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Maastricht University

Faculty of Medicine and Life Sciences
School for Life Sciences
Master of Biomedical Sciences

Master's thesis

Exploring the impact of plant density on hemp microbiome composition

Wannes Dupont

Thesis presented in fulfillment of the requirements for the degree of Master of Biomedical Sciences, specialization Molecular Mechanisms in Health and Disease

SUPERVISOR :

dr. Sofie THIJS

MENTOR :

Mevrouw Leila PAQUAY

2020
2021

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The impact of plant density on hemp microbiome composition

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The impact of plant density on the hemp microbiome

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Keywords: *Cannabis sativa, microbiome, growth promotion, 16S*

ABSTRACT

Hemp is acknowledged as a sustainable source of natural fiber with multiple uses. However, previous research has shown a large variety of fiber quality and yield between and within the field. We want to increase yield and quality while aiming for a net durable solution. Past research indicates that higher plant density is associated with higher fiber quality and a higher yield efficiency. Additionally it has been proven that the microbiome of plants has the potential to make agriculture more effective and sustainable overall. This is largely attributed to three effects: increased nutrient uptake, protection against pathogens, and affecting plant development. Within a plant microbiome different compartments are characterized with distinctive microbiome compositions with potentially different physiology. Until now, no research has been conducted to associate plant density and microbiome composition in *Cannabis sativa.* In this project, we set up an experiment with 2 varying plant density (1 plant per pot, and 6 plants per pot) to study the impact of plant density on microbiome composition and plant growth. Our data did not measure a significant difference in physical parameters for plants from different density treatments. However the data does indicate that plant density shifts microbiome composition within the root endosphere. This research forms the basis for more mechanistic understanding using manipulative experiments

Introduction

Cannabis sativa is a plant with multiple industrial features and is referred to as hemp when it is cultivated for industrial purposes. Hemp fiber is a sustainable source of natural fiber that can be used for textile, bioplastics and many other applications. Shivs, the wooden core of the stem, is used for animal bedding and construction material. Hemps also have favorable properties for biofuel production. Cannabis seeds have an interesting nutritional composition as they consist of 30% oil and 25% protein and contain dietary fibers, vitamins, and minerals. Additionally, hemp produces essential oils (cannabinoids) of which some are proven to have therapeutic uses(1, 2). The rationale of hemp as a sustainable source for natural fiber is largely contributed to the limited requirements to grow including irrigation, fertilization, soil consistency and fast plant growth compared to other fiber sources such as cotton. Hemp fiber contains a high level of cellulose making it an exceptional strong natural fiber. Within hemp fiber two types of fiber can be distinguished, primary and secondary fibers. The primary fibers are developed during the lengthening of the stem (primary growth) and originate from near the top of the plant. The secondary fibers are developed during the thickening of the stem (secondary growth) and are significantly shorter and contain more lignin than primary fiber. These two fibers have different uses. The longer primary fiber is mainly used in the textile industry, while the shorter secondary fiber is suitable for other industries like the paper industry. The yield and quality of both types is heavily reliant on the growth conditions. For example, during a period of drought

shorter primary fibers are developed and as consequence thinner fiber bundles are established. Because the primary fiber is only developed during the stem lengthening process this 'weak' spot will persist throughout the plant's development (3). Another variable impacting primary fiber quality is plant density. At higher plant density the average stem thickness decreases and average stem length increases. Both of these factors favor primary fiber production. Therefore, a high plant density is associated with high-quality long primary fibers(4, 5). Although different variables have been associated with fiber development, the extent of their impact and the mechanism by which they impact the fiber development are still unclear(5).

Previous research has shown that the plant microbiome has an influence on plant development and health. The microbiome refers to a microbial community in a well-defined habitat. It includes both the microorganisms as their physiology (6). The plant microbiome is the collection of all microorganisms associated with that particular plant. Numerous research has shown that the plants' associated microbiome influence the plant development by providing increased mineral nutrient uptake, by protecting plants against pathogens and by producing substances like plant hormones or secondary metabolites that affect plant development directly which among others results in better coping with abiotic and biotic stress (7, 8). For example, nitrogen fixating rhizobacteria of soybean provide the plant with an accessible source of nitrogen (9). Folman et al. reported that *Lysobacter spp.* was efficient in controlling soil-borne pathogens by producing extracellular enzymes and other metabolites (10). different species of Basidiomycota, Oomycota, and Ascomycota are characterized to promote plant growth and protect plants under peculiar and abiotic stress circumstances.(11). **Microbiome-based agricultural strategy and products can therefore provide a sustainable solution to improve agricultural output.** One such product is a biofertilizer. It contains a variety of microbes that through their metabolic activities mobilize nutrients and make these nutrients more accessible for plants (12). This idea is not new, already in 1895, a biofertilizer "Nitragin", containing mainly nitrogen fixating taxa, was commercialized. At present, biofertilizers are a fundamental part of the current organic agriculture (13).

Within a plant microbiome, different compartments are recognized associated with the plant compartment or tissue that creates a micro-habitat for a certain microbial community. The composition of the community from different compartments varies due to niche differentiation (8). A widely recognized compartment is the rhizosphere. The rhizosphere refers to the area of soil closely surrounding the root tissue inhabited by microbes (fig. 1). Plant roots release chemicals that affect the physical and chemical characters of the soil and attract microorganisms(14). Plant roots therefore directly influence the composition and diversity of microbial communities around them(15). The rhizosphere is one of the most complex ecosystems, due to the high variance of species and intricate interactions. Some of these bacterial community members have a beneficial effect on the growth and development of plants by direct and/or indirect mechanisms. They are known as plant growth-promoting rhizobacteria (RGPR).

The habitat of microbes within the plant is called the endosphere and the microbes inhabiting it are known as endophytes. Endophytes are found to be ubiquitous and can either have benign or adverse effects on plant health. Some metabolites of endophytes are reported as antifungal and antibacterial chemotherapeutics (8). While other endophyte metabolites induce resistance to hostile environments or promote the pathogen defense system of the host (7). Some of these metabolites are phytohormones that influence the gene expression of the host plant and so impact plant development (16). Specific within hemp microbial community, microbiota have been found that impact secondary metabolite production(17). There is limited knowledge about the microbial community for hemp and to our knowledge no research has studied planting density variation in relation to microbiome community composition and growth promotion. We hypothesize that plant density will influence the microbiome composition in *Cannabis sativa* and that the altered microbiome composition and planting density influence jointly stem thickness in *Cannabis sativa*. In this study, we set up an experiment with two varying plant density (1 plant per pot, and 6 plants per pot). From bottom, middle, and top sections of 70-day-old plants, stem thickness, fresh weight and dry weight were recorded, and a sample collected for NGS sequencing. With the recording of physical

parameters of plants on different density we aim to reproduce previous found association of high density to increased stem length and decreased stem thickness. For the 2nd research question, we seek confirmation of characterization on microbiome compartments. And lastly, we aim to provide data on the impact of plant density on microbiome composition in different compartments. We found that the physical parameters recorded from both sample groups were not significantly different and that plant density impacts the microbiome composition in some compartments. This research forms the basis for more mechanistic understanding using manipulative experiments: e.g., growing in sterile container, same effect?

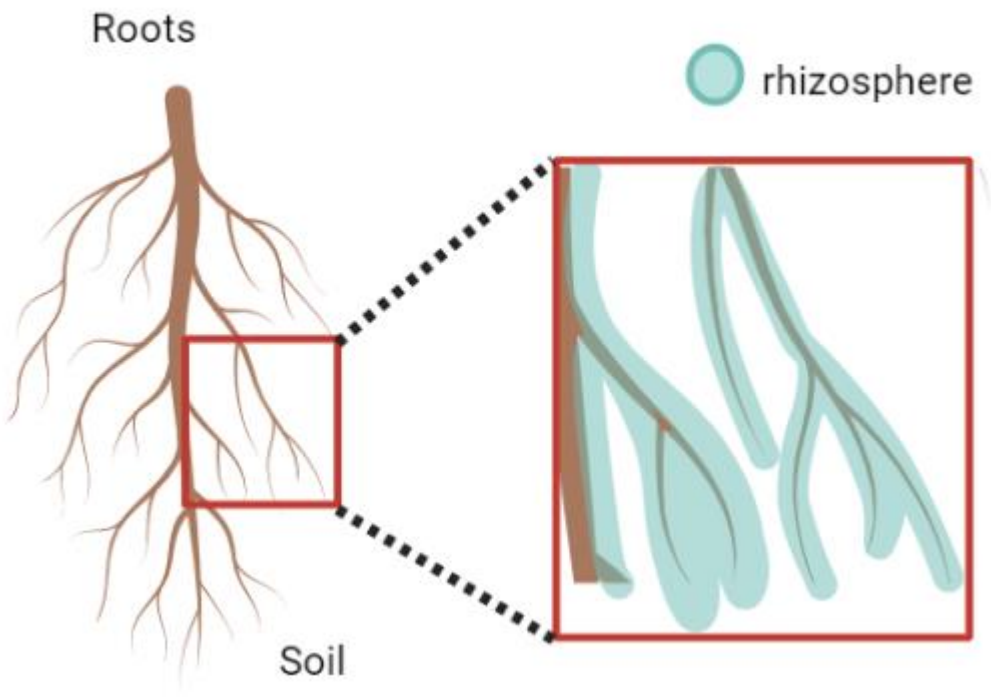

Fig. 1 – Visualization of the rhizosphere

EXPERIMENTAL PROCEDURES

Growing conditions

10 plants of Cannabis sativa (Cultivar: Felina 32) were grown in a controlled environment in growing chambers (conditions summarized in supplement). The growing substrate consisted out of a 4:1 mixture of Bril Argex substrate, day/night temperature of 28/22°C and a photoperiod of 12h. Plants were grown in either 1 plant per pot or 6 plants per pot and harvest took place 70 days after sowing. Six plants per experimental conditions were collected excluding the four outer plants to avoid border effects. However, if a plant had died, it was replaced by one of the outer plants. A general overview of sample processing is provided in fig. 2.

Plant sample collection

At harvest, flowers were removed and used in a parallel experiment. The leaves were separated from the stem and the fresh/dry weight determined. The stem was cut at the soil level to separate the shoot from the roots. A root section of 3 cm was cut starting from the soil level. It was shaken to leave only about 3mm of soil attached and put in a PBS buffer solution. The total stem length was measured, and the number of internodes was counted. After which the stem was cut into different sections: bottom (0-50cm), middle (4cm) and top (54-94cm). The bottom and top sections along with any leftover part were weighted, dried and weighted again to record fresh /dry weight The middle section was cleaned with Milli-Q water and put in a PBS buffer solution.

Surface sterilization and sample preparation before DNA extraction

From each plant sample three microbial samples were isolated: rhizosphere (RHS), root endosphere (ROE) and shoot endosphere (SHE). Rhizosphere samples were obtained by repeated washing steps of the root sections in a PBS solution. The washing buffer was then centrifuged, and the resulting pellet was defined as the rhizosphere. The root sections were sonicated to remove any microbial cells left on their surface. SHE samples consisted of the middle part of the stem. All these samples were flash-frozen in liquid nitrogen and stored at -80°C.

DNA extraction and Illumina sequencing.

DNA extraction from the rhizosphere samples was performed using an optimized version of the RNeasy Powersoil Total RNA protocol. DNA was extracted by chemical and mechanical shredding. Using NucleoMag Beads in a bead solution, with different cell disruptive solutions. Including: NaCl SDS solution (pH=7), aluminum ammonium sulfate dodecahydrate (pH=3.2), 5 M NaCl in 22 mM citric acid anhydrous salt, 29 mM trisodium citrate, dehydrate (pH=5). 250mg of soil was used for each sample. DNA extraction of both root and shoot was performed using the PowerPlant® Pro DNA Isolation Kit. The extraction process similar to rhizosphere DNA extraction but with steel beads and additional DNA quality improving products such as RNAse and 5 M Guanidine hydrochloride, 30 mM Tris, 9% isopropanol (pH 6.8). To target and amplify the V4 region of the 16S small subunit ribosomal gene following primers were chosen. The forward primer (515imp-f-illumad): TCG TCG GCA GCG TCA GAT GTG TAT AAG AGA CAG GTG YCA GCM GCC GCG GTA A. The reversed primer (806imp-r-illumad): GTC TCG TGG GCT CGG AGA TGT GTA TAA GAG ACA GGG ACT ACN VGG GTW TCT AAT. To target and amplify the internal transcribed spacer DNA of fungi following primers were used. The forward primer

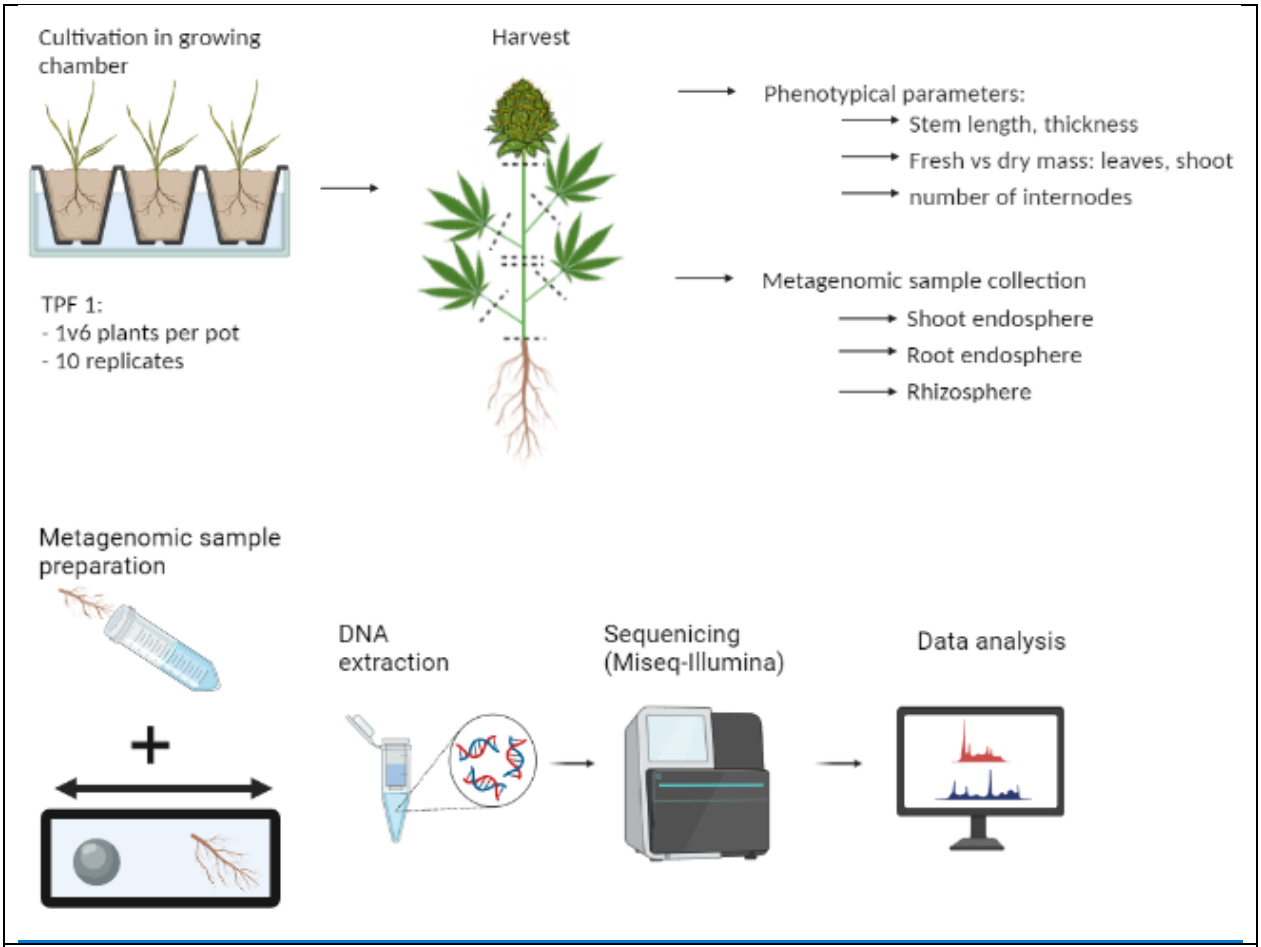

Fig. 1 – Sample processing scheme

(gITS86F): TCG TCG GCA GCG TCA GAT GTG TAT AAG AGA CAG GTG ART CAT CGA RTC TTT GAA. The reversed primer (ITS4R): GTC TCG TGG GCT CGG AGA TGT GTA TAA GAG ACA GTC CTC CGC TTA TTG ATA TGC. Amplification of the samples was executed using a Techne TC-5000 thermocycler (Bibby Scientific Limited, Staffordshire, UK) and under the following conditions: initial denaturation at 98°C for 3min, followed by 30 cycles of denaturation at 98°C for 30s, annealing at 53°C for 30s and extension at 72°C for 30s, with a final extension at 72°C for 7 min. PCR reactions were performed in 25µl reaction volumes using the Q5 Hot Start High-Fidelity DNA Polymerase (NEB, Massachusetts, USA). Each reaction contained 5 x Q5 Reaction buffer, 10 mM dNTPs, 10 µM of each primer, Q5 Hot Start High-Fidelity DNA Polymerase (2U/µl), 1µl of DNA sample. After confirmation by gel electrophoresis and PCR clean-up, a 2nd PCR reaction was performed. This 2nd PCR contained Nextera XT Index Primer 1 (N7xx) and Nextera XT Index Primer 2 (S5xx) in addition to 5 x Q5 Reaction buffer, 10 mM dNTPs, Q5 Hot Start High-Fidelity DNA Polymerase (2U/µl). For root and shoot samples plastid and mitochondrial PNA blockers are added. Results were checked on 1.5% agarose gel before continuing with indexing. Before and after the index PCR the samples were cleaned with AmPureXP magnetic beads (Agencourt, Beverly, MA, USA). The samples were then pooled in equimolar concentrations and sequenced at Biomed (Diepenbeek, BE) using an Illumina Miseq platform.

Bioinformatics sequence analyses

Processing of the metagenomic data was done in R. For the removal of primers and filtering on quality for bacterial reads dada2 package was used. For the fungal reads, cutadapt was used in addition to dada2. To construct AVS table bacterial reads were referenced to the Silva 138.1 16S database and fungal reads to UNITE general FASTA database(18, 19). Further processing was performed by removing contaminants of mitochondrial and chloroplast DNA and Singletons were removed.

analysis of the metagenomic data

With the Divnet alpha diversity was estimated for Shannon and Simpson indices. DivNet assesses the true diversity of an ecosystem by estimating the number of missing species. It then uses a mixed model (a statistical model containing both fixed effects and random effects) to test how much of the variation in alpha diversity estimates between different groups can be explained by the sample groups' membership(20). The Simpson diversity index represents the odds that 2 random individuals from an ecosystem belong to the same species and is mostly influenced by the evenness. The Shannon diversity index represents the uncertainty with which we can predict from which species will be one randomly selected individual in the community. The Shannon index increases with richness and evenness, and it puts more weight on the richness than on evenness and is therefore strongly influenced by rare species. Beta diversity analysis was measured by Bray-Curtis and Unifrac distance. Beta diversity is a measure of the dissimilarity between different samples. Different distance measures exist, including Bray-Curtis and the Unifrac distance. The Bray-Curtis distance is based on the abundance of the different taxa present in the compared samples. Unifrac incorporates the phylogenetic relatedness of those different taxa. Principal Component Analysis (PCoA) is a dimension reduction method that allows to explore and visualize the dissimilarity/similarity between samples. The Adonis test is a permutational multivariate analysis of variance. It uses a linear model to explain the proportion of the variance in a distance matrix that can be imputed to a combination of factors. It makes use of permutations to give a significance level to the results. Samples from the different compartments are clustered together along the 1st PCoa axis. Compositional differences were analyzed using the deseq2 (3.13) R package. Deseq2 does the normalization of counts and divides it by the geometrical mean of all reads. This allows correction for library size and composition bias, which occurs when only a small number of genes are very highly expressed in one experiment condition but not in the other. The output is expressed as 2foldchange to portray overrepresented taxa in sample groups.

RESULTS

Physical parameter evaluation

The physical parameter from each plant at two different density treatments were recorded and

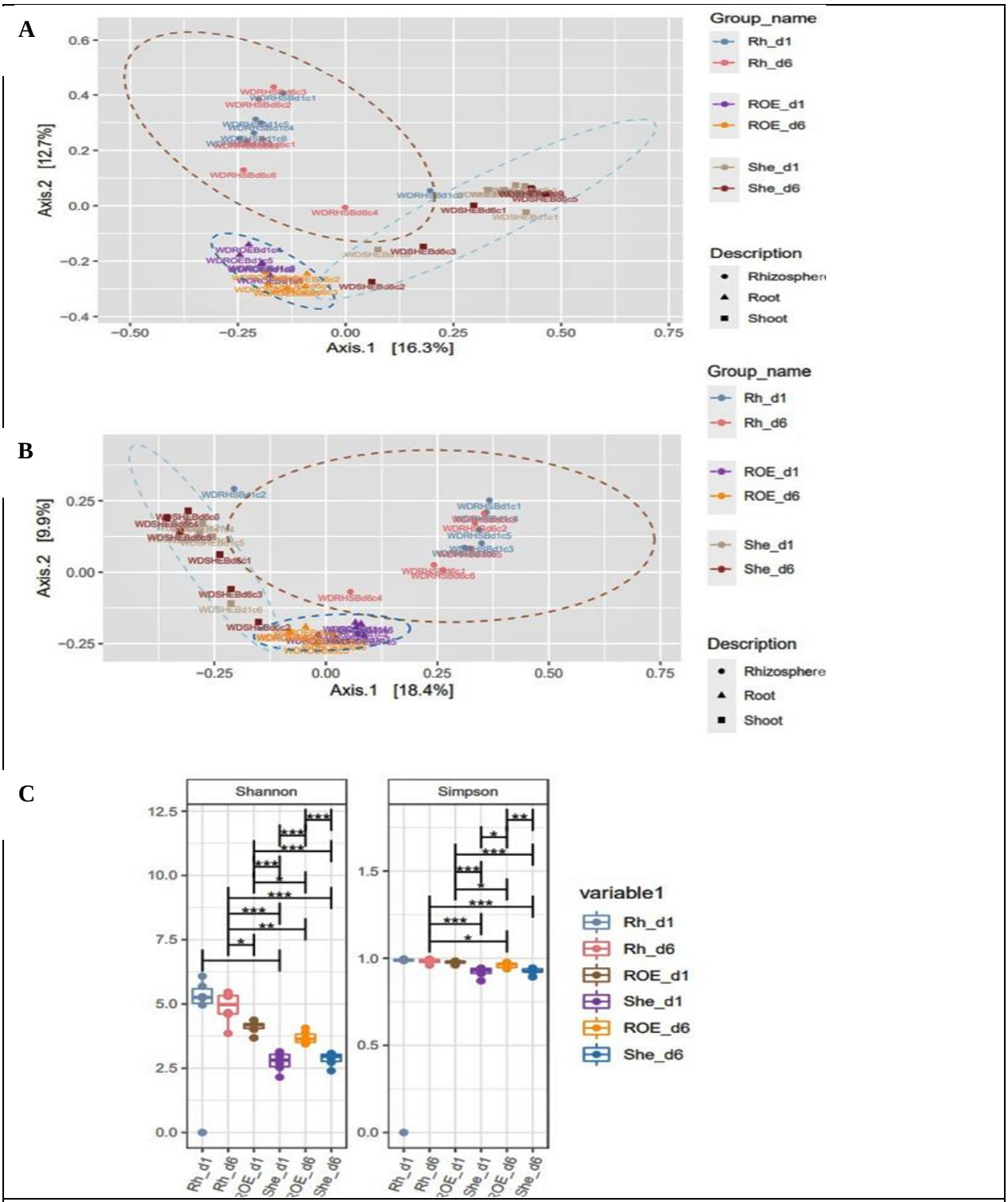

Fig. 3 – Alpha and beta diversity of bacterial communities in different compartments in hemp. **A** PCoA plot on the Bray Curtis dissimilarity between samples from different compartments calculated effect size (R^2) of 0.226 ($p<0.05$). This grouping of samples explains the total variation within the samples for 16.3%. **B** PCoA plot on unifrac distance between samples from different compartments, $R^2 = 0.298$ for unifrac ($p<0.05$). This grouping of samples explains the total variation within the samples for 18.4%. **C** comparison of the average Alpha diversity between bacterial communities from different sample groups. Rh: Rhizosphere sample ROE: Root endosphere sample She: Shoot endosphere sample d1: density 1 plant per pot d6: density 6plants per pot. *: $p<0.05$ **: $p<0.01$ ***: $p<0.001$

evaluated. No significant difference was found for any physical parameter (fig. 3).

Microbiome composition per compartment

To characterize the microbiome compositions from different compartment for bacterial taxa the alpha diversity and beta diversity from every sample was calculated and visualized (fig. 4). For alpha diversity two different indices were calculated, the Shannon and Simpson diversity indices. For both indices a significant difference between communities from different compartments is observed, while no significant differences are observed between communities within the same compartment. With exception to the root endosphere samples, a significant difference was observed between the two density treatments. Beta diversity was calculated with bray-curtis dissimilarity and unweighted unifrac distance. For both parameters samples originating from the same compartment are clustered together regardless of density treatment. The PcoA plot indicates that this specific way of grouping the samples accounts for 16.3 and 18.4% of the total variation for respective bray Curtis and unifrac distance. The clustering was statistically confirmed with an Adonis test, with a calculated effect size (R^2) of 0.226 for bray Curtis ($p<0.05$) and $R^2 = 0.298$ for unifrac ($p<0.05$).

Microbiome composition per density treatment.

For each compartment, the samples from each density treatment, 1 plant per pot or 6 plants per pot, were compared. For the root endosphere samples, alpha diversity was significantly different (fig. 4A). The beta diversity was again measured with Bray-Curtis distance and unifrac distance. For the Adonis test, the R^2 value indicates that at least part of the variation, Bray-Curtis (15,2%, p-value < 0,05) and unifrac (13,8, p-value < 0,01), is explained by the grouping of samples. In the PCoA plots the clustering of samples is illustrated (fig.4B) and a separation of samples by density treatment along the primary axis. Bray-Curtis and unifrac both display a single but different outlier. For rhizosphere no significant difference in composition could be measured for either bacterial or fungal taxa (fig supplemented). To further illustrate the differences between microbiomes the deseq2 package was used to calculate and normalize overrepresented taxa in different sample groups. The Log2 fold change of taxa was defined between samples by density treatment (table 1). The change is expressed as the difference from 6 plants per pot to 1 plant per pot. Also, the log2 fold change normalizes the difference between 2 values, meaning that when a taxon has a higher abundance in 6 plants per pot the log2foldchange is positive. When a taxon has a lower abundance in 6 plants per pot compared to 1 plant per pot, the log2foldchange is negative. In total, 16 taxa were represented significantly differently in the density groups for root endosphere samples. From these taxa, 11 were significantly overrepresented in 6 plants per pot while 5 taxa were overrepresented in 1 plant per pot. The relative abundance for each taxon was presented to characterize samples (fig.5).

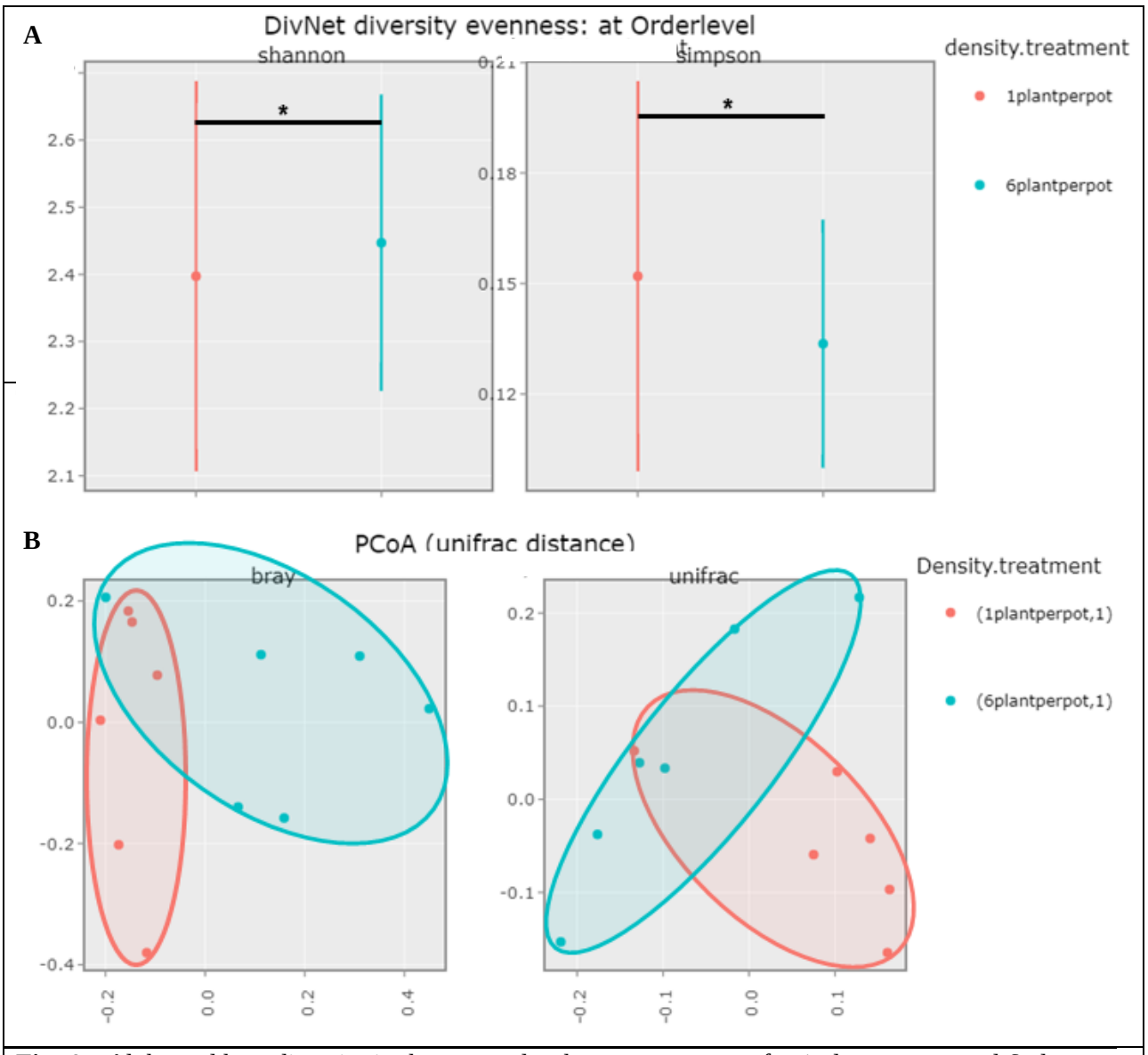

Fig. 4 – Alpha and beta diversity in the root endosphere compartment for 1 plant per pot and 6 plants per pot. **A** alpha diversity metrics Shannon and Simpson index on order taxa level. Both show a significant different alpha diversity between 1 plant per pot and 6 plant per pot ($p<0.05$). **B** beta diversity, samples are clustered by sample group. For Bray Curtis $R^2 = 0.15$ ($p<0.024$) and Unifrac distance $R^2 = 0.14$ ($p<0.004$). *: $p<0.05$.

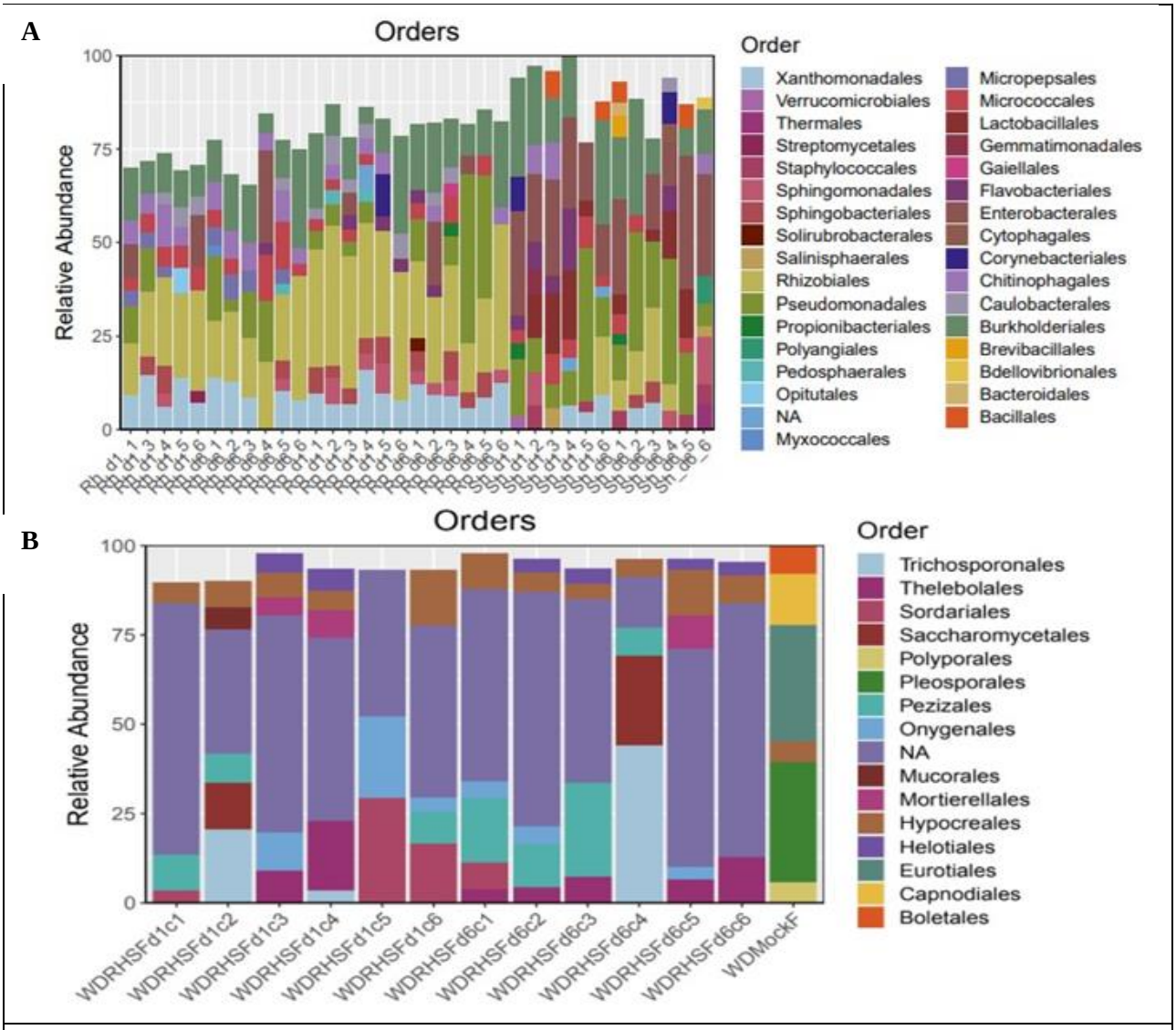

Fig. 5 – Relative abundance of taxa in different compartments of the hemp plants. A single bar represents the microbiome composition from a unique sample. Taxa are on order level classification, taxa < 2% were filtered out. **A** bacterial taxa found in rhizosphere, root and shoot samples. **B** fungal taxa found in rhizosphere samples. NA: Not available, Rh: rhizosphere sample, Ro: Root sample, Sh: shoot sample.

DISCUSSION

Hemp fiber can play an important role in the transition to more sustainable industry. And a more stable and improved quality of the yield of fibers would contribute greatly to this cause. Higher plant density is already associated with a higher efficiency fiber yield and with a higher primary fiber yield(5). Although the mechanism behind it is not yet understood. Until now, no research has associated microbiome composition with an increased plant density in *Cannabis sativa* or associated the microbiome composition with fiber yield and quality. Previous research has shown a wide spectrum of impact on plant health and development associated with the microbiome (8, 21). This research forms the basis for more mechanistic understanding using manipulative experiments. Our data indicates no significant difference in physical parameters for plants from different plant density treatments. Which is contradictive with previous research, where higher density is associated with a smaller stem diameter and higher plant length(5). The microbiome compositions from different compartments are

characterized by both alpha and beta diversity. This is in line with previous research and is likely to be attributed to niche differentiation(6). Further, our data indicates that the rhizosphere microbiome is not significantly impacted by plant density. However, we did observe a significant different microbiome composition in root endosphere due to plant density.

No difference in physical parameters

It was indicated in past studies that high plant density is associated with high-quality fiber due to an increase of plant length and a decrease in stem thickness(4, 5). However, our data could not confirm these findings, no significant difference was found for any physical parameter. Competition between plants is directly related to plant density. With higher plant density, competition between plants for nutrients increases. This causes either an asymptotic or parabolic relationship between plant density and yield. Where a parabolic relationship is caused by a limitation of available nutrients (22). Our results indicate that the competition between plants at a higher density was not a growth limiting factor. Further experimenting under different conditions could provide more insight: e.g., soil types, nutrient availability, higher plant density, etc.

Confirmation on the characterization of microbiome in different compartments.

Our data supports the previous findings that microbiome compositions from different compartments within Cannabis sativa are dissimilar. For the beta diversity analysis, we opted for unweighted unifrac distance instead of weighted. Weighted unifrac takes into account the abundance of a taxa, which makes unweighted unifrac more sensitive for rare species within a sample so small differences could be detected.

Plant density shifts the root endosphere microbiome composition.

The beta diversity analysis showed that the density of the hemp plants impacts the microbiome composition in the root endosphere. We found that multiple taxa overrepresented in the higher density group are related to bacterial taxa with beneficial traits including nutrient mobilization, organic decomposition, pathogen inhibition, etc. (table 2). Further analysis is needed to determine if the found taxa are actually beneficial. For example, taxa like *Pseudomonas* contain species both beneficial and pathogenic. In addition, if this data is cross-referenced with the physical parameters of the plant, it could provide more insight into the actual impact of certain taxa on plant growth and development. The density of the host plant did not significantly contribute to the rhizosphere composition. Previous research has indicated that the foremost factors that impact the microbiome of the rhizosphere are host genotype, historical land use, and soil type (23). Other research has indicated

Table 2 – Overrepresented taxa in the rhizosphere in '6 plant per pot' to '1 plant per pot'

Taxa name	**Taxonomic level**	**Function**
Abditibacterium	Genus	metabolism optimized for survival in low-nutrient habitats, correlated with extreme resistance against antibiotics and toxic compounds. (3)
Acidobacteria	Class	drought tolerance for host plant species; disease suppression; growth promotion and disease inhibition; nitrogen and sulfur metabolism(4)
Actinobacteria	Class	litter degrading, symbiotic, endophytic, or as pathogenic microorganisms(4)
Burkholderiales	order	disease suppression; growth promotion and disease inhibition(4)
Xanthobacteraceae	family	degradation of organic compound(5)
Pseudomonas	Genus	Very diverse: including pathogens and beneficial species (4)
Azospirillum	Genus	Nitrogen fixating (included in biofertilizers)(6)
Sphingomondales	Order	disease suppression; growth promotion and disease inhibition(4)
Massillia	Genus	growth promotion(7)
Rhodospirillales	Order	disease suppression; growth promotion and disease inhibition(4)

that plant growth-promoting taxa derived from the rhizosphere have an essential role in plant health and development. The conclusion that plants density does not impact the rhizosphere microbiome, suggests that microbiome functions like nutrient mobilization are also not impacted. This further indicates that the rhizosphere microbiome is not a limitation on maximum plant density.

Exclusion of the shoot endosphere samples

The shoot endosphere samples were excluded from the analysis due to too low a number of reads. This was the result of the amplification of the plant's mitochondrial and chloroplast DNA which has a bacterial origin. In previous experiments, it was found that the host-derived plastid and mitochondrial sequences can cover up to 95% of all sequenced reads. This would limit the sequencing depth of the target 16S amplification of the bacterial taxa (24). To avoid this, PNA blockers were used. PNA blockers are artificially synthesized peptide nucleic acid oligomers with a high affinity and specificity to a DNA sequence. It is used to correct amplification bias by clamping a DNA sequence essentially hiding it from DNA polymerase. In this project, two blockers were used mPNA and pPNA to mitigate the amplification of mitochondria and chloroplasts. However, in this case, they were ineffective. To ensure that shoot endosphere samples can be incorporated in future analysis the method of blocking chloroplast and mitochondrial DNA has to be addressed. In previous research, the PNA blockers were proven successful for different plants. It is possible that the mitochondrial DNA of *Cannabis sativa* differs more from other cultivated plants and is, therefore, less sensitive for used PNA blockers (25).

Contaminants

Some taxa that were overrepresented in the ‘1 plant per pot’ density treatment group correspond to taxa found in contaminated water including *flaviobacter* and *legionella*. This could be a result of contamination during the sample preparation as the PBS solution was mixed up with milliQ water. When such accidents happen in the future and that it is not possible to obtain new samples, a solution could be to sequence along with the mixed-up solution with the samples to identify the contaminant to be able to exclude them during the bioinformatics preprocessing.

Optimization of R pipeline

The optimization of the R analysis pipeline during this internship will provide a more efficient and uniform way to process and analyze metagenomic data in future projects. The generated data for TPF2 is ready to be processed and analyzed and will further contribute to mapping the hemp microbiome and provide more insight into potential beneficial taxa for increased fiber quality and yield. Moreover, the metagenomic data from TPF1 and TPF2 will be evaluated with the corresponding physical data to find a correlation between taxa and plant health and development, fiber quality, and yield.

Future perspectives

The future perspectives of this project are finding taxa that correlate with increased fiber quality and yield. When such taxa are identified the impact needs to be validated. The most straightforward approach would be to culture the identified taxa and add them on hemp seeds, in the soil or maybe even spray them on the plants. As was shown in the literature, only a fraction of microorganisms can be cultivated in lab conditions which could prove a challenge in the future to validate the isolated impact of taxa but also for a more in-depth study of their physiological and physical properties.

CONCLUSION

Our results indicate that plant density induces a significant change in microbial composition in the root endosphere while the microbial community in the rhizosphere seems unaffected. Within the root endosphere samples at different plant densities several taxa were found to be represented differently. Some of these taxa are in literature associated with growth promotion, however more in-depth analysis is needed to confirm the effect of the different taxa.

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Acknowledgements – I gratefully acknowledge Leila Paquay and dr. Sofie Thijs for helpful comments on the draft of the manuscripts, the critical reflection and discussion on the conceptual framework. I would like to acknowledge the Centre for environmental Sciences (CMK) and more in particular dr. Sofie Thijs and Leila Paquay for the internship opportunity and for the support and directions during the internship.

Author contributions – –ST and LP conceived and designed the research. WD and LP performed experiments and data analysis. WD wrote the paper