceae	Terrabacter
OTU166	0.60045	-0.62115	TRUE	FALSE	FALSE	High	0.232398	Bacteria	Proteobacteria	Alphaproteobacteria	Rhizobiales	Rhizobiaceae	Phyllobacterium
OTU1722	-0.41977	0.434249	FALSE	TRUE	FALSE	Low	-0.00708	Bacteria	Actinobacteria	Actinobacteria	Propionibacteriales	Nocardioidaceae	Nocardioides
OTU174	0.42025	-0.43474	TRUE	FALSE	FALSE	High	0.007676	Bacteria	Bacteroidetes	Bacteroidia	Flavobacteriales	Weeksellaceae	Chryseobacterium
OTU175	-0.4945	0.511547	FALSE	TRUE	FALSE	Low	-0.10027	Bacteria	Actinobacteria	Actinobacteria	Propionibacteriales	Nocardioidaceae	Kribbella
OTU197	0.457352	-0.47312	TRUE	FALSE	FALSE	High	0.053945	Bacteria	Proteobacteria	Alphaproteobacteria	Caulobacterales	Caulobacteraceae	Brevundimonas
OTU2005	0.4609	-0.47679	TRUE	FALSE	FALSE	High	0.058369	Bacteria	Firmicutes	Bacilli	Bacillales	Paenibacillaceae	Paenibacillus

OTU2022	-0.46176	0.477683	FALSE	TRUE	FALSE	Low	-0.05944	Bacteria	Firmicutes	Clostridia	Clostridiales	Peptostreptococcaceae	Paeniclostridium
OTU210	0.620506	-0.6419	TRUE	FALSE	FALSE	High	0.25741	Bacteria	Actinobacteria	Thermoleophilia	Gaiellales	Gaiellaceae	Gaiella
OTU2120	0.462074	-0.47801	TRUE	FALSE	FALSE	High	0.059833	Bacteria	Proteobacteria	Alphaproteobacteria	Sphingomonadales	Sphingomonadaceae	Sphingorhabdus
OTU2123	0.425441	-0.44011	TRUE	FALSE	FALSE	High	0.01415	Bacteria	Bacteroidetes	Bacteroidia	Chitinophagales	Chitinophagaceae	NA
OTU213	0.522131	-0.54014	TRUE	FALSE	FALSE	High	0.134729	Bacteria	Bacteroidetes	Bacteroidia	Flavobacteriales	Weeksellaceae	Chryseobacterium
OTU22	-0.62515	0.646709	FALSE	TRUE	FALSE	Low	-0.2632	Bacteria	Firmicutes	Bacilli	Bacillales	Bacillaceae	NA
OTU235	0.457974	-0.47377	TRUE	FALSE	FALSE	High	0.05472	Bacteria	Proteobacteria	Gammaproteobacteria	Pseudomonadales	Moraxellaceae	NA
OTU2366	-0.43928	0.454422	FALSE	TRUE	FALSE	Low	-0.0314	Bacteria	Firmicutes	Bacilli	Bacillales	Paenibacillaceae	Paenibacillus
OTU240	0.461943	-0.47787	TRUE	FALSE	FALSE	High	0.05967	Bacteria	Proteobacteria	Alphaproteobacteria	Caulobacterales	Caulobacteraceae	Brevundimonas
OTU241	0.566333	-0.58586	TRUE	FALSE	FALSE	High	0.189852	Bacteria	Planctomycetes	Planctomycetacia	Planctomycetales	Rubinisphaeraceae	SH-PL14
OTU248	0.420403	-0.4349	TRUE	FALSE	FALSE	High	0.007867	Bacteria	Actinobacteria	Actinobacteria	Micrococcales	Cellulomonadaceae	Cellulomonas
OTU249	0.415936	-0.43028	TRUE	FALSE	FALSE	High	0.002296	Bacteria	Proteobacteria	Alphaproteobacteria	Rhizobiales	Devosiaceae	Devosia
OTU254	0.421829	-0.43637	TRUE	FALSE	FALSE	High	0.009645	Bacteria	Bacteroidetes	Bacteroidia	Sphingobacteriales	Sphingobacteriaceae	Sphingobacterium
OTU259	-0.46653	0.482613	FALSE	TRUE	FALSE	Low	-0.06539	Bacteria	Actinobacteria	Thermoleophilia	Solirubrobacterales	67-14	NA
OTU26	-0.45439	0.470055	FALSE	TRUE	FALSE	Low	-0.05025	Bacteria	Actinobacteria	Actinobacteria	Micrococcales	Brevibacteriaceae	Brevibacterium

OTU266	0.494341	-0.51139	TRUE	FALSE	FALSE	High	0.100073	Bacteria	Proteobacteria	Gammaproteobacteria	Steroidobacterales	Steroidobacteraceae	Steroidobacter
OTU27	0.513448	-0.53115	TRUE	FALSE	FALSE	High	0.123901	Bacteria	Actinobacteria	Actinobacteria	Pseudonocardiales	Pseudonocardiaceae	Lechevalieria
OTU287	0.53635	-0.55484	TRUE	FALSE	FALSE	High	0.152461	Bacteria	Proteobacteria	Alphaproteobacteria	Sphingomonadales	Sphingomonadaceae	Altererythrobacter
OTU3	-0.60621	0.627113	FALSE	TRUE	FALSE	Low	-0.23958	Bacteria	Actinobacteria	Actinobacteria	Micrococcales	Micrococcaceae	Pseudarthrobacter
OTU328	0.453205	-0.46883	TRUE	FALSE	FALSE	High	0.048774	Bacteria	Proteobacteria	Alphaproteobacteria	Sphingomonadales	Sphingomonadaceae	Sphingomonas
OTU371	-0.54689	0.565753	FALSE	TRUE	FALSE	Low	-0.16561	Bacteria	Proteobacteria	Gammaproteobacteria	Betaproteobacteriales	Burkholderiaceae	Variovorax
OTU38	-0.42525	0.439918	FALSE	TRUE	FALSE	Low	-0.01392	Bacteria	Verrucomicrobia	Verrucomicrobiae	Chthoniobacterales	Chthoniobacteraceae	Candidatus_Udaeobacter
OTU388	-0.43633	0.451372	FALSE	TRUE	FALSE	Low	-0.02772	Bacteria	Actinobacteria	Actinobacteria	Micrococcales	Intrasporangiaceae	Oryzihumus
OTU396	0.422862	-0.43744	TRUE	FALSE	FALSE	High	0.010933	Bacteria	Bacteroidetes	Bacteroidia	Flavobacteriales	Weeksellaceae	Chryseobacterium
OTU40	-0.42201	0.436566	FALSE	TRUE	FALSE	Low	-0.00988	Bacteria	Actinobacteria	Actinobacteria	Micrococcales	Micrococcaceae	Paenarthrobacter
OTU406	0.531302	-0.54962	TRUE	FALSE	FALSE	High	0.146166	Bacteria	Bacteroidetes	Bacteroidia	Chitinophagales	Chitinophagaceae	Terrimonas
OTU435	-0.47056	0.486782	FALSE	TRUE	FALSE	Low	-0.07041	Bacteria	Firmicutes	Bacilli	Bacillales	Planococcaceae	Sporosarcina
OTU448	0.478162	-0.49465	TRUE	FALSE	FALSE	High	0.079896	Bacteria	Bacteroidetes	Bacteroidia	Chitinophagales	Chitinophagaceae	Taibaiella
OTU449	0.415224	-0.42954	TRUE	FALSE	FALSE	High	0.001408	Bacteria	Actinobacteria	Actinobacteria	Micrococcales	Microbacteriaceae	Pseudoclavibacter

OTU468	-0.48364	0.500321	FALSE	TRUE	FALSE	Low	-0.08673	Bacteria	Bacteroidetes	Bacteroidia	Sphingobacteriales	Sphingobacteriaceae	Mucilaginibacter
OTU476	-0.51658	0.534393	FALSE	TRUE	FALSE	Low	-0.12781	Bacteria	Firmicutes	Bacilli	Bacillales	Paenibacillaceae	Ammoniphilus
OTU49	-0.51474	0.53249	FALSE	TRUE	FALSE	Low	-0.12551	Bacteria	Firmicutes	Bacilli	Bacillales	Bacillaceae	NA
OTU493	0.428223	-0.44299	TRUE	FALSE	FALSE	High	0.017619	Bacteria	Proteobacteria	Gammaproteobacteria	Betaproteobacteriales	Burkholderiaceae	NA
OTU553	0.566683	-0.58622	TRUE	FALSE	FALSE	High	0.190288	Bacteria	Bacteroidetes	Bacteroidia	Cytophagales	Microscillaceae	NA
OTU565	-0.4473	0.462727	FALSE	TRUE	FALSE	Low	-0.04141	Bacteria	Actinobacteria	Actinobacteria	Propionibacteriales	Nocardioidaceae	Marmoricola
OTU589	0.430213	-0.44505	TRUE	FALSE	FALSE	High	0.020101	Bacteria	Chloroflexi	Chloroflexia	Thermomicrobiales	JG30-KF-CM45	NA
OTU608	0.480535	-0.4971	TRUE	FALSE	FALSE	High	0.082855	Bacteria	Actinobacteria	Acidimicrobiia	Microtrichales	Ilumatobacteraceae	NA
OTU613	-0.46569	0.481747	FALSE	TRUE	FALSE	Low	-0.06434	Bacteria	Actinobacteria	Actinobacteria	Micrococcales	Intrasporangiaceae	Phycicoccus
OTU614	0.475353	-0.49174	TRUE	FALSE	FALSE	High	0.076393	Bacteria	Proteobacteria	Alphaproteobacteria	Sphingomonadales	Sphingomonadaceae	Sphingopyxis
OTU617	0.494215	-0.51126	TRUE	FALSE	FALSE	High	0.099916	Bacteria	Proteobacteria	Alphaproteobacteria	Acetobacterales	Acetobacteraceae	Roseomonas
OTU620	0.49016	-0.50706	TRUE	FALSE	FALSE	High	0.094858	Bacteria	Chloroflexi	Chloroflexia	Thermomicrobiales	JG30-KF-CM45	NA
OTU633	0.421663	-0.4362	TRUE	FALSE	FALSE	High	0.009438	Bacteria	Proteobacteria	Gammaproteobacteria	Betaproteobacteriales	Burkholderiaceae	Polaromonas
OTU64	0.500504	-0.51776	TRUE	FALSE	FALSE	High	0.107759	Bacteria	Proteobacteria	Alphaproteobacteria	Sphingomonadales	Sphingomonadaceae	Sphingomonas
OTU640	0.47849	-0.49499	TRUE	FALSE	FALSE	High	0.080306	Bacteria	Firmicutes	Bacilli	Bacillales	Paenibacillaceae	Paenibacillus

OTU66	0.666149	-0.68912	TRUE	FALSE	FALSE	High	0.31433	Bacteria	Proteobacteria	Gammaproteobacteria	Xanthomonadales	Xanthomonadaceae	Pseudoxanthomonas
OTU678	-0.42324	0.437834	FALSE	TRUE	FALSE	Low	-0.0114	Bacteria	Actinobacteria	Actinobacteria	Micrococcales	Micrococcaceae	Arthrobacter
OTU70	0.435955	-0.45099	TRUE	FALSE	FALSE	High	0.027261	Bacteria	Proteobacteria	Alphaproteobacteria	Caulobacterales	Caulobacteraceae	Brevundimonas
OTU74	-0.46922	0.485398	FALSE	TRUE	FALSE	Low	-0.06874	Bacteria	Actinobacteria	Actinobacteria	Micrococcales	Dermabacteraceae	Brachybacterium
OTU755	0.415037	-0.42935	TRUE	FALSE	FALSE	High	0.001175	Bacteria	Bacteroidetes	Bacteroidia	Flavobacteriales	Weeksellaceae	Chryseobacterium
OTU764	-0.51819	0.536056	FALSE	TRUE	FALSE	Low	-0.12981	Bacteria	Firmicutes	Bacilli	Bacillales	Planococcaceae	Lysinibacillus
OTU789	0.453513	-0.46915	TRUE	FALSE	FALSE	High	0.049158	Bacteria	Actinobacteria	Actinobacteria	Pseudonocardiales	Pseudonocardiaceae	Allokutzneria
OTU793	0.425375	-0.44004	TRUE	FALSE	FALSE	High	0.014067	Bacteria	Bacteroidetes	Bacteroidia	Cytophagales	Spirosomaceae	Dyadobacter
OTU796	0.462753	-0.47871	TRUE	FALSE	FALSE	High	0.060681	Bacteria	Proteobacteria	Gammaproteobacteria	Xanthomonadales	Xanthomonadaceae	Stenotrophomonas
OTU856	0.433782	-0.44874	TRUE	FALSE	FALSE	High	0.024552	Bacteria	Proteobacteria	Alphaproteobacteria	Azospirillales	Azospirillaceae	Azospirillum
OTU888	0.425873	-0.44056	TRUE	FALSE	FALSE	High	0.014688	Bacteria	Proteobacteria	Alphaproteobacteria	Rhizobiales	Beijerinckiaceae	Methylobacterium
OTU916	0.414177	-0.42846	TRUE	FALSE	FALSE	High	0.000103	Bacteria	Proteobacteria	Alphaproteobacteria	Sphingomonadales	Sphingomonadaceae	Sphingomonas
OTU952	0.419104	-0.43356	TRUE	FALSE	FALSE	High	0.006247	Bacteria	Bacteroidetes	Bacteroidia	Cytophagales	Spirosomaceae	Dyadobacter
OTU96	0.547326	-0.5662	TRUE	FALSE	FALSE	High	0.166149	Bacteria	Actinobacteria	Actinobacteria	Corynebacteriales	Mycobacteriaceae	Mycobacterium
OTU980	-0.44234	0.457591	FALSE	TRUE	FALSE	Low	-0.03522	Bacteria	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	Lachnoclostridium_5

Table S6: SPLSDA components of bacterial taxa in 2019 soil.

OTU	High	Low	Contrib. High	Contrib. Low	Contrib	GroupContrib	importance	Kingdom	Phylum	Class	Order	Family	Genus
OTU1001	-0.57474	0.599725	FALSE	TRUE	FALSE	Low	0.020973	Bacteria	Proteobacteria	Gammaproteobacteria	Xanthomonadales	Rhodanobacteraceae	Dokdonella
OTU1013	-0.58466	0.610076	FALSE	TRUE	FALSE	Low	0.041271	Bacteria	Planctomycetes	Planctomycetacia	Isosphaerales	Isosphaeraceae	NA
OTU1034	0.581928	-0.60723	TRUE	FALSE	FALSE	High	-0.03569	Bacteria	Verrucomicrobia	Verrucomicrobiae	Chthoniobacterales	Chthoniobacteraceae	Chthoniobacter
OTU1086	0.567742	-0.59243	TRUE	FALSE	FALSE	High	-0.00666	Bacteria	Bacteroidetes	Bacteroidia	Chitinophagales	Chitinophagaceae	Taibaiella
OTU1168	-0.59929	0.625346	FALSE	TRUE	FALSE	Low	0.071216	Bacteria	Acidobacteria	Acidobacteriia	Acidobacteriales	Acidobacteriaceae_(Subgroup_1)	Occallatibacter
OTU117	0.600977	-0.62711	TRUE	FALSE	FALSE	High	-0.07467	Bacteria	Bacteroidetes	Bacteroidia	Cytophagales	Microscillaceae	Chryseolinea
OTU118	-0.624	0.651134	FALSE	TRUE	FALSE	Low	0.121787	Bacteria	Proteobacteria	Alphaproteobacteria	Sphingomonadales	Sphingomonadaceae	Sphingomonas
OTU120	0.601974	-0.62815	TRUE	FALSE	FALSE	High	-0.07671	Bacteria	Bacteroidetes	Bacteroidia	Cytophagales	Hymenobacteraceae	Adhaeribacter
OTU1276	-0.59781	0.6238	FALSE	TRUE	FALSE	Low	0.068185	Bacteria	Planctomycetes	Planctomycetacia	Pirellulales	Pirellulaceae	NA
OTU1316	0.603909	-0.63017	TRUE	FALSE	FALSE	High	-0.08067	Bacteria	Verrucomicrobia	Verrucomicrobiae	Verrucomicrobiales	Verrucomicrobiaceae	NA
OTU133	0.613149	-0.63981	TRUE	FALSE	FALSE	High	-0.09958	Bacteria	Actinobacteria	Actinobacteria	Micrococcales	Microbacteriaceae	Agromyces

OTU1352	-0.56975	0.594526	FALSE	TRUE	FALSE	Low	0.010777	Bacteria	Actinobacteria	Actinobacteria	Streptosporangiales	Streptosporangiaceae	Streptosporangium
OTU156	0.610579	-0.63713	TRUE	FALSE	FALSE	High	-0.09432	Bacteria	Acidobacteria	Blastocatellia_(Subgroup_4)	Pyrinomonadales	Pyrinomonadaceae	RB41
OTU158	0.642088	-0.67	TRUE	FALSE	FALSE	High	-0.15879	Bacteria	Gemmatimonadetes	Gemmatimonadetes	Gemmatimonadales	Gemmatimonadaceae	NA
OTU160	-0.62679	0.654043	FALSE	TRUE	FALSE	Low	0.127492	Bacteria	Actinobacteria	Actinobacteria	Micrococcales	Intrasporangiaceae	Terrabacter
OTU164	0.581948	-0.60725	TRUE	FALSE	FALSE	High	-0.03573	Bacteria	Verrucomicrobia	Verrucomicrobiae	Pedosphaerales	Pedosphaeraceae	NA
OTU169	0.625765	-0.65297	TRUE	FALSE	FALSE	High	-0.12539	Bacteria	Proteobacteria	Gammaproteobacteria	Gammaproteobacteria_Incertae_Sedis	Unknown_Family	Acidibacter
OTU191	0.625606	-0.65281	TRUE	FALSE	FALSE	High	-0.12507	Bacteria	Proteobacteria	Gammaproteobacteria	Steroidobacterales	Steroidobacteraceae	NA
OTU210	0.593012	-0.61879	TRUE	FALSE	FALSE	High	-0.05837	Bacteria	Actinobacteria	Thermoleophilia	Gaiellales	Gaiellaceae	Gaiella
OTU22	-0.63823	0.665978	FALSE	TRUE	FALSE	Low	0.150898	Bacteria	Firmicutes	Bacilli	Bacillales	Bacillaceae	NA
OTU228	0.635147	-0.66276	TRUE	FALSE	FALSE	High	-0.14459	Bacteria	Proteobacteria	Gammaproteobacteria	Gammaproteobacteria_Incertae_Sedis	Unknown_Family	Acidibacter
OTU259	-0.60961	0.636118	FALSE	TRUE	FALSE	Low	0.092342	Bacteria	Actinobacteria	Thermoleophilia	Solirubrobacterales	67-14	NA
OTU285	-0.61033	0.636869	FALSE	TRUE	FALSE	Low	0.093813	Bacteria	Proteobacteria	Alphaproteobacteria	Rhizobiales	Xanthobacteraceae	Pseudolabrys
OTU288	0.591638	-0.61736	TRUE	FALSE	FALSE	High	-0.05556	Bacteria	Actinobacteria	Acidimicrobiia	Microtrichales	Iamiaceae	Iamia
OTU305	-0.57715	0.602246	FALSE	TRUE	FALSE	Low	0.025917	Bacteria	Acidobacteria	Acidobacteriia	Solibacterales	Solibacteraceae_(Subgroup_3)	Candidatus_Solibacter
OTU337	0.690017	-0.72002	TRUE	FALSE	FALSE	High	-0.25687	Bacteria	Proteobacteria	Alphaproteobacteria	Sphingomonadales	Sphingomonadaceae	Altererythrobacter

OTU352	-0.56521	0.589782	FALSE	TRUE	FALSE	Low	0.001474	Bacteria	Proteobacteria	Gammaproteobacteria	Betaproteobacteriales	A21b	NA
OTU358	-0.5679	0.592588	FALSE	TRUE	FALSE	Low	0.006976	Bacteria	Actinobacteria	Actinobacteria	Corynebacteriales	Mycobacteriaceae	Mycobacterium
OTU378	-0.58111	0.606375	FALSE	TRUE	FALSE	Low	0.034013	Bacteria	Verrucomicrobia	Verrucomicrobiae	Chthoniobacterales	Chthoniobacteraceae	Candidatus_Udaeobacter
OTU38	-0.59992	0.626005	FALSE	TRUE	FALSE	Low	0.072509	Bacteria	Verrucomicrobia	Verrucomicrobiae	Chthoniobacterales	Chthoniobacteraceae	Candidatus_Udaeobacter
OTU388	-0.62988	0.657271	FALSE	TRUE	FALSE	Low	0.133823	Bacteria	Actinobacteria	Actinobacteria	Micrococcales	Intrasporangiaceae	Oryzihumus
OTU428	-0.62304	0.650128	FALSE	TRUE	FALSE	Low	0.119816	Bacteria	Firmicutes	Bacilli	Bacillales	Bacillaceae	Bacillus
OTU431	-0.6274	0.654674	FALSE	TRUE	FALSE	Low	0.12873	Bacteria	Actinobacteria	Actinobacteria	Propionibacteriales	Nocardioidaceae	Nocardioides
OTU435	-0.6247	0.651858	FALSE	TRUE	FALSE	Low	0.123208	Bacteria	Firmicutes	Bacilli	Bacillales	Planococcaceae	Sporosarcina
OTU440	0.57491	-0.59991	TRUE	FALSE	FALSE	High	-0.02133	Bacteria	Proteobacteria	Gammaproteobacteria	Xanthomonadales	Xanthomonadaceae	Lysobacter
OTU460	-0.57049	0.595296	FALSE	TRUE	FALSE	Low	0.012286	Bacteria	Proteobacteria	Alphaproteobacteria	Rhizobiales	Xanthobacteraceae	NA
OTU476	-0.63264	0.660144	FALSE	TRUE	FALSE	Low	0.139456	Bacteria	Firmicutes	Bacilli	Bacillales	Paenibacillaceae	Ammoniphilus
OTU489	-0.57449	0.599468	FALSE	TRUE	FALSE	Low	0.020469	Bacteria	Verrucomicrobia	Verrucomicrobiae	Chthoniobacterales	Chthoniobacteraceae	Candidatus_Udaeobacter
OTU49	-0.70814	0.738923	FALSE	TRUE	FALSE	Low	0.293947	Bacteria	Firmicutes	Bacilli	Bacillales	Bacillaceae	NA
OTU491	-0.59729	0.623258	FALSE	TRUE	FALSE	Low	0.067122	Bacteria	Verrucomicrobia	Verrucomicrobiae	Pedosphaerales	Pedosphaeraceae	ADurb.Bin063-1

OTU495	-0.57416	0.599127	FALSE	TRUE	FALSE	Low	0.019799	Bacteria	Proteobacteria	Alphaproteobacteria	Acetobacterales	Acetobacteraceae	Acidicaldus
OTU51	0.662108	-0.6909	TRUE	FALSE	FALSE	High	-0.19976	Bacteria	Proteobacteria	Gammaproteobacteria	Betaproteobacteriales	Burkholderiaceae	NA
OTU510	0.569647	-0.59441	TRUE	FALSE	FALSE	High	-0.01056	Bacteria	Gemmatimonadetes	Gemmatimonadetes	Gemmatimonadales	Gemmatimonadaceae	NA
OTU52	-0.65289	0.681277	FALSE	TRUE	FALSE	Low	0.180899	Bacteria	Firmicutes	Bacilli	Bacillales	Planococcaceae	NA
OTU546	0.590302	-0.61597	TRUE	FALSE	FALSE	High	-0.05282	Bacteria	Bacteroidetes	Bacteroidia	Chitinophagales	Chitinophagaceae	NA
OTU613	-0.6297	0.657078	FALSE	TRUE	FALSE	Low	0.133444	Bacteria	Actinobacteria	Actinobacteria	Micrococcales	Intrasporangiaceae	Phycicoccus
OTU647	0.58998	-0.61563	TRUE	FALSE	FALSE	High	-0.05216	Bacteria	Bacteroidetes	Bacteroidia	Chitinophagales	Chitinophagaceae	Parasegetibacter
OTU670	-0.57142	0.596262	FALSE	TRUE	FALSE	Low	0.014181	Bacteria	Actinobacteria	Thermoleophilia	Gaiellales	Gaiellaceae	Gaiella
OTU709	-0.56741	0.592078	FALSE	TRUE	FALSE	Low	0.005976	Bacteria	Chloroflexi	Ktedonobacteria	Ktedonobacterales	JG30-KF-AS9	NA
OTU725	-0.56483	0.58939	FALSE	TRUE	FALSE	Low	0.000704	Bacteria	Gemmatimonadetes	Gemmatimonadetes	Gemmatimonadales	Gemmatimonadaceae	Gemmatimonas
OTU73	-0.6406	0.668451	FALSE	TRUE	FALSE	Low	0.155748	Bacteria	Proteobacteria	Alphaproteobacteria	Rhizobiales	Xanthobacteraceae	Bradyrhizobium
OTU764	-0.63065	0.658065	FALSE	TRUE	FALSE	Low	0.135379	Bacteria	Firmicutes	Bacilli	Bacillales	Planococcaceae	Lysinibacillus
OTU81	0.5997	-0.62577	TRUE	FALSE	FALSE	High	-0.07205	Bacteria	Acidobacteria	Blastocatellia_(Subgroup_4)	Pyrinomonadales	Pyrinomonadaceae	RB41
OTU882	-0.58217	0.607482	FALSE	TRUE	FALSE	Low	0.036183	Bacteria	Proteobacteria	Alphaproteobacteria	Rhizobiales	Rhodomicrobiaceae	Rhodomicrobium

OTU884	-0.58147	0.606754	FALSE	TRUE	FALSE	Low	0.034756	Bacteria	Actinobacteria	Thermoleophilia	Solirubrobacterales	67-14	NA	

Corresponding address for this report:
Postbus 16
6700 AA Wageningen
T 0317 48 07 00
www.wur.nl/plant-research

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