Universiteit Utrecht

MAJOR RESEARCH PROJECT REPORT

MICROBIOLOGY WITHIN GREEN ARCHITECTURE

IMPACT OF ACTIVE HYDROPONIC BIOWALLS ON THE INDOOR AIR MICROBIOME

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Abstract

The contemporary shift towards a more sustainable society and built environment has given rise to a trend of retrofitting buildings with vertical plant walls, rooftop- and indoor gardens. This paper addresses the influence of active hydroponic biowalls (kind of vegetation wall) on the indoor aerial microbiome. A slit sampler was used to measure the concentration and species of microbes on LB- and PD agar plates. Experiments were conducted at vegetation dominated buildings (greenhouses of a botanical garden) and in a controlled environment with potted plants. Lastly a custom made biowall was built in the controlled environment to measure its effects on the indoor microbiome. It was expected that buildings with vegetation systems contribute to a rich and stable indoor air microbiome, but also provide more habitat for possible pathogens. The data in this research show that indoor vegetation and biowalls do not significantly increase the microbial load of indoor air. A significant relation was found between the number of microbes and the relative humidity. Further analysis of air-samples using potted plants revealed that two of the four determined organisms were pathogens, although also here the concentrations were not more abundant than the control samples. The built biowall showed oscillating patterns of abundance and diversity in the first days after starting the experiment, but the conditions at day 35 were quite similar to the starting conditions. The data in this research show no potential to ameliorate the indoor microbiome, but do show that biowalls can be safely used, from a microbiological perspective. The outcomes of this research may be significant for further research on the health of indoor air microbiomes, micro biodiversity and the ability of building services to work together with ecological systems.

Layman summary

Fine particles and volatile organic compounds (VOC's) cause poor indoor air quality. These substances are released by building materials, paints, furniture, printers and human activities and cause discomfort, headache and contribute to chronic diseases. A solution for this problem, sometimes called the 'sick building syndrome', is to properly ventilate the building. Due to practical reasons, design choices, maintenance and energy usage, the level of ventilation is often not high enough. Plants can degrade harmful substances and vegetation may therefore be used together with ventilation systems. Enhancing this air cleaning potential, air blowers may be used to generate an airflow through the growth medium of plants. Indoor vertical plant walls that use air blowers are called biowalls. So far, however, the impact of these walls on microbial composition and abundance has hardly been assessed. Therefore, this research seeks to find an answer on what effect indoor vertical plant walls have on the composition of bacteria and fungi in indoor air. Depending on the number and types of bacteria and fungi this answer may be positive, neutral or negative towards the health of humans.

In this research air samples were taken in buildings with a large collection of plants (botanical greenhouses) and in a controlled closed environment (i.e. an incubator) before and after introduction of plants or a custom built biowall. The abundance and diversity of the microbes in the indoor air samples was determined. These data were compared to the soil- and leaf samples of the plants in the case of the incubator experiments. It turned out that relative humidity of indoor air significantly increased fungal spore load. Moreover, abundance and diversity of fungi, yeasts and bacteria in the air increased after placing and watering plants in the incubator but this was a short-term effect. In the experiment with the longest duration the conditions at day 35 were similar to the starting conditions. Together, this study suggests that the indoor air microbiome and abundance is not affected by biowalls. This "not harmful" outcome does not hamper the use of biowalls to improve indoor air quality

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Section 1

Introduction

The world is rapidly urbanizing. It is predicted that 68% of all people across the planet will live in cities by 2050 (United Nations, 2018). People in western societies spend already up to 90% of their time in an Indoor environment (Soreanu, Dixon, & Darlington, 2013). Therefore, benefits derived from being in natural environments, such as sensory input, fresh air, psychological restoration (A. E. van den Berg, Hartig, & Staats, 2007) and microbial uptake and interaction are less experienced by the average citizen. Because of the increasing time the average citizen spends in an indoor environment, it is important to optimize this indoor environment. This fact is recognized by (Bluyssen et al., 2016; Thomsen, Sønderstrup-Andersen, & Müller, 2011) who indicate that Indoor Environmental Quality (IEQ) is able to play an important role in wellbeing and productivity.

Applying living (plant) walls in buildings may contribute to IEQ by ecosystem services like, but not limited to, noise reduction, water recycling, temperature regulation, biodiversity, cleaning air and pollutant removal. The emergence of scientific research and application of living walls for indoor and outdoor purposes, especially in the last decade, sparks the nascent living wall industry. Although their application is not yet reliable and affordable in all situations as of today (Riley, 2017), insights from these developments provide lessons for the current evolving industry. These lessons range from optimizing ecosystem services to maintenance, ecology and suitable construction materials. Vegetation walls may contribute to the sustainability and resiliency of the built environment in the face of climate change and improve the indoor air quality (IAQ).

A large body of research on houseplants and green walls focusses on removal capacity of indoor air pollutants. Around 90% of city dwellers in Europe are exposed to concentrations of pollutants that are higher than the air quality levels deemed harmful to health (European Environmental Agency). Especially Volatile Organic Compounds (VOC's) are often researched. VOC's (s.a. benzene, formaldehyde) can be released by building materials, paints, furniture, printers

and human activities (Gilbert & Stephens, 2018). Together with fine particles, VOC's seem important drivers in causing poor indoor air quality (IAQ), impacting human health through causing discomfort, acute and chronic diseases. The ''Sick Building Syndrome'' (SBS), which entails ocular, nasal, cutaneous irritations, allergies, respiratory dysfunction, head- ache and fatigue is a typical indicator of poor indoor air quality (Soreanu et al., 2013). Ventilation with outdoor air is the main solution to diffuse these high concentrations, however, heating and ventilation of indoor air uses energy (Waring, 2016).

Wolverton et al (1984; 1989; 1997), showed that the microbial communities associated with the rootzone (rhizosphere) in certain common houseplants are able to remove toxins like benzene, formaldehyde and trichloroethylene from the air. The processes involved in air cleaning are called phytoremediation and biofiltration. ''Phytoremediation can be defined as the use of plants to remove pollutants from the air, water and soil. Biofiltration is defined as the process of drawing air through organic material (such as moss, soil and plants), resulting in the removal of organic gases such as VOC's with a mechanical system involved.'' (Moya, van den Dobbelsteen, Ottelé, & Bluyssen, 2018). Pollutants are handled by plants in several ways (figure 1). They may be degraded by microorganisms in the root zone by rhizodegradation or degraded inside plant tissues via enzymatic catalysis. They can be filtered from polluted air or water and accumulate in harvestable tissues by phyto (rhizo) extraction or released from plants by phytovolatisation (via evaporation or plant transpiration). Plants can also be used to stabilize pollutants within a site by limiting erosion, leaching or runoff. (Soreanu et al., 2013)

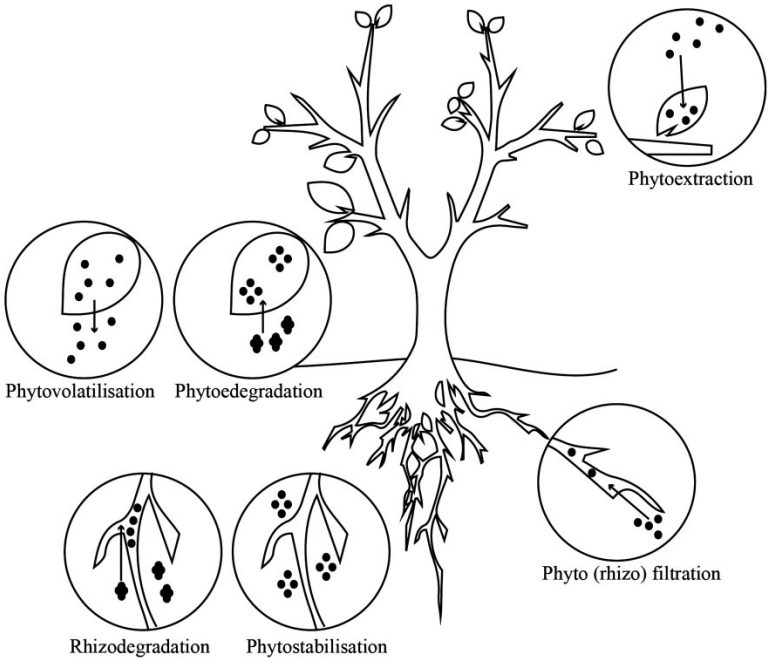
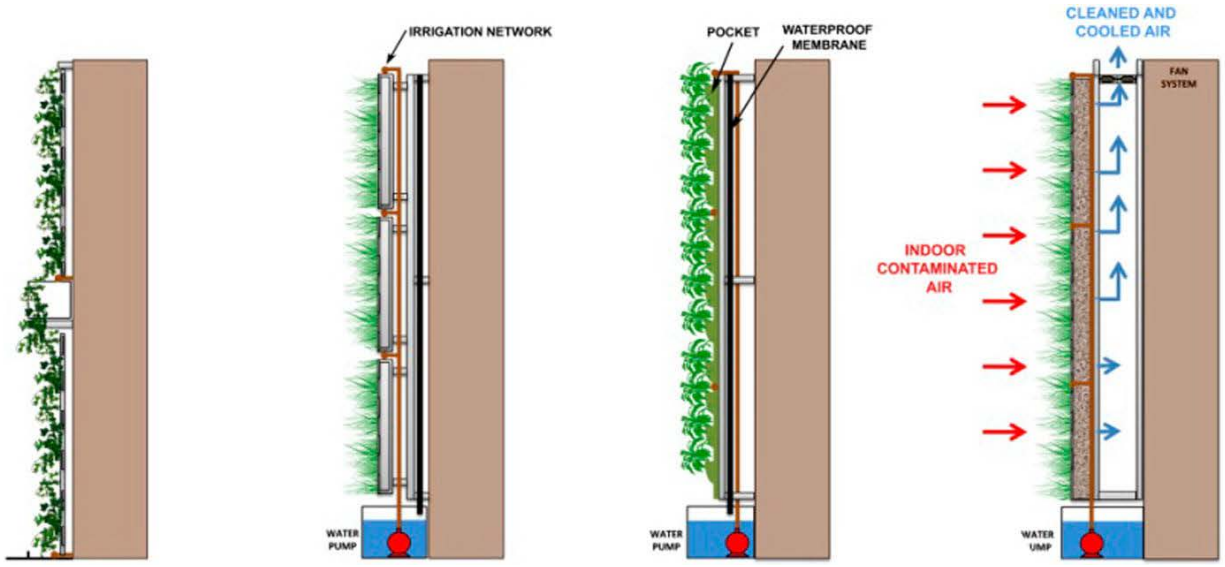


Figure 1. Processes of phytoremediation (Moya et al., 2018)

The microbiome within buildings is often seen as detrimental to human health, as various microbes act as pathogens for humans. Buildings, and especially hospital environments are therefore kept as sterile as possible (Vucemilo, Vinkovic, Tofant, Simpraga, & Pavicic, 2005). However,

humans evolved under constant exposure to environmental microbes. Only a few centuries ago have our lifestyles started to change dramatically, altering the composition, abundance and diversity of microbial communities which we are exposed to in our everyday lives (Mhuireach et al., 2016). An increasing body of research shows findings that a rich microbial diversity, especially in childhood, is needed to train the human immune system (Adams et al., 2016; Gilbert & Stephens, 2018; Peccia & Kwan, 2016). Apart from outside air, indoor microbiomes originate mainly from human skin, pets and plants (G. Berg, Mahnert, & Moissl-Eichinger, 2014a; Gilbert & Stephens, 2018; Mahnert, Moissl-Eichinger, & Berg, 2015a). Members of the plant microbiome are able to play an important role in the microbial composition of indoor environments. It is said that they are able to stabilize the ecosystem, enhance biodiversity and help avoid outbreaks of pathogens. (G. Berg et al., 2014a) Thus, vegetation in buildings could play a role towards a health promoting indoor microbiome, reducing asthma and allergies.

In contrast to the laboratory experiments conducted by Wolverton et al (1984; 1989; 1997), Waring (2016) shows by calculations that potted plants are unable to deliver clean air within buildings, due to their low clean air delivery rate (CADR) in relation to standard ventilation debits. Therefore, this research focusses on the use of vertical greening systems within buildings. The distinction between diverse vertical greening systems is not always clear. In general, green façades are created by letting plants grow across the façade by plants planted at the base of the wall or in planter boxes. As opposed to green facades, living wall systems grow directly on the walls or in a separate system attached to the wall, and are not rooted at the base of the wall (Moya, 2015). Indoor living wall systems are called biowalls and can be used as biofilters (figure 2). The plants within greening systems can be grown in soil or in hydroponic conditions. While soil offers several benefits for plant life, soil in itself is not needed to sustain plants. Plants essentially need water, light, air, nutrients and support. Hydroponics is a method for growing plants that does not require conventional soil. (Darlington, Arsenault, 2012) In hydroponics a structure offers plants support, while a running nutrient-rich medium interacts with the plants' root system.



Green façade | Living wall (panel system) | Living wall (cloth system) | Biowall (active system)

Figure 2. Types of vertical greening systems

(Pérez-Urrestarazu, Fernández-Cañero, Franco-Salas, & Egea, 2015)

In biowalls ambient air is pulled through the plant's growth medium. When dirty air comes in contact with the growth media, contaminants dissolve in water and are metabolized by beneficial microbes in the plant's rhizosphere (figure 3) (Mikkonen et al., 2018). Whereas hundreds of plants per m^2 would be needed to clean $1m^3$ air of a room of $20m^2$ (volume $50m^3$), a biowall of $3m^2$ would be required (Waring, 2016). Suitable plant species for biowalls are discussed in the blue box (figure 4).

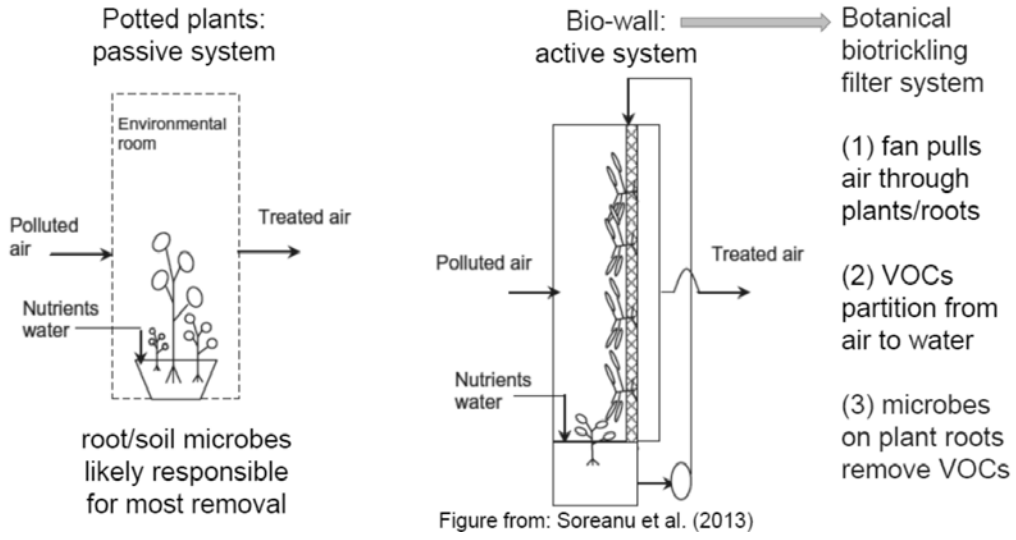
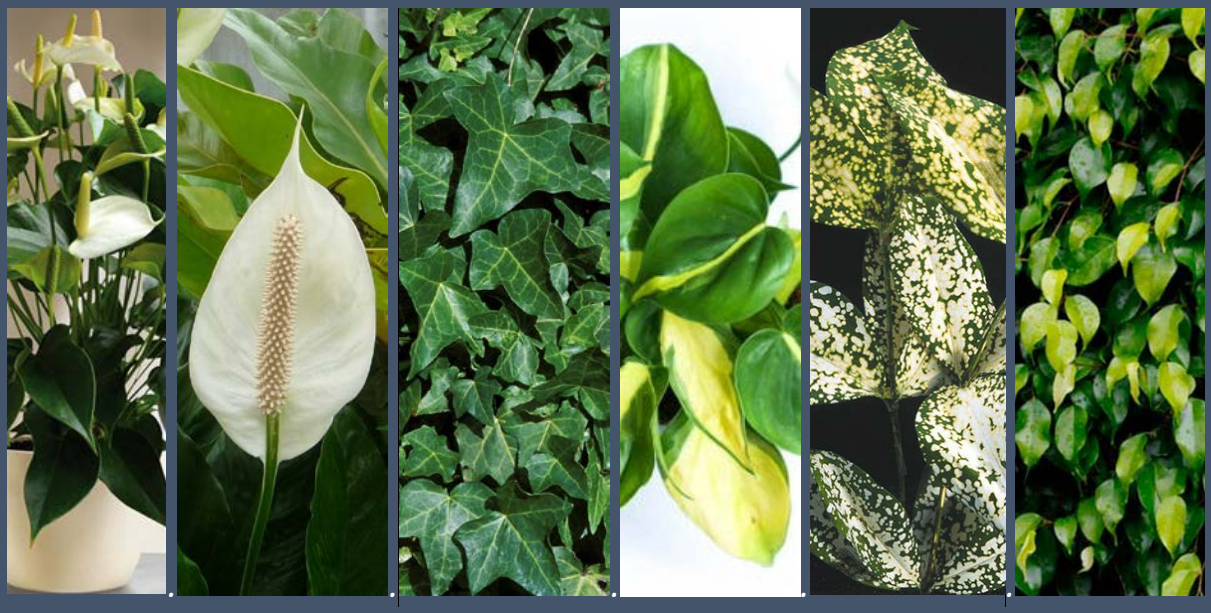


Figure 3. Potted plants vs Biowall (Waring, 2016; Soreanu et al, 2013)

There are several houseplants that are suitable to live in indoor vertical plant walls. Plants used for this purpose often fall in the general category of 'foliage' plants. They include varieties of Ficus, Dracaena, Philodendron and Syngonium podophyllum. (Darlington & Arsenault, 2012) Other plants suitable to live in indoor vertical (hydroponic) plant walls, are Adiantum raddianum, Rhododendron obtusum, Marraya sp., Vriesea splendens, Dieffenbachia picta, Plagiomnium cuspidatum, and Taxiphyllum deplantanum (Soreanu et al., 2013). Also, certain plants are chosen for their aesthetics (continual flowering), durability and ease of maintenance, such as Anthurium spp. In respect to botanical biofiltration, performance depends on interactions between pollutants, plants and microorganisms. Plants with high stomatal conductance and lower sensitivities to the pollutants are deemed most suitable (Moya et al, 2018). Although Dracaena and Spathiphyllum are the most studied plants, better biofiltration results can be achieved with Hemigraphis alternata, Trades- cantia pallida, Hedera helix, Asparagus densiflorous, Hoya camosa and Crassula portulacea (Soreanu et al., 2013).



Anthurium *Spatiphyllum* *Hedera helix* *Philodendron* *Dracaena* *Ficus*

Figure 4. Suitable plant species (Image sources; see reference list)

Although lots of research has been done on the VOC removal capacity of indoor vegetation, very little information exists on the microbial amelioration of indoor air by indoor plants to improve human health. The purpose of this research paper is uncover the basic potential of biowalls to impact and possibly enhance the indoor air microbiome. In this study the main emphasis is put on gathering data about the abundance and diversity of micro-organisms released by plants in several set-ups. Measured parameters of plants, soil and air included microbial abundance and diversity on different growing media, temperature and relative humidity. The aim of this research is to acquire more knowledge on the aerial microbiome within buildings with vegetation systems. Instead of destroying and mitigating the indoor microbiome, this research searches to promote a health benefitting microbiome.

Section 2

Materials and methods

Active hydroponic biowall

Test set-up - building a suitable active hydroponic biowall system

The test set-up was built inside a plant incubator featuring plant support meshes, lighting system, temperature control, relative humidity control, and air blowers. A metal mesh along the rear panel of the incubator was introduced for structural support. Then, in order to create a non-airtight barrier, a synthetic felt was introduced in front of the metal mesh and fastened. To limit airflow to the area behind the vegetation wall, a plastic sheet was placed in front of the synthetic felt, fastened airtight with tape along the edges of the walls and ceiling. A wooden casing for the water reservoir was made and placed on the floor of the incubator, above the pressurized air compartment. To create a watertight water reservoir, a layer of plastic was draped over the wooden casing. The actual green wall element was built as a separate element, entailing its own metal frame and metal mesh as backbone. As growth media and water storage, a 12 cm thick layer of rock-wool was placed in front of this metal mesh, leaving out cavities for the already developed root system of the plants. To ensure that the growth media stayed in place, it was sandwiched between the backbone and a layer of synthetic felt (with openings for the plants) and a metal mesh with gaps 10 cm x 10 cm, using rope to tie the construction together. Careful to limit damage, the soil was washed away from the root system of the plants (anthurium species) After soaking the root system in water for a while, the plants were carefully introduced to the biowall. A watering system was made with a 1100 l/h 12W water pump, a water meter, and ducts, faucets, connectors, splitters and plugs to lead the water up to the top of the vegetation wall. Two pipes with regular perforations were used to distribute water evenly to the growth media.

Culturing

Preparing cultivation media

Potato Dextrose Agar (PDA) samples were prepared by mixing 19.5 g PDA with 250 ml of water. The mixture was sterilized at 121°C, using an autoclave, for 20 minutes. The PDA mixture and 250 ml double concentrated water agar were heated 5 minutes in a microwave till both media were uniformly liquid. Both ingredients were mixed and poured into 9 cm diameter petri dishes in a laminar flow cabinet. The dishes were closed after the agar medium had solidified and were stored in a 4°C cold room.

Luria Broth Agar (LBA) samples were prepared by heating 250 ml double concentrated LB and 250 ml double concentrated water agar 5 minutes in a microwave till both media were uniformly liquid. In a safety cabinet both ingredients were mixed, and poured into 9cm diameter petri dishes. After the samples were solidified the lids were closed and the samples were labeled and stored in a 4°C cold room.

Culturing samples

Culturing samples was done using a modular climate chamber by Snijders, set on 30 °C, 55 % relative humidity, in the dark. Samples were placed in the climate chamber for three days.

Sampling

Air sampling

Air samples were taken with a slit sampler connected to a vacuum pump for 20 minutes. Before sampling, the air sampler and vacuum pump were cleaned with 70 % ethanol. Approximately 400 L air was sampled at a height of 40 cm during the sampling period. In parallel, a microlite climate sensor was used to measure temperature and relative humidity in real time. Samples were stored in a 4 °C cold room.

Soil sampling

Soil samples were taken before plants were transferred to a climate-controlled room. 1 g of soil was put in a 50 ml tube and diluted with 10 ml demi-water. The tube was vortexed for 10 seconds and

left for 5 hours to settle. The supernatant was pipetted into a 50 ml tube. Abundance of microorganisms per milliliter of water was corrected for the water content of the soil sample. This was corrected by drying the soil for 48 hours on 60 °C. In a safety cabinet, 100 µl per sample was inoculated on a 9 cm diameter PDA and LBA agar plate. Samples were incubated for three days at 30°C and 55% rh in an incubator, photographed, and stored in a 4°C cold room.

Leaf sampling

Leaf samples were taken before plants were transferred to a climate-controlled room. Two small leaves (approx. 20 cm^2) were cut off In a flow-cabinet and pressed on a LBA and PDA plate, gently rubbing the leaf with an inoculating stick. Samples were incubated for three days at 30°C and 55% rh in an incubator, photographed, and stored in a 4°C cold room.

Air flow analysis

A smoke machine was introduced in the test setup to analyze the flow of air within the climate-controlled room. The smoke machine was placed in different positions within the incubator, with a camera capturing the distribution of smoke in the closed environment. The set-up was tested using different speeds of the air fans regulating air movements within the incubator (See appendix 5 for more details).

Identification

Counting organisms

Colony counting was done with a mechanical tally counter based on morphology. Agar plates with near confluent growth were handled by counting a representative quarter or eighth of the agar plate.

Yeast identification test

Colonies from agar plates were transferred twice to fresh agar plates to purify the cultures. Yeasts were identified using the Remel RapID™ Yeast Plus System. PDA and LBA samples were used in this test, though Sabouraud Dextrose Agar (SDA) – Emmons formulation, was most recommended. Colony material was taken using a cotton swab and suspended in 2 ml RapID Inoculation Fluid to

achieve a specified turbidity. When needed, the suspensions were mixed thoroughly. The suspension was transferred to the RapID Yeast Plus Panels, tilted 45 degrees, and gently rocked from side to side to evenly distribute the suspension. Maintaining a level, horizontal position, the panels were tilted towards the reaction cavities. Inoculated panels were incubated for 4 hours at 30 °C. Reagent A and B were added, after which the reaction of the wells was scored. Microcodes obtained from the report forms were entered into an online database (ERIC) for identification.

DNA Extraction

Mycelium was grown overnight in 700 µl of PD respectively LB in 2 ml tubes. After pelleting at 10.000 g for three minutes and washing twice with water, two glass beads per sample were added and the samples were immersed in liquid nitrogen for a few seconds. The sample was then further homogenized 2 times 1 minute using a Tissuelyser machine (setting 25.0, clamps turned around second time). The homogenate was resuspended in 600 µl lysis buffer and incubated at room temperature for ten minutes. 150 µl 3 M KOAc solution pH 5.5 was added, vortexed briefly and spun at 10.000g for one minute. Supernatant was transferred to a new tube, spun again and transferred again to a new tube. An equal volume of isopropanol was added, mixed by inversion, and pelleted at 10.000 g for two minutes after which the supernatant was discarded. The sample was washed with 300 µl 70% ethanol and pelleted at 10.000 g for one minute. The ethanol was discarded, the sample resuspended in 50 µL of TE buffer, of which 1 µl was used for a 20 µl PCR volume.

PCR

The ITS fragment was amplified by PCR using the universal primers ITS 1 (5'TCC GTA GGT GAA CCT GCG G 3'), and ITS 4 (5'TCC TCC GCT TAT TGA TAT GC 3) (Mulet et al., 2001). The PCR involved 1 cycle at 95°C for 5 min, followed by 30 cycles with a denaturation step at 95°C for 30 s, an annealing step at 55°C for 1 min, and an extension step at 72°C for 1 min, followed by 1 cycle at 68 °C for 6 mins. The PCR mastermix contained 212,35 µl H_2O, 29,04 µl 10x Taq Buffer, 2 mM dNTPs, 5,81 µl of 10 times diluted forward primer, 5,81 µl reverse primer (also 10 times diluted), 7,26 One Taq (1U/µl). Caution was taken to keep the mastermix below room temperature and was always stored at -20°C. 21,09 µl of mastermix was added to 1 µl DNA with a nucleotide concentration of 0,179 mM. Gel electrophoresis was done using 1.5% agar medium with TAE buffer.

Greenhouses and incubators

Abundance and diversity of micro-organisms in air of botanical greenhouses

Air samples were taken at five different locations at the greenhouses of the Botanical gardens at Utrecht University. Choice of locations was based on differences in micro climate, diversity and type of plants, and vegetation area index (figure 4). The latter was estimated by dividing the surface area of plants divided by the total surface area. Within each greenhouse, samples were taken at three different locations using a slit-sampler and PDA and LBA plates. Samples were taken in a randomized pattern timewise, beginning two hours after watering the plants in the greenhouses. Samples were taken in duplo.

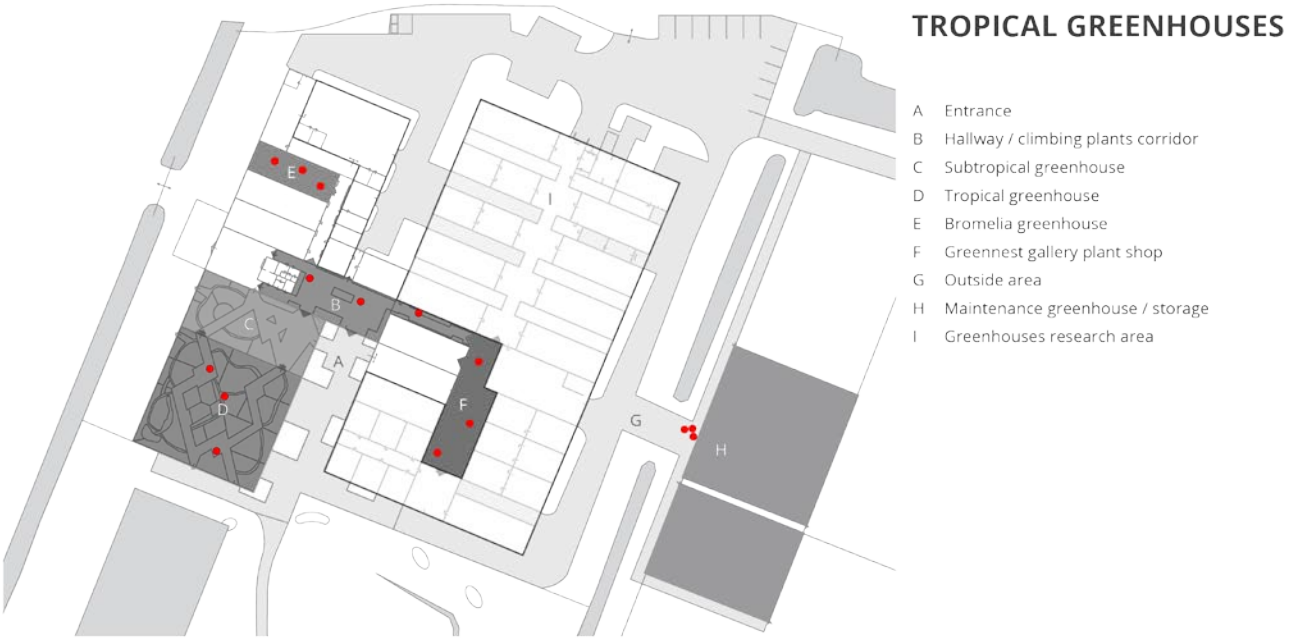


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Figure 5. Test locations to determine indoor air microbial load depending on climate and plant diversity and concentration. (Botanical Gardens Utrecht, 2018; T.J. Hillenius, 2018)

1(D) Tropical greenhouse (mean: 19,9 °C, 92% relative humidity, vegetation area index of 80).
2(B) Central hallway (air mixture of corridor, several greenhouses and outside air, mean: 18,9 °C, 76% relative humidity, vegetation area index of 40).
3(E) Bromelia corridor (winters minima 10 °C, mean during measurements: 18,2 °C, 77% relative humidity, vegetation area index of 60).
4(F) Plant store (Large diversity of house plants, mean: 20,0 °C, 70% relative humidity, vegetation area index of 20)
5(G)Outside environment (control, mean: 7,2 °C, 77% relative humidity, vegetation area index of 10)

Abundance and diversity of micro-organisms in a climate-controlled room with plants or an active hydroponic biowall

The climate-controlled incubator was cleaned with 70% ethanol. After absorbent paper was placed on the bottom, halamid was sprayed into the incubator and left to settle for two days, with the incubator turned off. After cutting leaves for leaf samples and extracting soil for soil samples, a total of 8 plants were placed in the incubator or in the hydroponic wall. The incubator setup was 20°C, 60% relative humidity, 12h light, 12h dark. Air samples were taken outside the incubator, inside the incubator without plants and inside the incubator after placing the plants. Samples were stored in a 4°C cold room and photographed after the duration of the experiment.

Statistics

Spss was used to statistically analyze the data using $p < 0.05$. Colony forming units per 400 L of air on PDA and LBA were summed up. Data was analyzed for normal distribution by using the Shapiro Wilk test. Homogeneity of variance was tested by using a Levene's test. Differences in colony forming units between outdoor air and greenhouses were tested using a Kruskal Wallis test. Correlation between fungal and yeast / bacterial species and environmental factors (temperature, relative humidity and vegetation index) was tested by doing a Pearson correlation in the case of a normal distribution and a Spearman correlation in the case of a non-normal distribution.

Section 3

Results

Abundance and diversity of micro-organisms in air of botanical greenhouses

The air of four greenhouses at the botanical gardens at Utrecht University was sampled to analyze the abundance and diversity of micro-organisms. The greenhouses were chosen for their differences in climatic conditions (figure 6). The plant store was relatively warm and dry, whereas the B*romelia* greenhouse fluctuated a lot in relative humidity. This was likely due to ventilation by an automated window opening system. The tropical greenhouse was very humid and warm, while the central hallway was most diverse in temperature. This was likely because of the mixing of air from different greenhouses and the outside environment. Then colony forming units of the different morphologies were counted. Diversity of fungi, yeasts and bacteria was the same in the different greenhouses and in outside air (table 1). Outside air showed the lowest number (av. 213) of fungal colonies, whereas the other locations showed similar numbers of fungi (table 1, figure 6). However, the differences between outside and indoor air were not statistically significant. The tropical greenhouse showed four times more colonies of yeasts and bacteria when compared to the control sample (av. 20) (figure 6 and 7). Also in this case, statistical analysis revealed that yeast / bacterial counts were not significantly different between the various locations. In addition, correlation between fungal and yeast / bacterial species and environmental factors (temperature, relative humidity and vegetation index) was only significant in the case of the number of fungal colony forming units and the relative humidity (figure 7).

Table 1. Airborne microbes within greenhouses of the botanical garden. Numbers are indicated with standard deviations.

SPACE	Fungi Mean Abundance	Yeasts / Bacteria Mean Abundance	Fungal Diversity	Yeasts / Bacteria Diversity	TEMP (°C)	RELATIVE HUMIDITY (%)	+- % PLANT VOLUME
TROPICAL GREEN-HOUSE	483 +- 200,2	83 +- 105,0	11,7 +- 1,4	5,5 +- 2,7	19,9 +- 1,5	92,3 +- 6,1	80
BROMELIA GREEN-HOUSE	601 +- 520,7	42 +- 41,1	9,8 +- 3,1	5,5 +- 1,4	18,3 +- 1,2	76,3 +- 9,8	60
CENTRAL HALLWAY	507 +- 419,0	39 +- 32,0	12,3 +- 1,4	6 +- 1,3	18,9 +- 2,0	76,2 +- 7,8	40
PLANT STORE	1049 +- 1752,4	37 +- 22,0	10 +- 3,6	5,5 +- 2,4	20 +- 1,0	70,1 +- 6,5	20
OUTSIDE	213 +- 243,4	20 +- 8,6	11,5 +- 1,9	5,5 +- 1,6	7,2 +- 2,1	76,9 +- 11,1	10

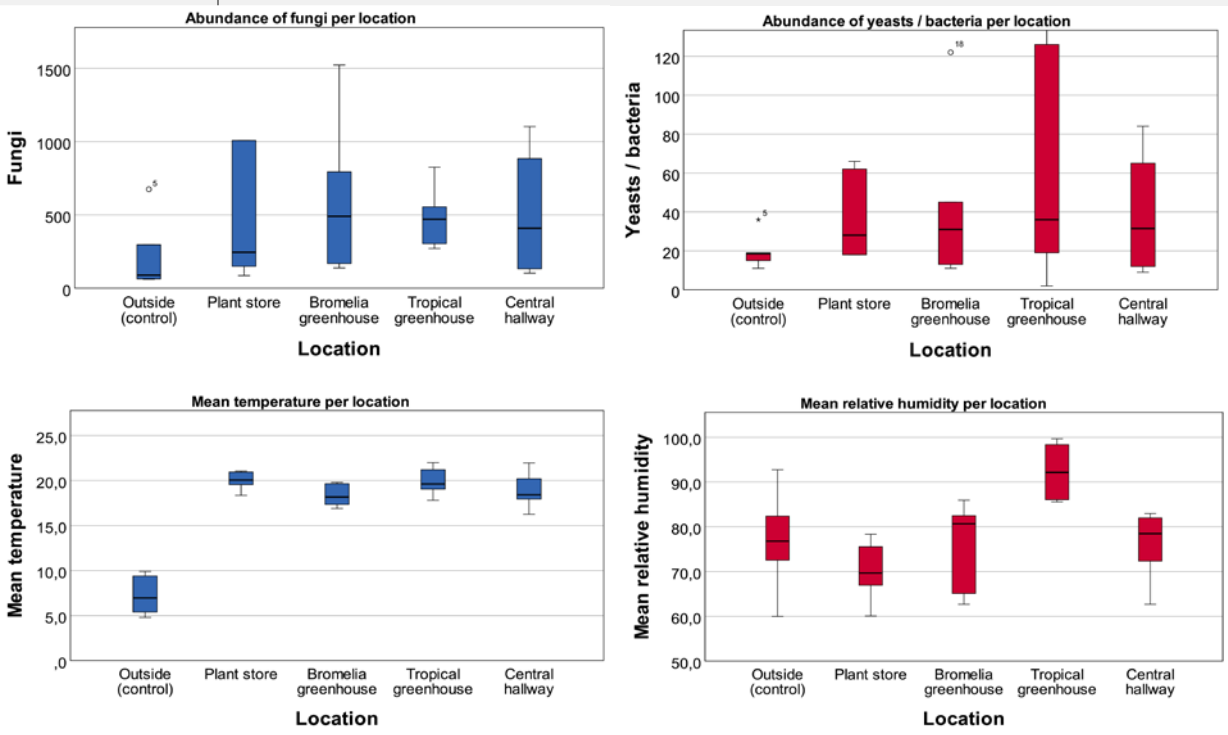


Figure 6. Boxplots of abundance and climatologic differences in outdoor air and greenhouses.

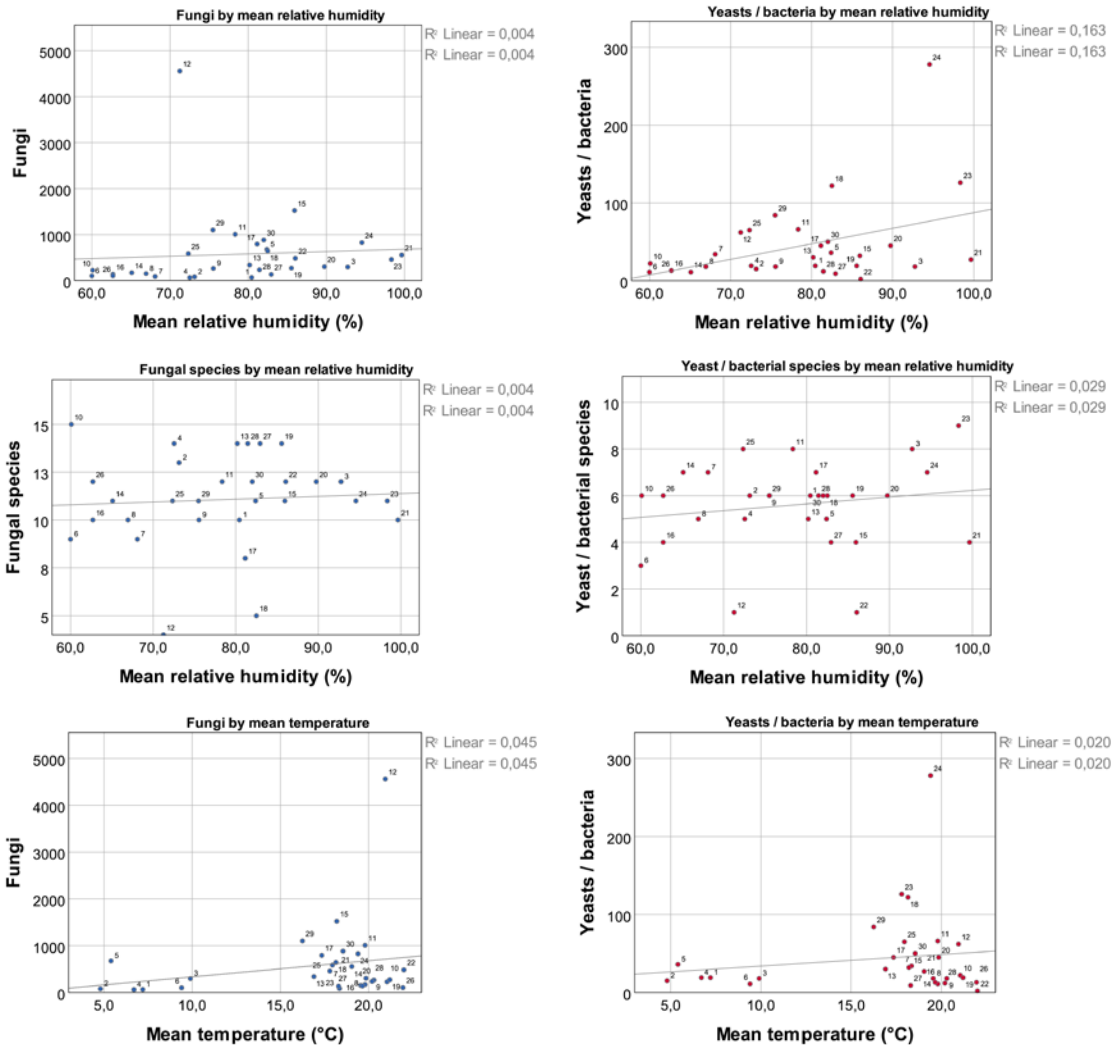


Figure 7. Correlations of abundance and diversity of microbes to mean temperature, relative humidity and vegetation area index.

Abundance and diversity of micro-organisms in a climate-controlled room with plants

The effect of plants on the number of fungi and yeasts / bacteria in the air was assessed. To this end, plants were placed in an incubator for several days and colony forming units were assessed at day 1, 3 and 5 not using replicates. A trend was observed that the number of colony forming units was decreasing in time (figure 8). From the chart it also apparent that there was a peak of microbes before (control) and after placing and watering the plants on day one. Lower numbers were found next days, suggesting microbes settle during the incubation. Iterations of the experiment showed large fluctuations in measurements. Moreover, after a few iterations a control measurement outside the

incubator was included which turned out fairly high in respect to the measurements in the incubator (figure 8).

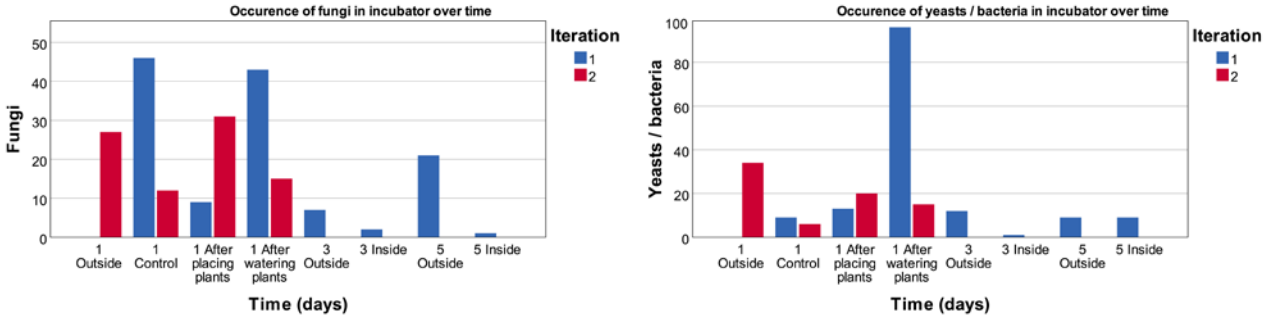


Figure 8. Abundance of fungi and yeasts / bacteria in air outside and inside incubator on day one, three and five.

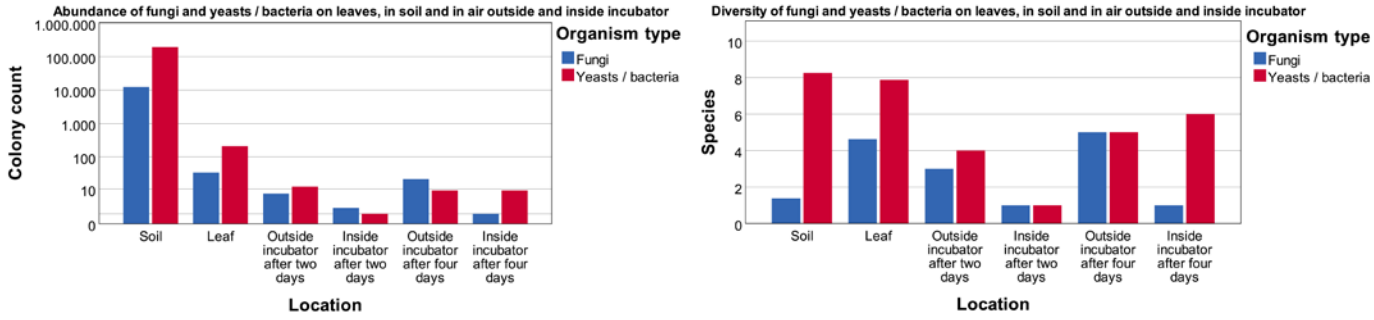
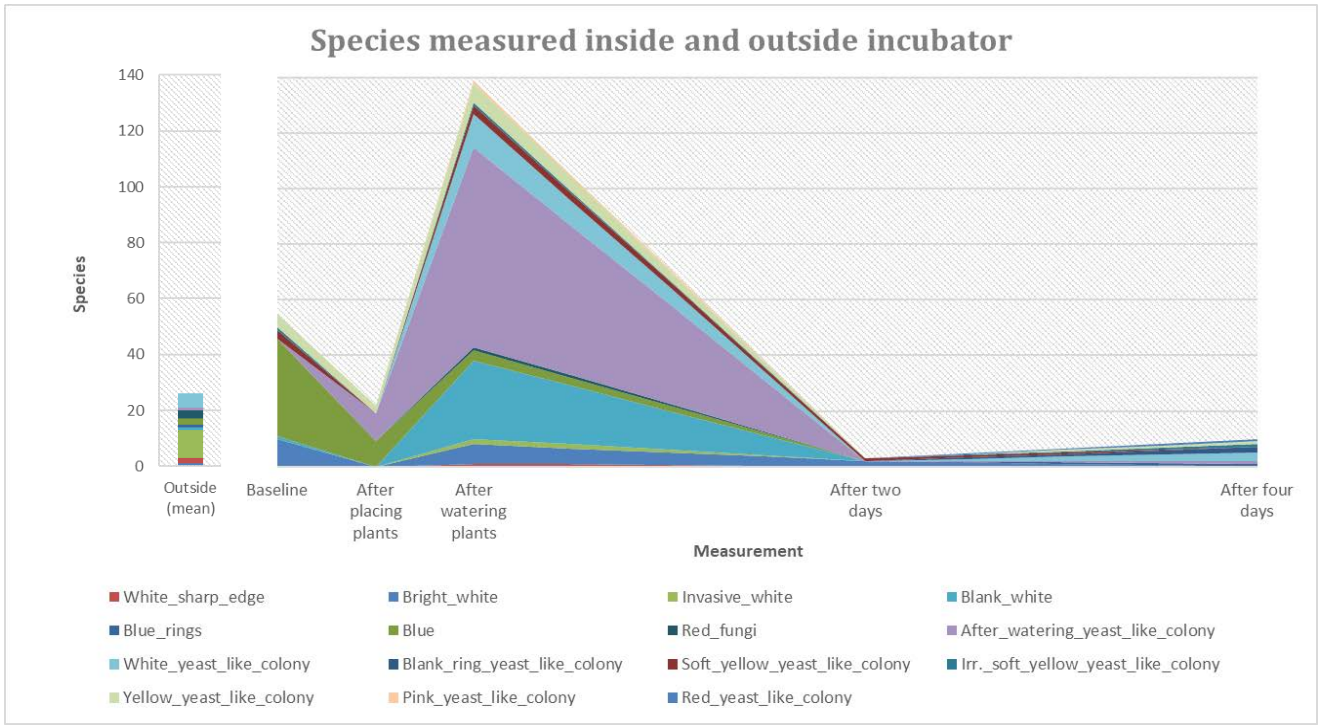


Figure 9. Bar-charts of abundance and diversity of fungi and yeasts / bacteria on leaves, in soil and in air outside and inside incubator. Note the logarithmic scale on the abundance graph. Soil-data are means of colonies and species per gram of dried soil x1,65*10^3 of 8 soil samples (appendix 9). Leaf-data are means of colonies and species per two small leaves of approx. 20 cm^2 each of 8 anthurium plants.

In the next set of experiments, samples were taken from leaves and soil around the plants and the air outside and inside the incubator. Counting colony forming units revealed that soil contained up to 400.000 colony forming units. Leaf microbes peaked at 200, while microbes in the air peaked at 22 (figure 9). Measurements show comparable numbers in diversity of microorganisms on leaves and in soil and air. What was interesting, was that air in the room outside of the incubator was more abundant and diverse in fungal microorganisms than the air inside, while similar number of yeasts / bacteria were observed inside and outside the incubator. Together these data indicate that plants do not increase microbial load in the air. Watering may have a temporal effect on microbial load in the air.



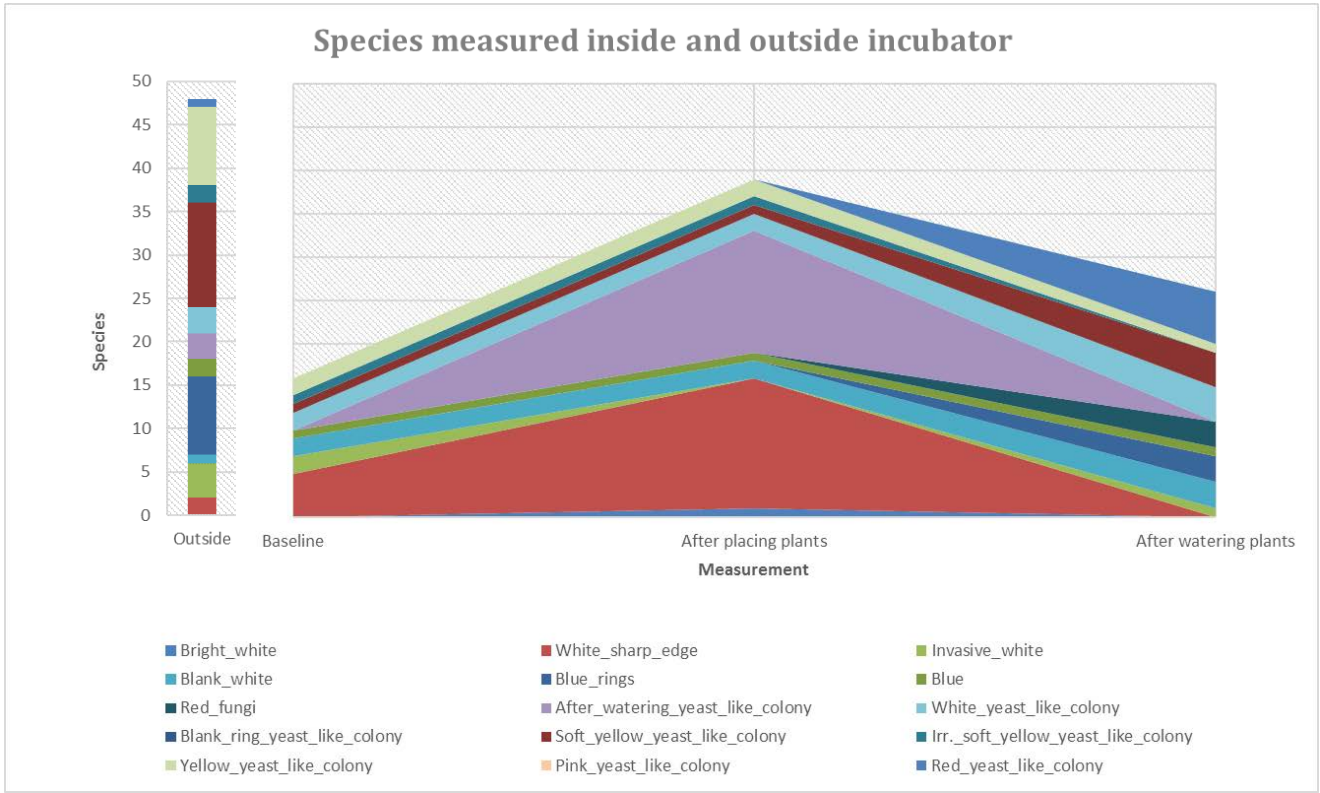


Figure 10. Stacked area charts of species profiles of fungi and yeasts / bacteria in air outside and inside incubator.

Closer analysis of species profiles inside the incubator revealed a peak in micro-organisms after placing or watering the plants, mainly due to one yeast species (figure 10). Moreover, diversity seemed to increase too after watering the plants. In order to assess where the microbes found in the

air originated from, an analysis of species profiles was conducted (figure 11). What can be seen is that certain species occur on all different measuring locations. Most species of the microbiome of the air inside the incubator are also found on the leaf microbiome (although leaf microbiome was most diverse in this data). The yeast species that was abundant after placing/watering the plants in the incubator, was likely to originate from the soil, as it was quite abundant in that species profile.

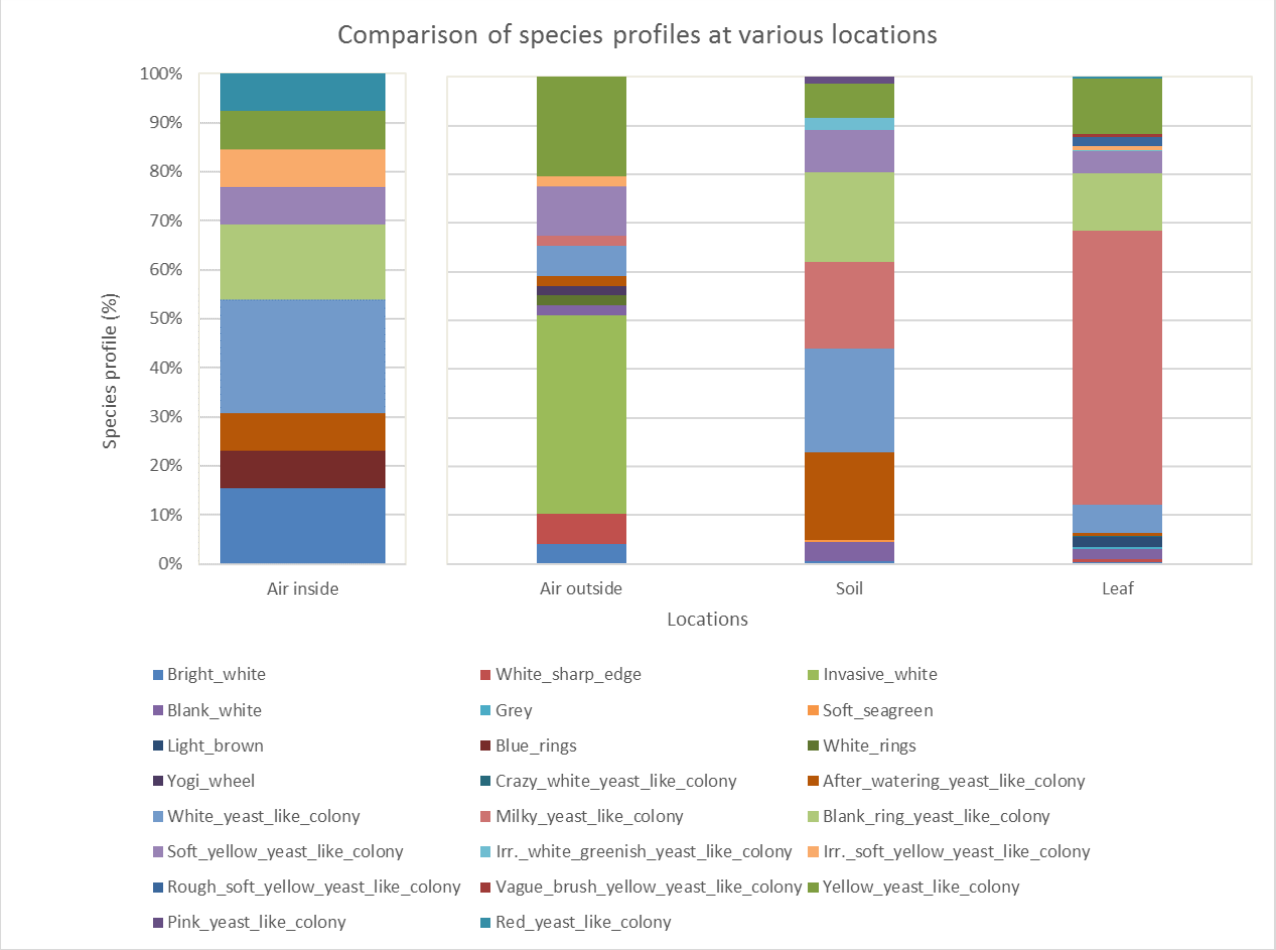


Figure 11. Species profiles of fungi and yeasts / bacteria on leaves, in soil and in air outside and inside incubator. For each species profile two measurements were averaged.

PCR and sequencing results

Samples taken after watering the plants were selected for further research. A PCR was conducted to determine the species of all 10 morphologies of one of the air samples. After DNA extraction and PCR only four organisms showed bands (figure 12). The ITS from the forward and reverse strains of these four organisms was sequenced. In two of the four cases a 100% match was found, F. proliferatum and P. citrinum respectively. One of them being a plant pathogen and the other one a

commonly occurring filamentous fungus. Of the remaining two cases one organism was determined on the class Agaricomycetes and the other on the genus Schizophyllum. Running a PCR on Cox1 gene could determine those organisms to the species level (Molitor, Inthavong, Sage, Geremia, & Mouhamadou, 2010).

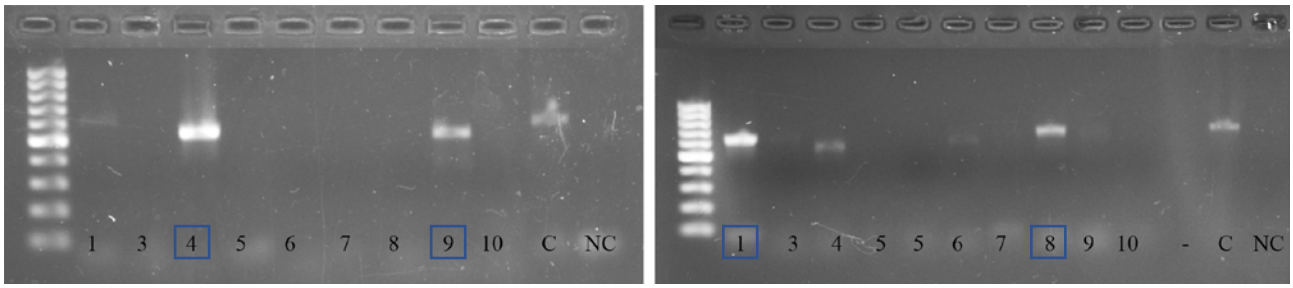


Figure 12. Results of PCR using gel electrophoresis.

Table 2. Sequencing results obtained from PCR of airborne microbes in incubator after watering the plants on day one of the experiment.

	1	**2**	**3**	**4**
Division	**Basidiomycota**	**Ascomycota**	**Basidiomycota**	**Ascomycota**
Class	**Agaricomycetes**	**Sordariomycetes**	**Agaricomycetes**	**Eurotiomycetes**
Order	Polyporales	**Hypocreales**	**Agaricales**	**Eurotiales**
Family	Meruliaceae	**Nectriaceae**	**Schizophyllaceae**	**Trichocomaceae**
Genus	Bjerkandera	**Fusarium**	**Schizophyllum**	**Penicillium**
Species	B. adusta 99.66%	**F. proliferatum 100%**	S. radiatum 99.15%	**P. citrinum 100%**
Type	Plant pathogen, appears on dead wood. Investigated use for bioremediation.	Fungal plant pathogen infecting asparagus.	Emerging Fungus From Human Respiratory Tract	A commonly occurring filamentous fungus.
Notes	+ 3 other species within 98-99.66% (Thanatephorus cucumeris, Marasmius cohaerens, Trichaptum abietinum)	+ 5 other species within 98-99.99%	+ S. commune within 98-99.00%	+ 2 other species within 98-99.99%.

Abundance and diversity of micro-organisms in air of a climate-controlled room with an active hydroponic biowall

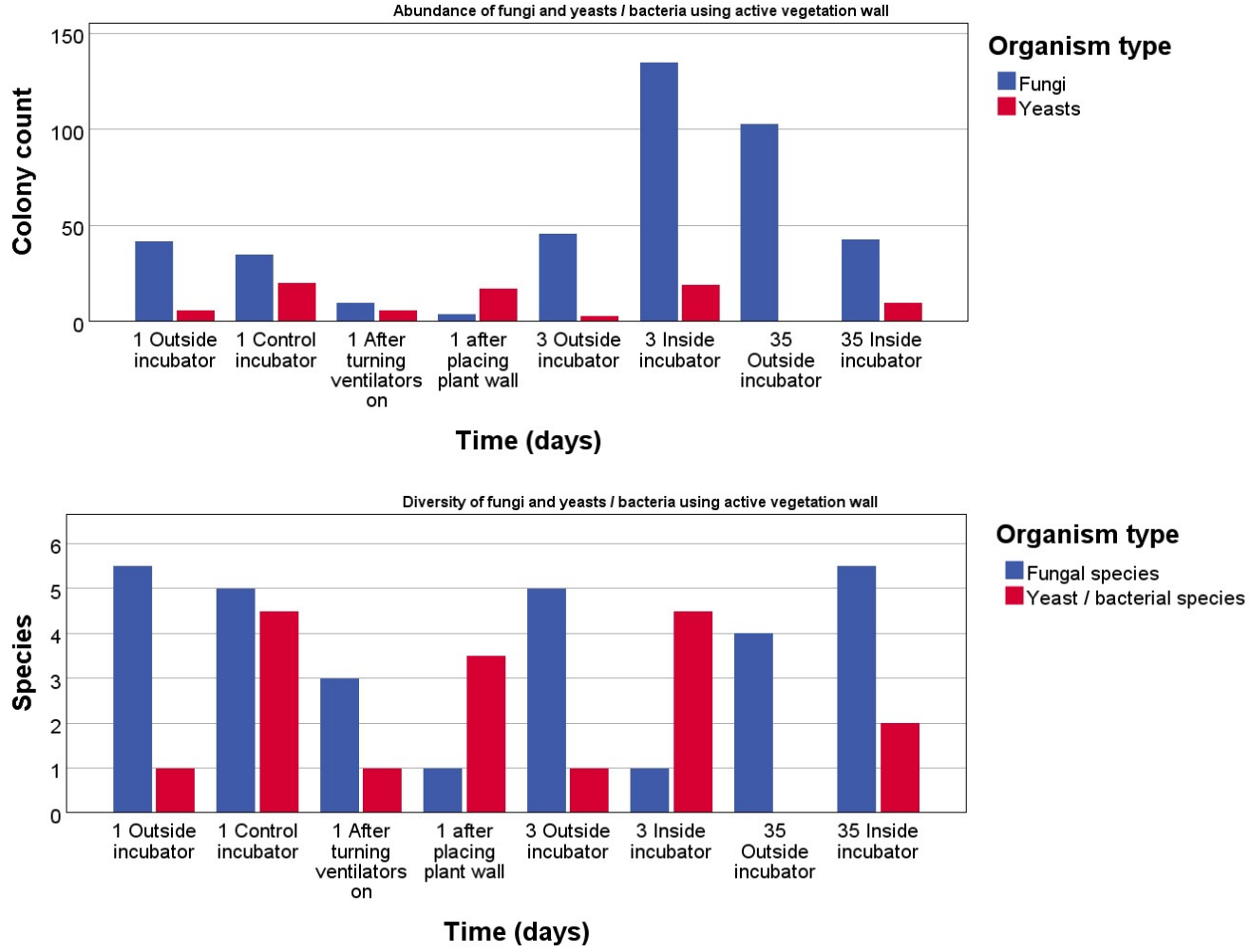


Figure 12. Bar-charts of abundance and diversity of fungi and yeasts / bacteria in air outside and inside incubator on day one, three and five.

After designing, collecting, constructing and testing the hydroponic biowall, an experiment was conducted to investigate its effects on the microbial load in the long term. Due to the dependence of the test setup on consistent watering, the cleaning time of the incubator was shorter. This might be a reason the microbial load of yeasts / bacteria in the baseline measurement at day 1 of the experiment turned out higher than that outside of the incubator (figure 12). Interestingly, after turning the ventilators on, the amount of microorganism measured decreased by a large amount. Oscillations in abundance and diversity can be seen between the measurements on the first day and the third day.

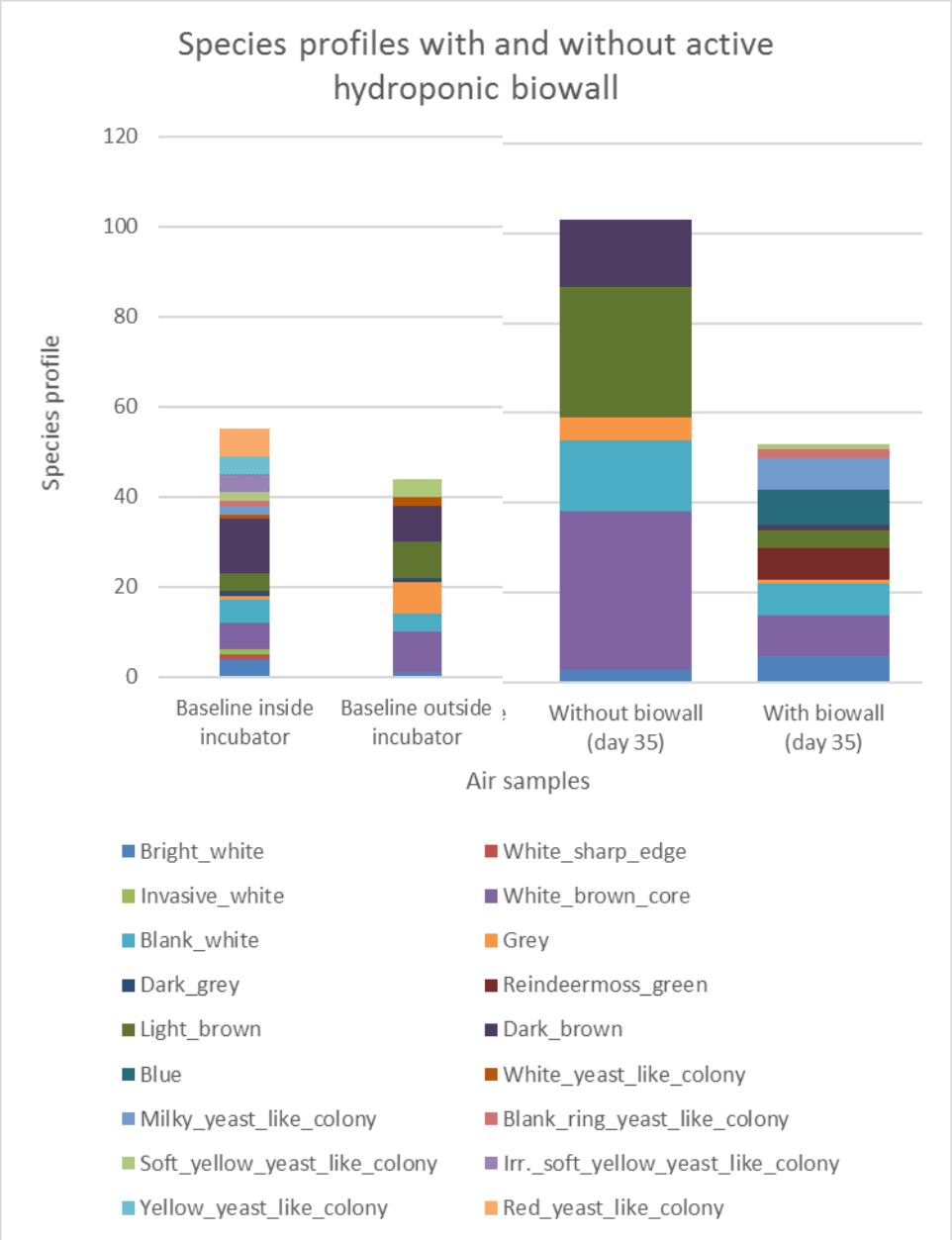


Figure 13. Species profiles of microbial load with and without active hydroponic biowall.

After 35 days of running the active hydroponic biowall the diversity of fungi in indoor air stayed the same, while the diversity of bacteria had decreased (both when compared to day one and day three; figure 13). Although there were peculiar oscillations in abundance and diversity of microorganisms at day three compared to outside the incubator, the conditions at day 35 were quite similar to the starting conditions. It must be noted that the baseline inside the incubator turned out high in diversity, which might be caused by the testing phase of the biowall. The biowall seemed also to perform well to preserve the micro biodiversity over time.

Chapter 4

Discussion

Active biowalls and other types of vegetation walls have potential characteristics to improve sustainability of the built environment. While the removal capacity of VOC's by biowalls has been studied extensively, its ability to alter the abundance and diversity of microorganisms in indoor air remains unclear.

The results of this study suggest active biowalls do not significantly alter the microbial characteristics of indoor air. In the experiment with the longest duration the conditions at day 35 were quite similar to the starting conditions. The biowall seemed to perform well to preserve the micro biodiversity over time. There was a significant positive relationship between the amount of colony forming units of fungi and the relative humidity observed in vegetation dominated buildings. Abundance and diversity seemed to increase after placing and watering plants but this appears to be a short term effect.

Abundance and diversity of microorganisms in air of botanical greenhouses.

In this study, abundance of fungi between greenhouses and outside air could not be shown to be statistically different due to large standard deviations. Especially large fluctuations were seen in the measurements of the plant store. Possible explanations for this might be the relatively large human activity causing air disturbance, or by mixing of air mixed from several small scale research-greenhouses in the plant store's near vicinity. Extensive ventilation with outdoor air (*Bromelia* greenhouse) and high humidity (tropical greenhouse) could also have contributed to high standard deviations. It was expected that there would be overlap in abundance with outdoor air due to ventilation of the greenhouses. This was underlined by previous research done by Ercilla-Montserrat et al. (2017) which indicated that the most important source of fungal spores in the air within a

greenhouse, was the outdoor environment. In contrast to this line of thought, It is known that plants and potting soil have their own distinct microbiome (Ortega, Mahnert, Berg, Müller, & Berg, 2016), (Philippot, Raaijmakers, Lemanceau, & Van Der Putten, 2013). Therefore, it seems reasonable that the large collections of plants inside greenhouses influence their close surroundings. This hypothesis was strengthened by a recent research greatly similar to our research. The researchers found that the impact of outdoor air was not significantly shaping the microbiome in indoor air of glasshouses in a botanical garden (Kozdrój, Frączek, & Ropek, 2019). Also compositions of airborne fungi and bacteria were different in the glasshouses compared to those in outdoor environments. Commonly found organisms in indoor spaces seem to be related to people and human activity, as well as HVAC systems, kitchens, bathrooms, building materials, pets, and the extent of ventilation with outdoor air (Gilbert & Stephens, 2018; Heo, Lim, Kim, & Lee, 2017; Kozdrój et al., 2019). The microbial groups found in the research by Kozdróy et al. commonly originated from plants, decaying plant matter and soil. Maintenance activities, air movement and other mechanical disturbances by the workers and visitors may have helped to disperse microbes in indoor air.

The temperature in the greenhouses was distinctly higher than the outside environment, whereas the relative humidity was consistently above 60% for all locations. Greenhouses with a very dry and warm climate and greenhouses with a dry and a cold climate were not researched, but would have enhanced the reliability of the results. These places, however, were not available in the same greenhouse complex. Also other research done on microbial load in the air of greenhouses found a lowest mean relative humidity of 70% in the same season of the year (Kozdrój et al., 2019). The fluctuations in environmental conditions could have contributed to the lack of statistical power in this study.

Counterintuitively most correlations between fungal and yeast / bacterial species and environmental factors (temperature, relative humidity and vegetation index) levered no significant results. However, there was a positive relationship between the number of fungal colony forming units and relative humidity. Several studies confirm the relation between temperature and relative humidity and microbial growth. Kozdrój et al. (2019) state that it is has been relatively well established that temperature correlates with bacterial growth, whereas a relative humidity lower than 60% is unfavorable for fungal growth. Also Frankel et al. (2012) found that indoor relative humidity was positively correlated with indoor fungal abundance. From qualitative examination of the correlations in this study it can be said that temperature and humidity positively correlated with fungal and yeast / bacterial abundance. Relative humidity correlated positively with both fungal as

yeast / bacterial diversity, while temperature correlated negatively with fungal and yeast / bacterial diversity. Interestingly, in this research abundant vegetation seemed only positively correlated with bacterial abundance. Moreover, it must be noted that several relationships between plants and their environments are reciprocal. By evapotranspiration and shade plants affect environmental conditions inside the built environment (Ward, Choudhary, Cundy, Johnson, & McRobie, 2015).

Abundance and diversity of microorganisms in a climate-controlled room with plants

Experiments were conducted in a climate-controlled environment to closely investigate the effect plants have on fungi and yeasts / bacteria in the air. Interestingly, abundance in the air decreased the longer the plants were placed in the incubator. This suggests microbes settle during the incubation, even though ventilators created a constant upstream flow of air. Research in a controlled indoor environment revealed how an isolated spider plant (Chlorophytum Comosum) increased the abundance of bacteria and fungi on surrounding surface areas within 6 months of plant isolation (Mahnert, Moissl-Eichinger, & Berg, 2015b). Surprisingly, in contrast to the surrounding surfaces, the abundance and diversity of micro-organisms in indoor air remained stable. This might help explain why there was no abundant growth on our air-samples.

After placing and watering plants abundance seemed to increase, possibly disturbance and watering has a temporal effect on the abundance of microorganisms in the air. The variability in measurements and the small scale of the experiment prohibit making conclusions on this matter. L.D. Stezenbach wrote in *Encyclopedia of Microbiology (2009)* that Indoor bioaerosols are generated and dispersed by mechanical and human activity, and according to Singh et al 1994 moisture is a major factor in the proliferation of bioaerosols Thus, the hypothesis that disturbances from placing and watering the plants has a temporal effect on the abundance of microorganisms in the air seems reasonable. As well as abundance, disturbances and watering the plants might also give a temporal raise in diversity of microorganisms in the air.

Literature shows that a microbiome shift on the surrounding surfaces can be observed within longer timeframes plant isolation in a cleaned sealed chamber (Mahnert et al., 2015a).In that experiment bacterial diversity increased over time, while fungal diversity decreased. This might be a result from the plant as well as from the prolonged altered microclimate within the incubator.

Measurements show comparable numbers in diversity of microorganisms on leaves and in soil and air, which seems counterintuitive, as the abundance of soil organisms is far greater than that of phyllosphere and the surrounding air. It is predicted that temperate uncontaminated soil contains as much as 10^6 different microbial communities per gram of soil (DeAngelis et al., 2009). Thus, the method of sampling could be better equipped to show microbial abundance than microbial diversity. This seems also to be the case for leaf microbial diversity, as it is said this area has a remarkable microbial diversity (G. Berg, Mahnert, & Moissl-Eichinger, 2014b). In our study most species of the air microbiome were also found on the leaf microbiome. Indeed, a substantial part of bacteria is shared between the leaf and air microbiome (Lindow & Brandl, 2003).

Four of the ten organisms that were selected for further research were determined, of which likely three plant pathogens and a commonly occurring filamentous fungi. Counting three plant pathogens within four organisms is a high ratio. All pathogens are commonly occurring, though harmful to human health, inducing fungal infections or lung inflammation (Liu et al., 2014; Nucci & Anaissie, 2007; Siqueira et al., 2016). DNA isolation and PCR were iterated multiple times; thus, the quality of DNA-extraction might be dependent on the species tested. To answer questions about the differences and similarities between microbial communities in soil, leaf and air, at least 100 microbes per medium should be determined. Thus, further research is needed to answer these questions.

Biowall as microorganismal health device of indoor air

After 35 days of running the active hydroponic biowall the abundance of bacteria decreased. This might support the hypothesis that human activity is a main source of bacteria in the indoor environment. It may also be the case that the specific conditions within the incubator were less favorable for bacteria then for fungi. After 35 days of running the active hydroponic biowall the diversity of fungi in indoor air stayed the same, while the diversity of bacteria had decreased (both when compared to day one and day three). This result is the same as the results obtained from a 4-m^2 modular biowall (Mallany, Darlington, & Dixon, 2002) and comparable to a ½ m^2 biowall (Irga, Abdo, Zavattaro, & Torpy, 2017). In that research, the additional species that were found after turning on the biowall were present in such small concentrations that their appearance was likely due to normal background variation. This indicates that if a biowall contributes to microbial diversity, it is of a very small magnitude. Oscillations in abundance and diversity after turning on the biowall were also seen in these experiments. It would be interesting to research the abundance

and diversity of microbes on surfaces nearby the hydroponic biowall, in order to give a more complete picture of the dispersal of microbes from these systems.

Concerning the role of the ventilators on microbial load it is possible that changing the velocity of the airflow could change the release of microorganisms in the airstream. The experiments were conducted with the ventilators on 80% of their maximum speed, at an velocity of +- 0.5 m/s. Research found that the maximum removal capacity of VOC's of a biofilter occurred at the highest airflow (Irga, Pettit, & Torpy, 2018), although the residence time within the biofilter column could also be an important factor. Other research found that air velocities of 1.0 m/s or higher might be required to release spores into the airstream (Pasanen, Pasanen, Jantunen, & Kalliokoski, 1991). Interestingly, after turning the ventilators on, the amount of microorganism measured decreased by a large amount. This might suggest microbes settle on surfaces during the incubation, even though ventilators created a constant upstream flow of air. Also it might be possible that the ventilators created a inhomogeneous air dispersal. An airflow analysis was conducted to analyze to which extent de air of the incubator was mixed homogenously. As can be seen from the photographs depicted in appendix 5, air is dispersed homogeneously after 30 seconds. This means that the observed decline in microbes measured was not due to inhomogeneous air mixing. Another possible explanation for this is that the upstream flow of air made it difficult for bioaerosols to be captured by the suction of the slit sampler. Fluid dynamics could offer elucidations to which extent this was the case. This was deemed beyond the scope of this research.

There are several types of biowall systems utilizing the potential of plants to clean air. Whereas the biowalls proposed by the company NEDLAW use air fans placed behind the greenery utilizing the rhizosphere, the company Cloudgarden uses air fans in front of the greenery, utilizing the phyllosphere. Biowalls with horizontal plant beds are best in terms of ensuring compatibility with loose growth media and to prevent maldistribution of watering (Alraddadi et al., 2016). Vertical plant walls using hydroponics tend to be more effective in terms of rhizosphere biofiltration, water and weight management (Alan Darlington & Arsenault, 2012; Irga et al., 2018; Riley, 2017). These soil-less systems are also less prone to degrading and demorphing growth media. As especially the rhizosphere is pointed out as a promising functional area of air-cleaning (Irga et al., 2018; Tarran & Torpy, 2007), and hydroponics enhances this functionality, the biowall system of Nedlaw was used as basis for the biowall design used in this research. Anthurium plants were chosen to be dwellers of the system, as they are suited for vertical walls, strong, and were readily available.

Depending on using the mean of the last two measurements or only the last measurement, the trends observed would be different. This indicates that the trends in this study are open to multiple interpretations. Though pathogens were found in the air of a room with isolated plants, only few colony forming units were discovered. Also during commissioning of the tested biowall both fungal and bacterial spores were observed to remain well below common safety guidelines (Faassen & Thierauf, 2016; Alphen 2014). The NVVA (1989) recommends no more of 500 cfu/m^3 per species of microorganisms and 10.000 cfu/m^3 in total. Although the abundance on the third day after starting the biowall was quite high, the amount was comparable to air samples taken outside the incubator. Moreover, measurements after 35 days show a little less abundant but more diverse microbiome. These results are comparable to previous research on this topic (A. Darlington, 2000; Dixon, 2015; Mallany et al., 2002; Wolverton & Wolverton, 1996).

Limitations

Agar plate air sampling was the minimal cost effective and most precise technique to measure the indoor air microbiome. Passive agar plate sampling gives a direct measure of the microbes settling on surfaces (Napoli, Marcotrigiano, & Montagna, 2012). This technique was not used, as it was more prone to physical disturbances of the measuring environment. It could be used in extended research on the settling of microbes. Samples were grown on agar plates using potato dextrose agar (PDA) and luria broth agar (LBA), to culture fungal as well as bacterial growth. The fraction of cultivable microbes on one or even two specific media is extremely low (G. Berg et al., 2014b). Next generation sequencing methods is the most effective technique for longer and deeper studies in this field, but this technique is also costly. In this research, air samples were taken with a slit sampler connected to a vacuum pump for 20 minutes. The results from this active method of sampling can best be interpreted as microorganisms dispersed in air, and inhaled by persons in the room (Napoli et al., 2012). As in the research consistently the same equipment and settings were used, this limited distortion between air samples. To increase the representatives of the air samples in larger environments (greenhouses), three air samples were conducted at different spots. Although sampling at nose-level would have been more realistic, a height of 40 cm was chosen due to practical reasons. This was deemed sufficient, as dispersal from surfaces was limited by a low level of human movement.

The data obtained from the samples was sorted by human observation. Although much more precise than automated software to detect CFU's, this method too had limitations. Each species reacts and grows different with external influences, like different growth media and competition. Also, within one species the variegation is quite large. For example, different growth stadia of one colony have different colors and young (small) colonies are harder to correctly determine and distinguish from each other than mature colonies. Some species form clear borders, while other species form unclear borders. Not only organisms are heterogenous in their growth and morphology, also from human observation and decision-making issues arise. For example, colors are subjective. They tend to fool the eye with different backgrounds or colors in the surroundings. Sorting was also difficult, for example when the morphology of a colony fits exactly the one species-definition, while the color fits the other one. When a new species was discovered, previous samples were not backchecked for presence of this new morphology. Counting was troublesome too, when several colonies are grown together or a conglomeration of extremely small colonies. Besides, it was hard to standardize the method of measuring, as this shifted a bit through time with motivation, tiredness and mood. Lastly, condensation and reflection of the petri dish lid troubled the accurate determination of the organisms.

The test set-up was built inside a plant incubator featuring plant support meshes, lighting system, temperature control, relative humidity control, and air blowers. This system was quite reliable in maintaining and mimicking day and night environmental conditions, but had its limitations. The largest being the effect of opening the door during experimenting to change samples. Also, exact airtightness could not be ensured, small leaks within the system might have brought microbes from the air outside of the incubator and building into the test environment. Furthermore, the climate control could not counterbalance the humidity produced by the biowall so that the relative humidity was often around 80%. According to Darlington et al. operating the water system at cooler temperature than the surroundings, prevented excessive humidity, limited the probability of pathogens and favoured VOC's removal (Soreanu et al., 2013).

In the general outline of the research, it became clear that the indoor environment is not a sterile environment and that introducing a large community of plants into the indoor environment seems to add microbes to the indoor air, although this could not be statistically verified. Additional experiments would be needed in order to generate a clearer picture of the influence of biowalls on the microbial load. Thought can be of increasing iterations, continuous sampling, and quantifying optimal relative humidity, temperature and ventilation flow. Besides the air, soil and leaf samples,

sampling surfaces of the incubator was found to be essential to research the dispersal of microbes originating from the plant wall. Moreover, spore loads, humidity and CO^2 control, topics related to maintenance, and occupants acceptance of this technology need to be investigated further (Soreanu et al., 2013). To truly understand the mechanisms of the pollution removal and amelioration remains difficult. Many factors influence process performance such as plant species, microorganism types, substrate types, light source and number of plants. Sophisticated techniques like next generation sequencing, improved sequencing of the fungal mycobiome and computational models might lead to a better understanding of the interactions between rhizosphere, leaf, air and surface microbial communities (Gilbert & Stephens, 2018; Nur et al., 2020), thereby offering methods to optimize vertical hydroponic biowalls for improving indoor air by removing harmful chemicals, regulate pathogen species and ameliorating with a diverse community of commensals and probiotics.

Section 5

Conclusion

In this research we show that buildings with extensive plant collections qualitatively have higher microbial abundance than outside air, although no difference could be found statistically due to high standard deviations. A significant positive relationship between the amount of fungal colony forming units and the relative humidity was observed. This was also confirmed by Frankel et al. (2012). Although determination of four observed microbes via PCR revealed two (possibly three) microbes were pathogens, it could not be determined that samples from potted plants in controlled environments differed from control samples. The amount of microorganisms after running the biowall for 35 days were comparable to air samples taken outside the incubator. From the results in this study it does not seem that biowalls can be used to regulate the indoor microbiome and that plants are even able to let pathogens proliferate. However, these results may be obtained due to lack of a large dataset and precise measuring equipment. Most studies found biowalls to be not harmful to indoor air quality (G. Berg et al., 2014a; Irga et al., 2017; Kozdrój et al., 2019; Mallany et al., 2002; Soreanu et al., 2013). The outcomes of this study can be used for research into architecture, ecology and human health. As current used cleaning and hygiene strategies (especially in hospitals) promote multi-resistant pathogens instead of beneficials, further research on vegetation within buildings is necessary. Further understanding of the microbiomes that surround us will make strategies and innovations for beneficial interactions and integrated ecosystem services possible, thus improving the sustainability of the built environment.

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Symposia

Symposium '**The science of gut feelings'**. Door: Prof John Cryan, Assoc. Prof Rochellys Diaz Heijtz, Dr. Laura Steenbergen, Orsha Magyar MSc. Micropia Amsterdam 2019

Appendices

Appendix A. Theoretical framework, vision

Architecture

Now and in the upcoming decades climate change urges us to rethink our built environment. Intensive rain and droughts together with raising temperatures give rise to several problems in the stony ecosystem of the city. One concept to cope with these is to work on 'Sponge cities', cities that are able to efficiently capture, store, and use water are able to withstand droughts longer, keep the city cool and provide drinking water to its inhabitants. The body of research done on mitigating the Urban heat island effect (Hiemstra, 2018) has grown in the last decade, and fairs dealing with the built environment increasingly promote an adjustment of our city's ecosystem to accommodate more greenery (vakbeurs openbare ruimte, jaarbeurs Utrecht, 2018). Underlying climate change, a paradigm shift towards a circular economy has begun to mitigate the environmental impact of material streams. The building industry as a whole contributes to almost 40% of the greenhouse gas emissions today (United Nations, 2017), and to …% of material usage, hence the need to transform the sector into more sustainable practice. Circularity in building materials and a shift in perception to see buildings as material libraries (Madaster) are expected to contribute to the circularity of the built environment.

To enhance the functioning and energy efficiency of buildings, computer technology accompanied with sensors is increasingly used (Edge, Amsterdam). Buildings can increasingly be seen as 'large computers beneath a roof' and an increasing amount of buildings with incorporated intelligent systems is emerging.

Due to the interlinkages between smart systems ('the internet of things' (IOT)) our cities are becoming increasingly intelligent and efficient. This usage of; computer technology, the need to integrate ecosystem services to form a sustainable civilization, and the need to fight climate change, lead to innovations in the built environment retrofitting buildings with smart vertical plant walls, rooftop- and indoor gardens.

Biology

Microbes are of high importance for ecosystem health, via the governance of ecosystem functions related to bio-chemical processes (Morin & Mcgrady-steed, 2004). The soil – housing an incredible abundance and diversity of microorganisms – promotes aboveground biodiversity by improving nutrient-pools and therefore regulating plant species dominance (Fulthorpe, MacIvor, Jia, & Yasui, 2018). Cross linkages related to microbiome functioning are found in the fields of microbiology, human biology and environmental biology. Plants derive so many benefits from the microbial community near their roots, that it is possible to speak of a holobiont; an assemblage of different species that form an ecological unit, instead of isolated individuals. As holobionts, plants are far better equipped to handle environmental stressors. For example: transplantation of soil with rich microbial diversity and high disease surpressiveness to a disease conducive soil, leads to transfer of disease suppressiveness (Philippot et al., 2013). Analogous in human biology indications of correlations are found between disturbed gut-microbiota and allergies and even depression. Recent treatments of FMT, which entails stool transfer from a healthy donor into a patient's intestine, show it is possible to restore a healthy balance between human host and microbes (Brugman et al., 2018). Via the Nervus Vagus the state of microbial communities in the gut influences processes in the human brain (symposium micropia, Amsterdam 2019). The evidence is growing that microbiota in the gut influences the nervous system by the substances the microbes produce, therefore the use of pre- and probiotics in preventing or treating neurologic diseases is a topic of great interest. Plant-associated micro-organisms and ubiquitus commensals could act as counterparts against pathogens, enhancing biodiversity and stabilizing the indoor microbial ecosystem (G. Berg et al., 2014b). As current used cleaning strategies, especially in hospitals, often promote

multi-resistant pathogens instead of beneficials, it is important to re-evaluate our relationship to our surrounding microbiomes. This 'paradigm shift in ecology' is required for humans as well for animals, our food production, and our environment. Fortunately, the broad approach of community ecology and studying holobionts is assisted by improvements in technology like next-generation sequencing. These allow a much better assessment of them. Moreover, we can develop innovations and strategies for beneficial interactions.

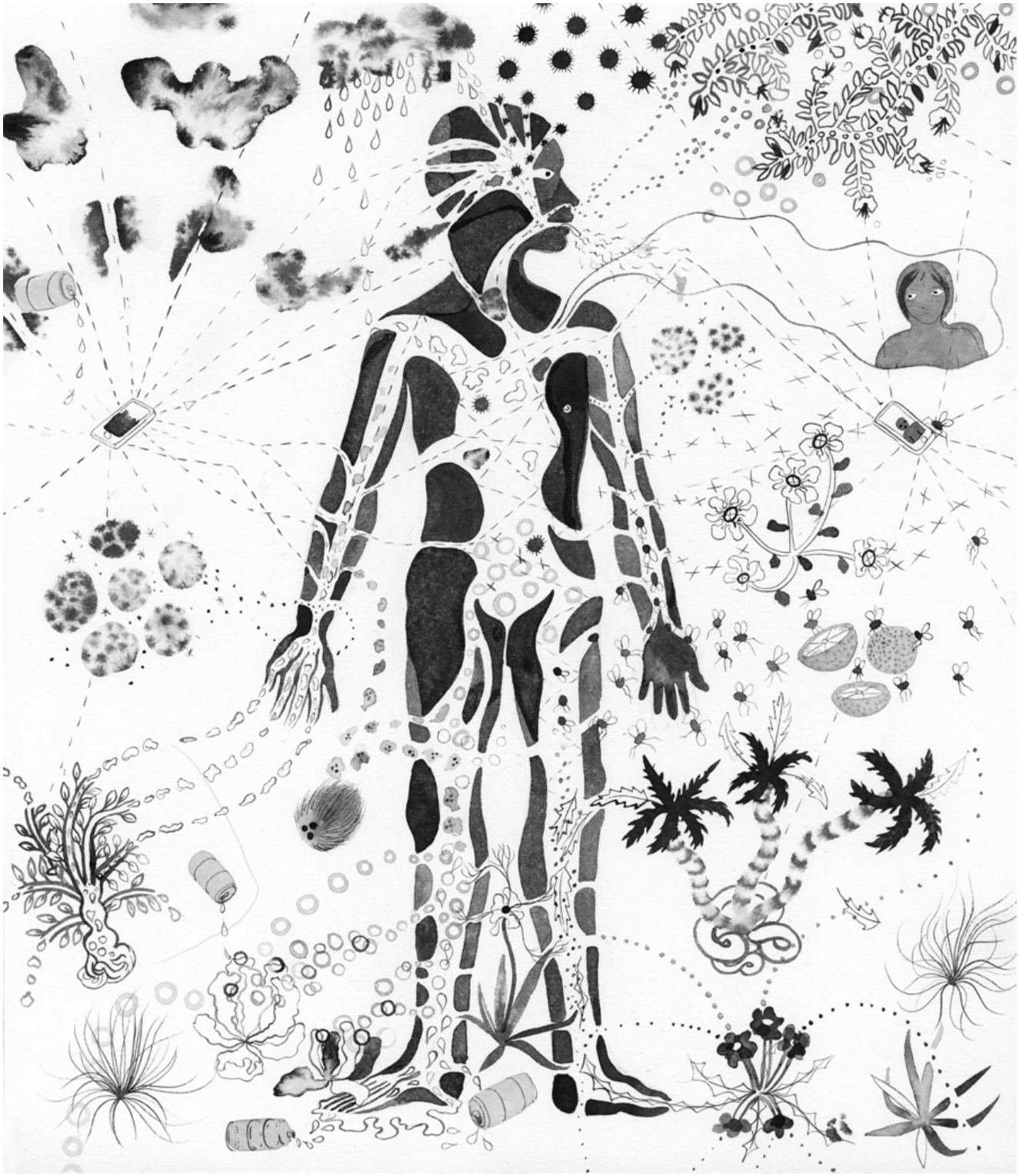
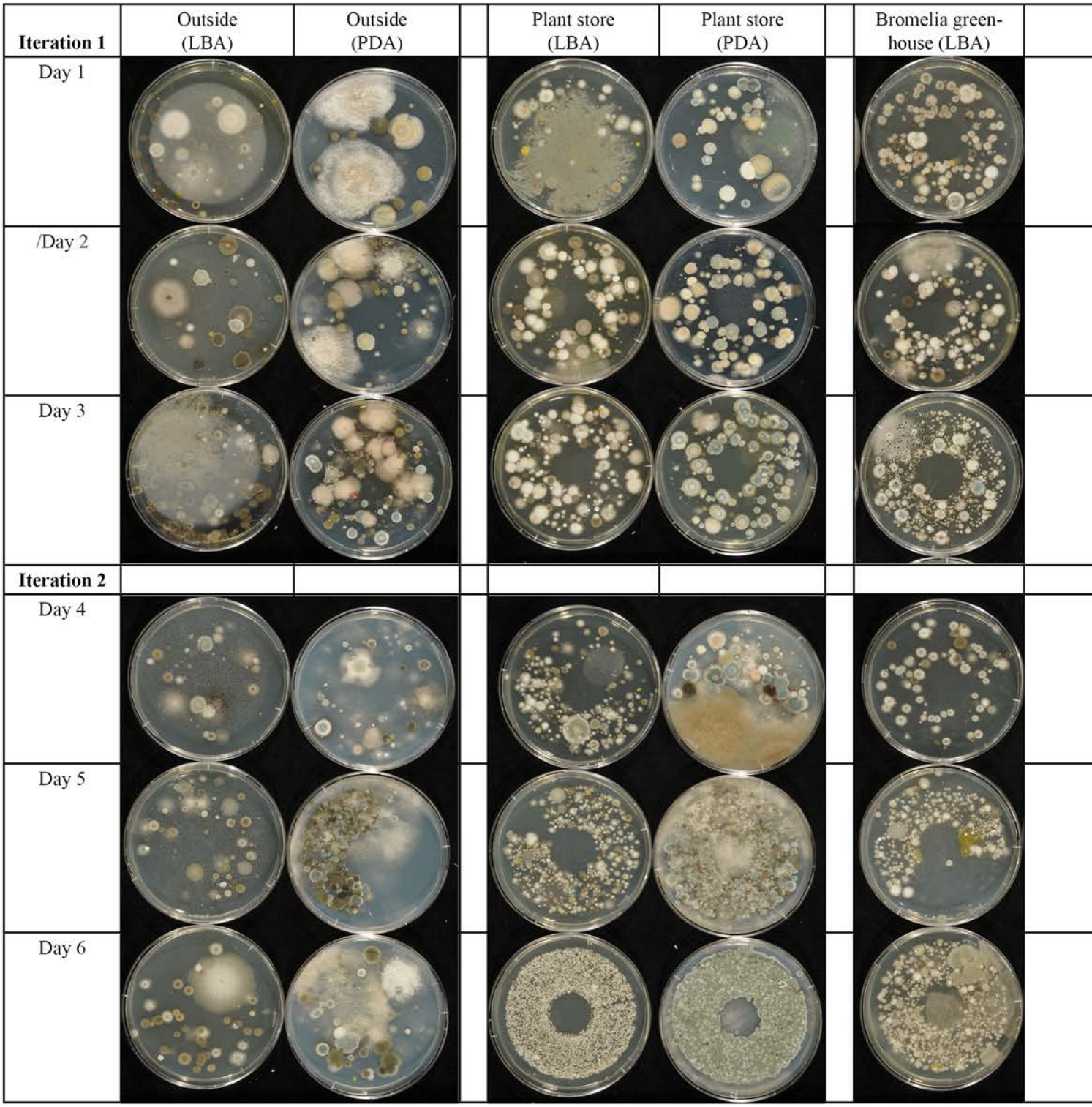
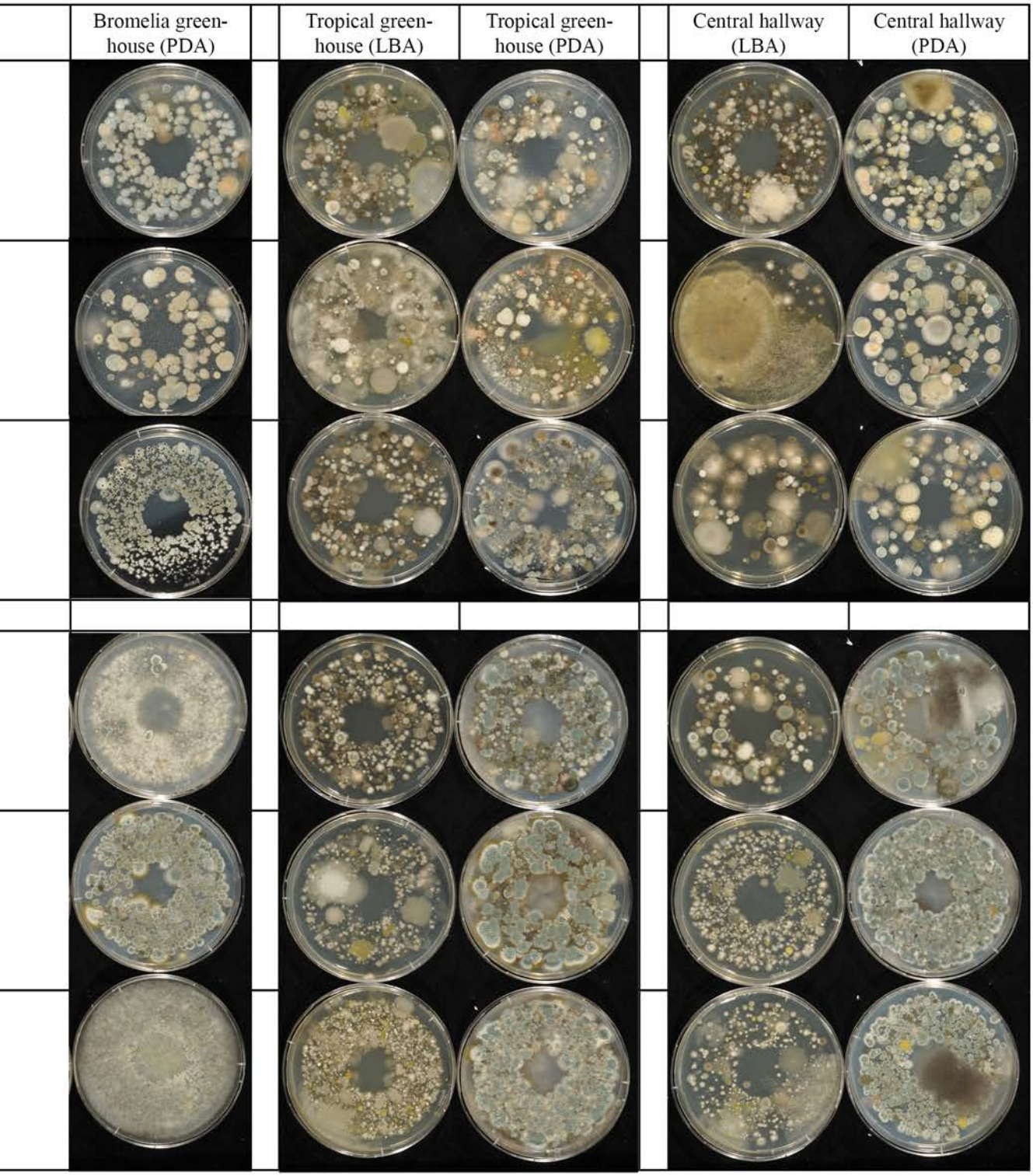


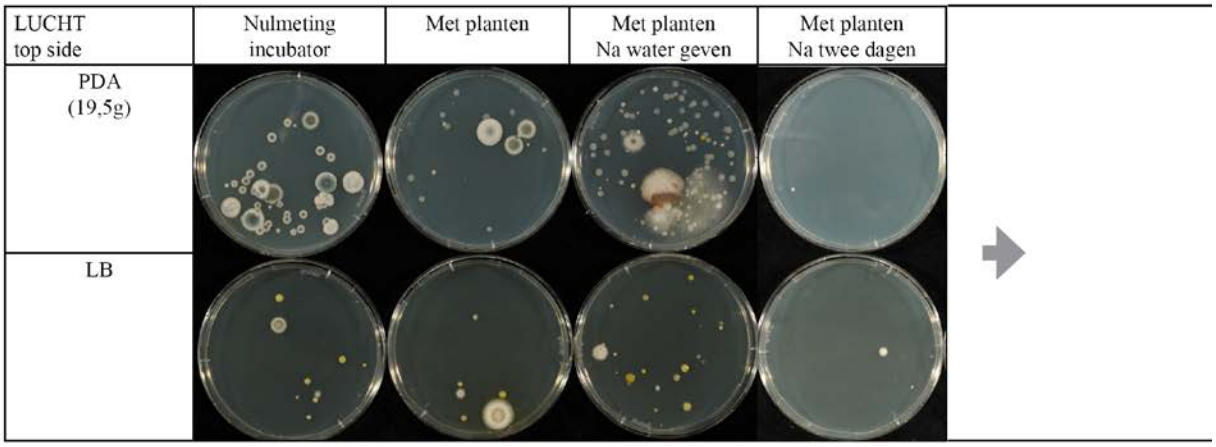
Figure 14. Holobionts and the gut-brain axis, in *Things Get In You.* M. Diddie

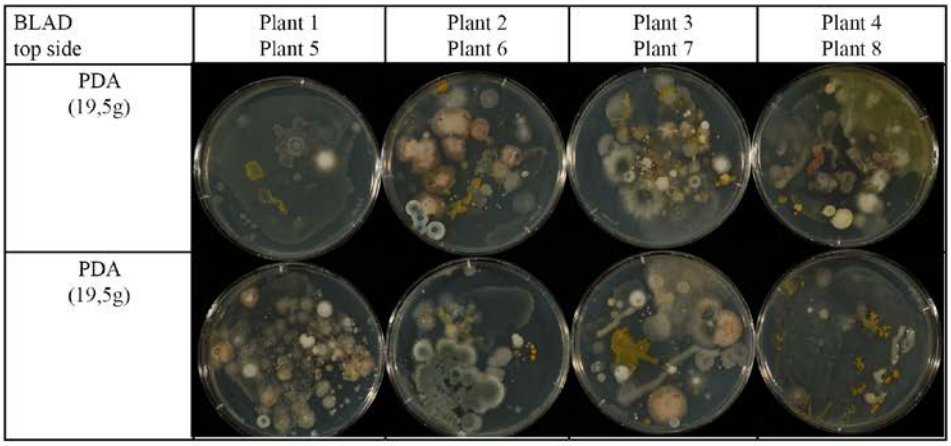
Appendix B. Photos experiment 1

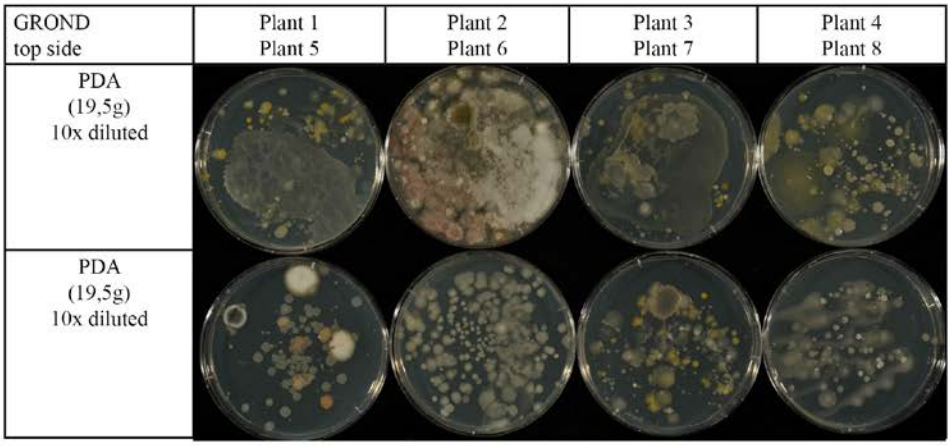



Bromelia green-house (PDA)
Tropical green-house (LBA)
Tropical green-house (PDA)
Central hallway (LBA)
Central hallway (PDA)

Appendix C. Photos experiment 2





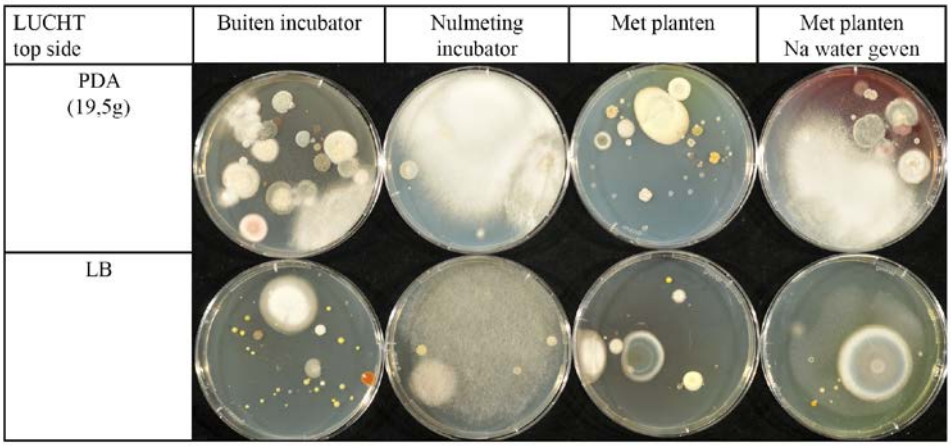


LUCHT bottom side	Buiten incubator Na twee dagen	Met planten Na vier dagen	Buiten Incubator Na vier dagen
PDA (19,5g)			
LB			

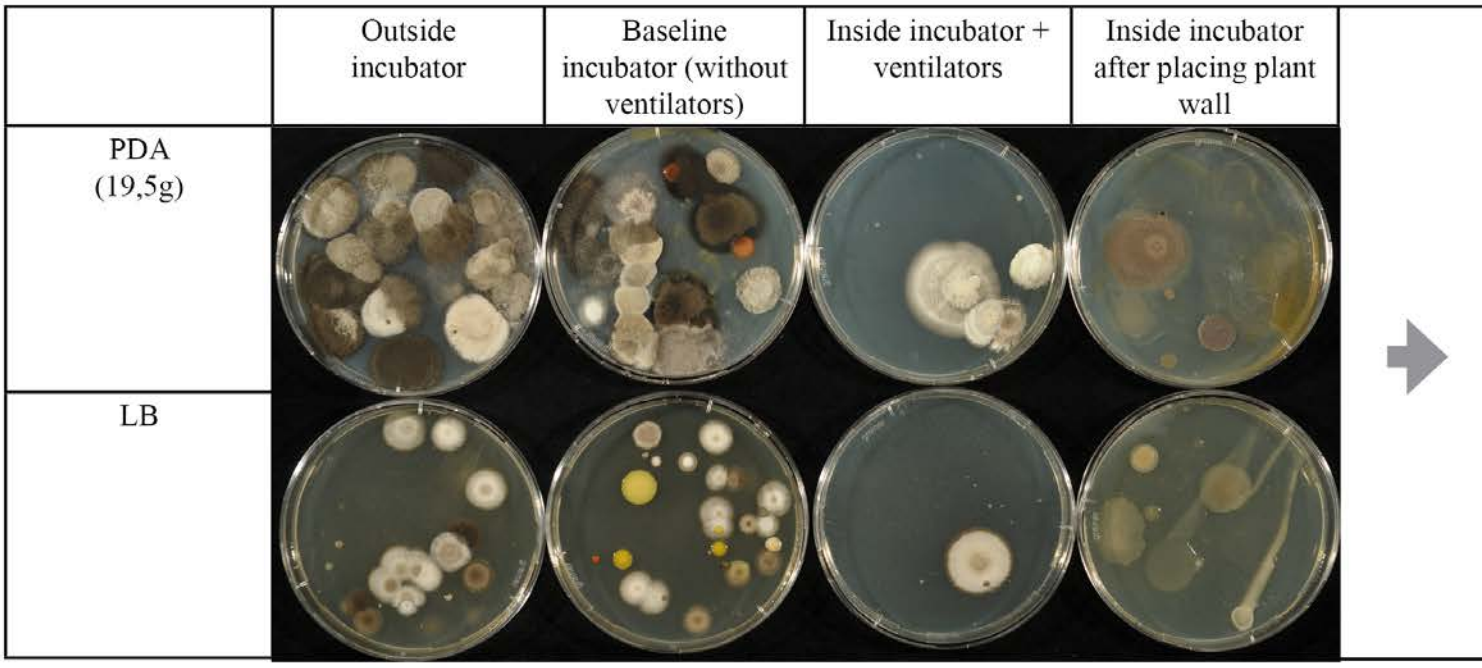
BLAD top side	Plant 1 Plant 5	Plant 2 Plant 6	Plant 3 Plant 7	Plant 4 Plant 8
LB				
LB				

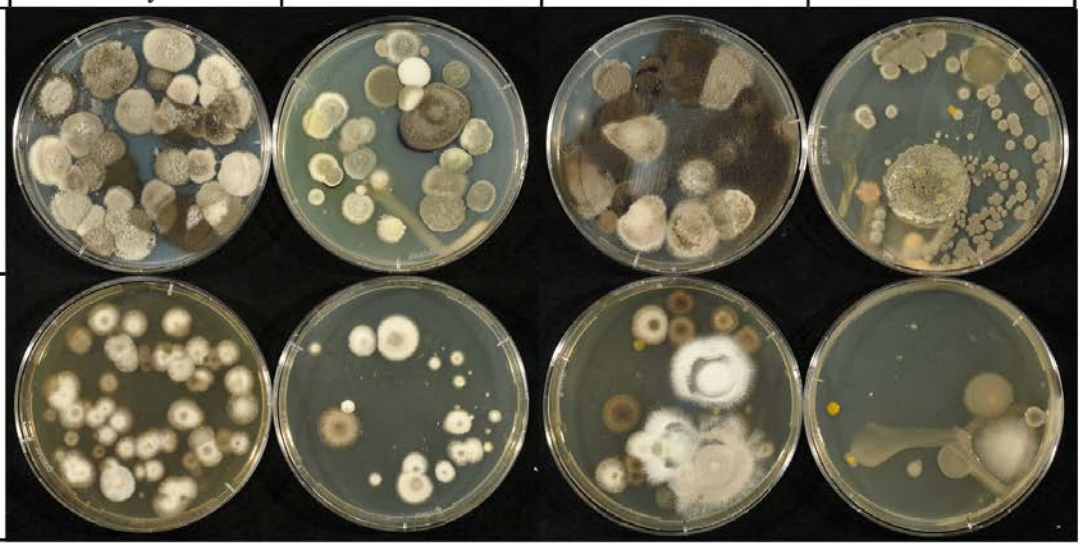
GROND top side	Plant 1 Plant 5	Plant 2 Plant 6	Plant 3 Plant 7	Plant 4 Plant 8
LB 10x diluted				
LB 10x diluted				

Iteration 2

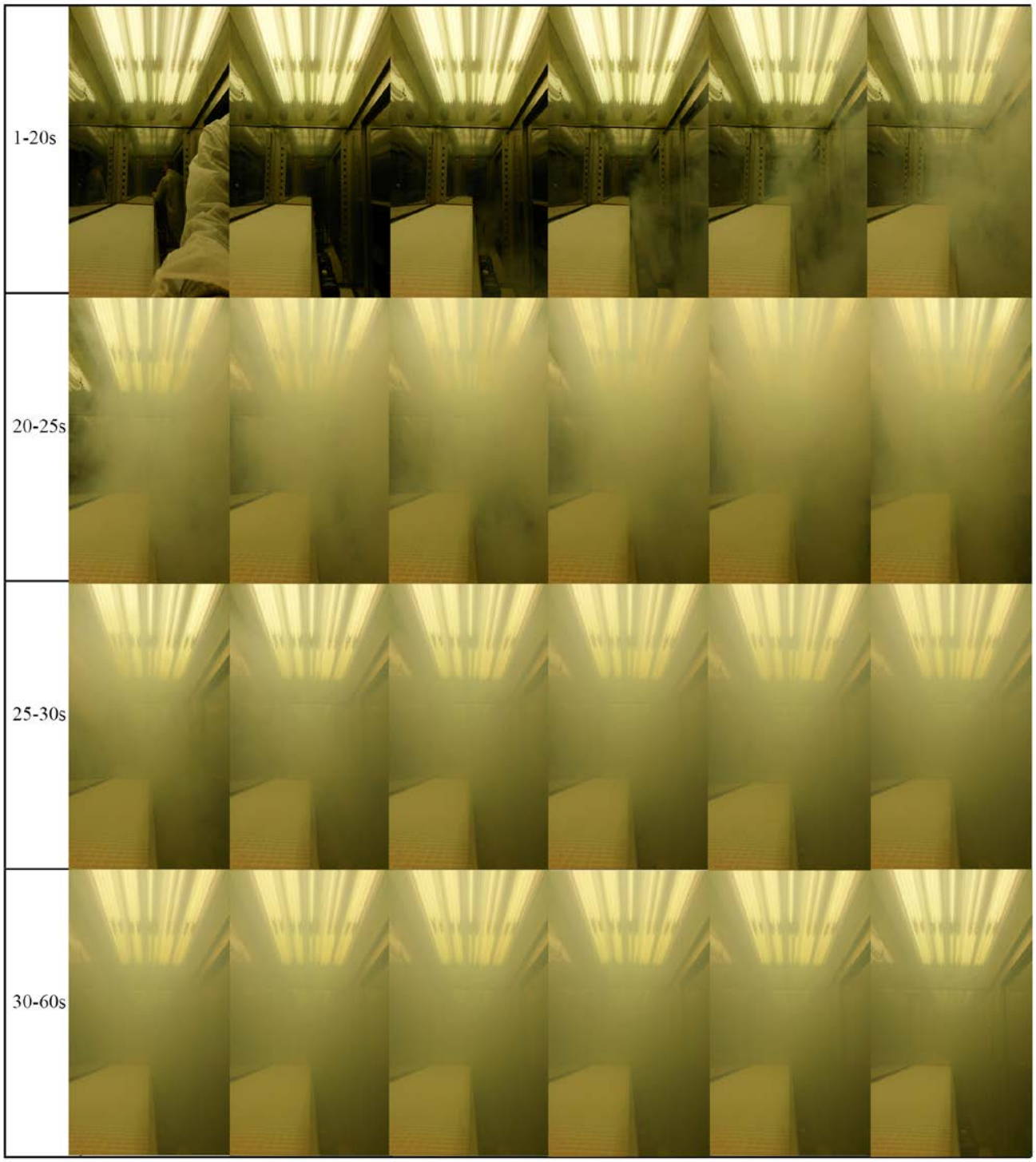


Appendix D. Photos experiment 3



	Outside incubator after two days	Inside incubator after two days	Outside incubator after 34 days	Inside incubator after 34 days
PDA (19,5g)				
LB				

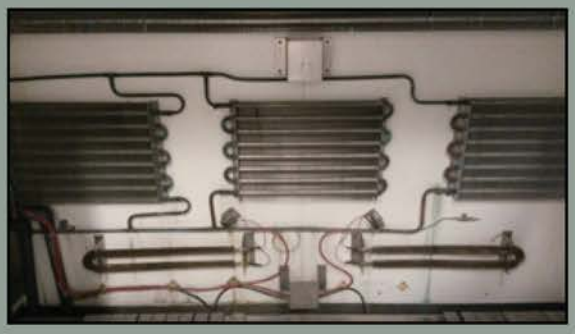
Appendix E. Development of air dispersal inside the incubator in seconds

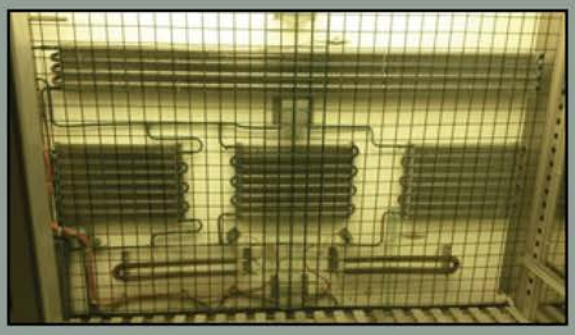


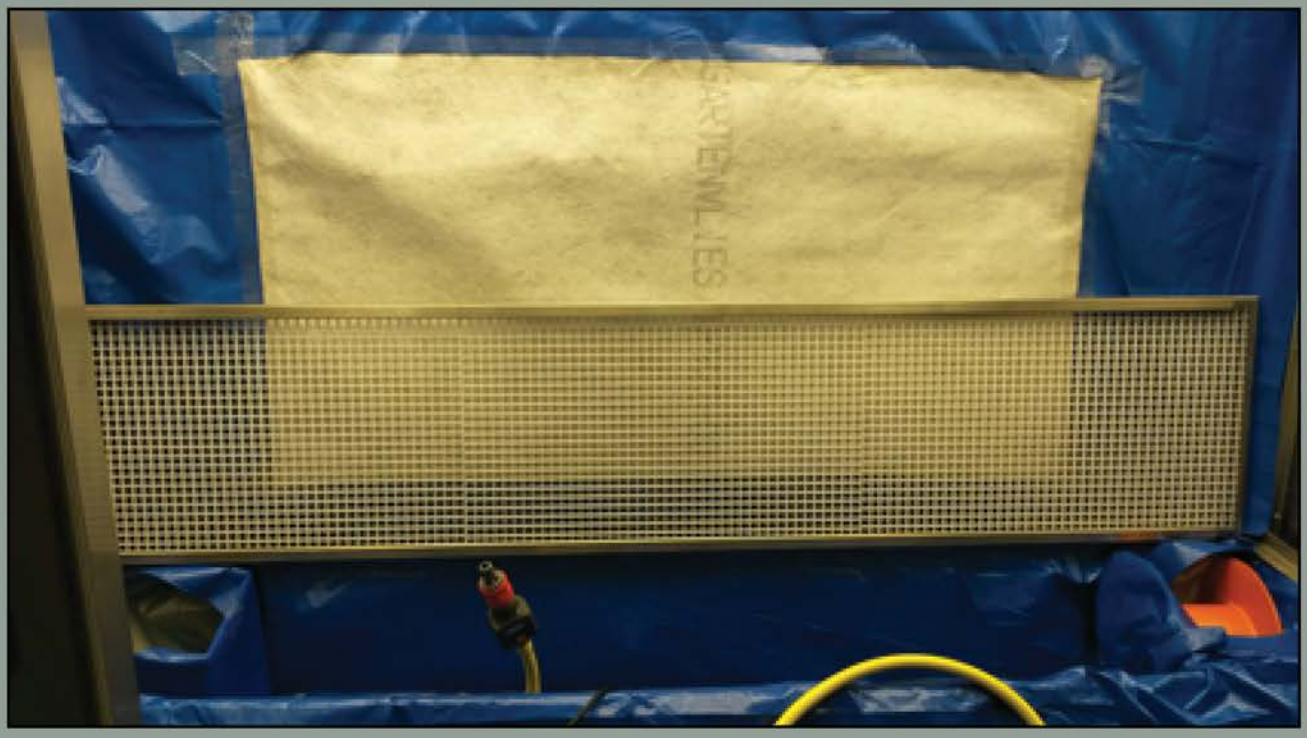
Appendix F. Choosing a suitable biowall

- The dimensions of the system should fit in the enclosed test space and be maximum 2m*2m.d structural requirements.
- The rhizosphere biofiltration needs air fans, an airtight separation between input and output air, an air permeable growth medium and continual watering keeping the rhizosphere moist.
- The watering system needs to distribute water evenly to the plants in the biowall, work continuously, use rainwater, provide nutrients, and optimally be turned off for one hour each week to ensure root health.
- The plants used in the test-setup should be used in common commercial vertical plant walls, be resilient to environmental changes, and preferably help cleaning the air of VOC's.
- The test space needs to be enclosed and air-exchange should be minimized.
- A test space oriented to the north keeps daylighting relatively equal, otherwise suited artificial lighting is needed.
- The test space needs to be smaller than 3m*4m to ensure that relevant test factors are not minimized by the
- Electricity
- Temperature control
- Lighting control

Appendix G. Building of the biowall in pictures







Appendix H. Collateral effects of using the biowall.

Climatological

-Humid air condensates at the cooling elements of the incubator.

- Condensated water drips down behind the vegetation wall, to the floor of the incubator, were it builds up as in a bath tub.

-When the ventilators are turning at a slower rate, the incubator has (most likely) more difficulty in cooling the incubator down.

-Slower air speeds and cooler cooling elements lead to the built up of ice on the back of the vegetation wall.

-Due to the condensation of water, the water storage below the vegetation wall gets emptier over time.

(Needs refilling from the floor of the incubator each week, 7 days is critical value) The system as a whole is watertight.

Biological

- Roots of the plants start invading the growth medium (rockwool) between the plants, and even start forming through the perforated back panel.

-The plants start forming a large amount of aerial roots in front of the vegetation wall.

-There is a built up of algae within the pipes and ducts of the watering system.

-Moss and algae start growing everywhere were there are humid conditions between the plants, on the synthetic felt and rockwool.

Appendix I. Table of soil organisms

Table 3. The concentrations of microorganisms in the soil

* Plant	Weight wet soil (g)	Added demi water (ml)	Water pipetted (ml)	Weight dried soil (g)	Demi water + soil moisture (ml)	Demi water needed per sample (ml)	Demi water needed per pipetted sample (ml)
1	1,00	10	7,4	0,34	10,66	+0,55	+0,4
2	1,01	10	8,2	0,44	10,57	+3,94	+3,1
3	1,58	10	8,0	0,39	11,19	+1,52	+1,1
4	1,06	10	7,8	0,38	10,68	+1,85	+1,4
5	1,15	10	8,5	0,37	10,78	+1,42	+1,1
6	1,10	10	7,1	0,34	10,76	+0,45	+0,3
7	1,08	10	7,7	0,34	10,74	+0,47	+0,3
8	1,55	10	8,5	0,34	11,21	+0	+0,3

To know the concentration of microorganisms per gram of soil, some calculations were needed. In the table it can be seen that demi water + soil moisture = added demi water + (weight wet soil – weight dried soil). Eg: 10 ml +(1,01 g - 0,44 g = 10,57 ml). Then the water needed to dilute to same concentration was calculated, taking the weakest concentration (0,34 g dry soil in 11,21 ml water) as baseline. Eg: 0,44 g * 11,21 ml / 0,34 g = 14,51. 14,51 ml - 11,21 ml = 3,94 ml should then be added to the original sample. However, in meanwhile the water is pipetted back. Using the calculated proportion of the original sample, the water needed per pipetted sample could be calculated. Eg: 8,2 ml * 14,51 ml / 10,57 ml = 11,26. 11,26 ml – 8,2 ml = 3,1 ml. The resulting concentration of the samples was 1,0 g dried soil / 33,0 ml water.

Before inoculating 100 microliter on a PDA plate and a LBA plate each, the sample was 10x diluted. In the data processing one PDA plate and one LBA plate are added together as one sample, the colony count was per 200 μL of soil solution (330 ml/g). This resulted in a colony count per gram of soil x1,65*10^3 in the graphs.