Pro gradu dissertation

The effects of urban indoor farming for intestinal microbiota richness and diversity

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Allergies and immune-mediated diseases are a rapidly growing threat in industrialized countries. During the last few decades, it has become clearer that people distancing from nature might have something to do with this increase. This ImmunoGarden study aims for finding natural solutions for preventing and possibly curing immune-mediated diseases, and the goal for this pro gradu study is to answer the question whether the human intestinal microbiota richness and diversity change due to urban indoor farming or not and do the changes in intestinal microbiota vary in between different research groups using different seedbeds. One of the used seedbeds is designed especially for this experiment, and it has safe and diverse forest- and plant microbiota added in it. The stool samples were collected from volunteers who had done urban indoor farming for three months using either compost soil or microbe-enriched seedbed, and different plants for farming. The 16S rRNA gene was isolated from the stool samples, multiplied in PCR, and analyzed further with Illumina sequencing. With this experiment the urban indoor farming did not significantly change the human intestinal microbiota richness or diversity, but the intestinal microbiotas of the research subjects in both research groups changed through time due to the increased hygiene levels in the beginning of COVID19-pandemic.

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Allergiat ja immuunivälitteiset sairaudet ovat nopeasti kasvava ongelma teollistuneissa maissa. Viime vuosikymmenten aikana on käynyt selväksi, että ihmisten etääntyminen luonnosta on yhteydessä immuunivälitteisten sairauksien yleistymiseen. Tämä ImmunoGarden -tutkimus tähtää immuunivälitteisten sairauksien luonnonmukaisten ennaltaehkäisykeinojen ja hoitomuotojen kehittämiseen, ja tämän pro gradu -tutkimuksen tarkoituksena on selvittää, muuttuvatko ihmisen suolistomikrobiston lajirunsaus ja monimuotoisuus kaupunkiympäristössä tapahtuvan sisäviljelyn seurauksena, ja ovatko suolistomikrobiston lajirunsaus ja monimuotoisuus erilaiset eri kasvualustoja käyttäneiden ryhmien välillä. Yksi kasvualustoista on varta vasten tätä koetta varten suunniteltu, ja siihen on lisätty monimuotoista metsä- ja kasvimikrobistoa. Vapaaehtoiset koehenkilöt harjoittivat kaupunkiviljelyä kolmen kuukauden ajan istuttamalla erilaisia viljelykasveja joko kompostimultaan tai mikrobirikastettuun kasvualustaan, ja heiltä kerättiin ulostenäytteet. Ulostenäytteistä eristettiin 16S rRNA-geenit, jotka monistettiin PCR:lla, ja niille tehtiin Illumina-sekvensointi. Tässä kokeessa kaupunkiympäristössä tapahtunut sisäviljely ei merkittävästi muuttanut ihmisen suolistomikrobiston lajirikkautta tai monimuotoisuutta, mutta ajan kuluessa suolistomikrobistot muuttuivat molemmilla tutkimusryhmillä COVID19-pandemiasta johtuvan hygieniatason nousun seurauksena.

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TERMS AND ABBREVIATIONS

TERMS

Commensal microbiota	Beneficial microbiota that lives in a symbiotic relationship with humans and has positive effects on human health. The microbiota receive benefit usually without harming the human cells as their hosts.
Culture-independent studies	Studies that depend on nucleic acid analysis instead of culture of bacteria of specimens.
Ecological niche	A definition of where and how an organism or population lives.
Prebiotic	A food ingredient stimulating the growth and activity of certain bacteria and thus improving human health.
Probiotic	A food ingredient that contains living micro-organisms that improve the well-being of the human hosts.

ABBREVIATIONS

16S rRNA	A gene that encodes the subunit of a bacterial ribosome that is used in recognizing microbiota. The nucleotide base sequence composition varies at certain regions, which include so called V regions that are conserved among all bacterial groups. Exploring the V

	regions provides taxonomic information as signatures of different bacterial groups.
ANOVA	Analysis of Variance, a statistical test that compares the means of three or more different groups, in which the same subjects are present in each of the groups.
ASV	Amplicon sequence variant.
OTU	Operational taxonomic unit.
SCFA	Short-chain fatty acid.
PAH	Polycyclic aromatic hydrocarbon, an air pollutant especially in urban environments. Usually released to air in exhaust gases.
PERMANOVA	Permutational multivariate analysis of variation, a statistical test to compare previously defined groups of data to test the null hypothesis that all the data groups have equivalent centroids.
PCoA	Principal Coordinate Analysis, a statistical, multivariate analysis method where dissimilarity can be analyzed as a proximity matrix.
TGF-β1	Transforming growth factor–β1, a widely effective cytokine that has a significant role for example in repairing tissue damage

1 INTRODUCTION

1.1 Standard human intestinal microbiota and its diversity

Humans have a very diverse microbial community in their intestines, a microbial community that is typical for omnivorous mammals (Tannock 2017). Human intestines include a vast variety of different, rather stabile microbes, a whole dynamic microbial ecosystem that consists mostly of anaerobic, Gram-negative bacteria (Maslowski and Mackay 2010). Only about 1 % of the intestinal microbiota consists of archaea or fungi (Eckburg et. al. 2005). The functions of the intestinal microbiota are controlled both with allogenic and autogenic factors (Tannock 2017). The most common bacterial communities in human intestines are *Firmicutes, Bacteroidota, Proteobacteria, Actinobacteria* and *Verrucomicrobia* (Tannock 2017).

From these five most abundant phyla the *Firmicutes* and *Bacteroidota* cover approximately 90 % of the human intestinal microbiota, and they have many functions inside the human body (Qin et. al. 2010). For example, *Firmicutes* include the most active carbohydrate digesters, and they play an important role in the energy intake (Tannock 2017). They are often referred to as the "bad guys" of the human intestinal microbiota, since they are active on glucose and fat metabolism, and are correlated with obesity and diabetes (Qin et. al. 2010). The *Firmicutes* feed on fat, protein, sugar, and starch, and thus are often the most abundant phylum for Western people (De Filippo et. al. 2010).

Bacteroidota are the main phylum of the Gram-negative bacteria inside the human intestines (Cani et. al. 2007). They are generally good for humans when they stay inside the intestines (Cani et. al. 2007). Still, they can derive pro-inflammatory molecules such as lipopolysaccharides (Cani et. al. 2007). This doesn't fit into the common thought of *Bacteroidota* being beneficial for human health, but can be

explained by the lower endotoxic activity of the lipopolysaccharides derived from *Bacteroidota* (Zhang et. al. 2009). The consensus about the phylum *Bacteroidota* is that they are good for human health and are important in breaking down the starch and cellulose (Zhang et. al. 2009).

Many older studies claim that there are about 100 billion individual microbial cells in one gram of the intestinal mass (Rosner 2014, Sender et. al. 2016). This means that the number of microbial cells in human body outnumbers the number of human cells by a tenfold (Tannock 2017). With the genetic variation the difference is even greater: there are around 100 times more different genes in the intestinal microbiota, than in human cells (Tannock 2017).

Nowadays these previously calculated numbers of human cells and microbial cells inside the human body are controversial (Sender et. al. 2016). The research methods have developed rapidly, and thus the numbers of cells can be calculated more carefully (Sender et. al. 2016). One recent study by Sender et. al. estimates the number of microbial cells in and on the human body to outnumber the number of the human host cells only by 2- or 3-fold (Sender et. al. 2016). Still, the colon massively dominates the positioning of the microbiota, and therefore the estimate of 10^{14} bacterial cells remain correct for the gastrointestinal tract (Sender et. al. 2016).

The composition of human intestinal microbiota varies hugely in between individuals (Spor et. al. 2011). Even though the variation rate is high, the compositions of intestinal microbiotas vary less in between human individuals than with humans and other mammal species (Tannock 2017). The main things that affect the individual intestinal microbiota composition are food, and some environmental factors such as travelling, the contact with nature or animals, and persons own physical activity (DeSalle and Perkins 2015).

The intestinal microbiota varies also according to age, and the vast majority of the changes happen in early childhood (DeSalle and Perkins 2015). The birth and breastfeeding are the critical turning points for a child developing a diverse microbial community: newborns and infants get their microbial exposure from their

caregivers during vaginal childbirth, breastfeeding, and skin-to-skin contact (Fehr et. al. 2020). The core of the human intestinal microbiota is formed in the time from birth to approximately 3 years of age (Yatsunenko et. al. 2012). Nowadays the growing trend of washing hands before touching the children, giving birth by cesarean sections, and bottle-feeding the babies have resulted in decrease of the babies' skin- and gut microbiota, increase in infant dysbiosis, and rapid increase of immune-mediated diseases in small children (Keag et. al. 2018, Fehr et. al. 2020).

When examining the Hadzas, hunter-gatherer -tribe in Tanzania, researchers have found that the human intestinal microbiota varies in between different seasons (Smits et. al. 2017). The 350 stool samples from the Hadza tribe, collected within a year of the experiment, revealed that the human intestinal microbiota has an annual cycle in its composition changes (Smits et. al. 2017). The annual cycle varies along with nutrition and the change of environmental factors, and the overall diversity tends to slightly decrease during summer (Smits et. al. 2017).

It is also expected that the composition of human intestinal microbiota is different in different parts of the intestines, but the research of this topic has huge challenges due to limitations of the research methods (Tannock 2017). When examining the results of the human intestinal microbiota research, one must also remember to be cautious about generalizing the results: the individual variation in human intestinal microbiota is so grand that the results must be presented in such a condensed form that most of the data disappears into its form of presentation (Tannock 2017).

Many physiological functions such as metabolism, organ functions, digestion and mucosal-barrier integrity rely on the symbiotic microbiota-host interplay (Maslowski and Mackay 2010). In nature the microbes live in communities, within strictly specified areas (Tannock 2017). Therefore, it can be deduced that most of the microbial species live in causal and systematic relationship with other microbial species (Costello et. al. 2009). Sometimes these microbial communities are referred to as "supraorganisms", because their functions are equal to the functions of a whole organism (Dominiquez-Bello et. al. 2019). The human intestinal microbiota is this

kind of ”supraorganism” that has a metabolic activity equal to liver (Dominiquez-Bello et. al. 2019).

The main function of the intestinal microbiota is to degrade the food mass that comes to the intestines, so that the human gets more nutrients into their energy metabolism (Frenhani and Burini 1999). The process that is controlled by the intestinal microbiota is called polymeric hydrolysis, where especially the poly- and oligosaccharides from the plant-based food ingredients are broken down (Frenhani and Burini 1999). The intestinal microbiota uses the food and nutrients coming to the intestines via digestion with their own energy metabolism, and they provide their metabolic products such as short-chain fatty acids (SCFA’s), propionic acid, butyric acid, water, carbon dioxide, ammonium, vitamins and some amines and phenols, for human metabolism to use as energy sources (Koh et. al. 2016, Rowland et. al. 2018, Sun et. al. 2017, Tannock 2017). Most of the microbial metabolic products are acid, and they set the pH value of the intestines to the range of 5.5–6.9 (Tannock 2017).

Besides the intestinal microbiota that is beneficial for the human beings, there are also other microbial species inside the intestines (Tannock 2017). These foreign microbial species usually come inside the intestines with food, and sometimes these species end up interacting with the original intestinal microbiota (Tannock 2017). For example, strawberries include 10^7-10^{10} bacterial genes in one gram of the berry, and have a very diverse microbial community, which enriches the intestinal microbiota during digestion (Olimi et. al. 2022).

1.2 The coevolution of human and its intestinal microbiota

Traditionally it has been thought that each species goes through its evolution process on its own (McFadden 2005). With humans and their intestinal microbiota this is unlikely true: many of the evolutionary steps are tightly connected to each other (Cerling et. al. 1998). Long ago some of the ancestral humans might have accidentally eaten something that included decomposing bacteria, these bacteria

started to process the food inside the humans' gut and thus released more nutrients for the humans to use in their energy metabolism (Edwards et. al. 2010). This incident provided a competitive advantage for the humans that had eaten the bacteria, because they had more energy in an easier form to use quickly (Edwards et. al. 2010).

There are two competitive hypotheses about the human-intestinal microbiota - coevolution (Morris et. al. 2012, Morris et. al. 2015). "The Red Queen Hypothesis" suggests that humans and their intestinal microbiotas are in an on-going conflict and trying to develop weapons against each other (Morris 2015). After the "Black Queen Hypothesis" the biological functions the microbiota provide are equal to human energy consumption, and when heading towards the equilibrium in energy production and consumption, the most economical way to organize the microbiota-host-interplay is to have one microbial species per one biological function (Morris et. al. 2012).

It is hard to investigate the differences in intestinal microbiotas in between modern humans and ancient humans due to the organic nature of the mass inside the intestines (Tannock 2017). No samples have been preserved from the ancient humans, and researchers can only guess what their intestinal microbiota could have been like (Tannock 2017). Some guidelines can be drawn when examining the intestinal microbiotas of the modern peoples that live like hunter-gatherers, but with the modern living context it is impossible to generalize the research results to also cover the ancient people (Tannock 2017).

The intestinal microbiome of coprolites, a tribe living in Belgium in the Middle Ages, was successfully sequenced from a specimen found inside a barrel, using microscopy, culture-dependent analysis, and *ad hoc* PCR sequencing (Appelt et. al. 2014). The specimen had some environmental microbiota in and on it, and thus the results cannot be thought as fully reliable, but they give some guidelines about the human intestinal microbiota in the Middle Ages (Appelt et. al. 2014). The most abundant phyla in the specimen intestinal microbiota were *Proteobacteria,*

Gemmatimonadetes, Actinobacteria and Bacteroidota, which all still belong in the most common bacterial phyla in human intestines (Appelt et. al. 2014, Tannock 2017).

1.3 Studying the human intestinal microbiota

Studying the human intestinal microbiota has been challenging and not in the center of interest for researchers because of the negative attitude towards it (DeSalle and Perkins 2015). For a really long time microbiota was thought to consist of only pathogens, and they were despised and feared even in the research field (DeSalle and Perkins 2015). The first mentioning about human intestinal microbiota research is from late 1800's, but the research methods then were too incomplete to reach any reliable results (Tannock 2017). Only after 1960's the methods had improved enough to make progress in researching the human intestinal microbiota (Tannock 2017).

During the last few decades, the attitude towards microbiota has changed for the better and researchers have discovered that the human existence is depending on the symbiosis with the beneficial microbiota (DeSalle and Perkins 2015, Tannock 2017). The observation about humans and microbiota having some common ancestors has also shifted the perspective of the research towards better understanding about the complex relationship in between them (DeSalle and Perkins 2015).

The "Human Genome Project" began almost 30 years ago, altering the ways of DNA sequencing and finally ending up in mapping the whole human genome (Green et. al. 2015). It widened the variety of the research methods for investigating the human microbiota, where the invention of the first culture-independent study methods can be considered as a massive breakthrough (Green et. al. 2015). Before that the culture-dependent study methods were the only ways to examine human intestinal microbiota, which was inefficient due to the huge variety in the microbial diversity (Tannock 2017).

Pretty soon the ”Human Genome Project” got the ”Human Microbiome Project” by its side, where the first mappings of the human intestinal microbiota were made (Tannock 2017). The mapping of the human intestinal microbiota remains incomplete, but the 16S rRNA genome sequencing has provided much more accuracy to it, when comparing to the cell culture methods that were the only ways of doing the microbiota research before (Bartram et. al. 2011, Tannock 2017).

Studying the functional, chemical, and physiological aspects of the human-microbiota -relationships would be vital in achieving the complete understanding of this symbiosis (Cresci and Bawden 2015). All the research of this field currently made is highly speculative since most of the studies have been done in animals or in limited parts of the intestines and therefore can't be generalized to cover humans whole gastrointestinal tract (Tannock 2017). Most of the human intestinal microbiota research is done by collecting stool samples and assuming the intestinal microbiota is similar in all parts of the intestines, but this is unlikely true (Tannock 2017). For example, due to the different functions, metabolic activity rates or pH-values in different parts of the intestines, they provide different living environments for the microbiota (Tannock 2017). With current research methods of the intestinal microbiota, it is not very easy to get microbial samples from the upper parts of the intestines, because the intestines would have to be emptied to get a sample and emptying the intestines would harm the microbiota and give biased results (Tannock 2017). Intestinal catheters are sometimes used to collect samples from human intestines, but the sampling speed is low, and thus the symbiotic relationship with the microbiota and their host cannot be examined (van Trijp et. al. 2021).

The intestinal microbiota composition can be measured in calculating the species overall richness and diversity (Chao et. al. 2016). Richness describes the total amount of microbial species in the sample and gives valuable information about the microbial community that lives in the specimen (Chao et. al. 2016). Diversity can be calculated for example as Shannon's diversity indexes from the microbial DNA- or

RNA-sequences (Chao et. al. 2016). The higher the Shannon index value is, the more diverse the microbial community in the sample is (Chao et. al. 2016).

1.4 Immunity and the immune-mediated diseases

Immunity covers all the physiological and chemical mechanisms that a human body uses to defend itself against all the possible threats (Coico and Sunshine 2015). These threats can be a vast variety of things: for example, pathogens, micro-organisms, microbial metabolic products, nutrients, chemicals, metals, drugs, pollen or animal hair and dandruff (Coico and Sunshine 2015). Most of the immunity mechanisms develop in early childhood, but it improve and adapt for the whole lifetime (Coico and Sunshine 2015).

Immunity can be divided into two categories, innate and adaptive immunity (Coico and Sunshine 2015). The innate immunity is developed inside the womb, and it covers isolation factors such as skin and mucosal barriers, physical reflexes such as coughing, along with phagocytes, interferons, tand proteolytic enzymes (Coico and Sunshine 2015). The adaptive immunity is developed at later life, and it is a combination of antigens and specialized immune cells such as lymphocytes and antigen-recognizing B- and T-cell receptors (Pancer and Cooper 2007). The mechanisms of innate immunity respond rapidly to the possible threats, even in seconds or minutes, but the adaptive immunity mechanisms are slower: they can take days or weeks to form a response (Coico and Sunshine 2015, Pancer and Cooper 2007).

The human immunity mechanisms are based on memory and recognition: the immune cells remember the possible pathogens or other factors causing the immune response and react to them faster and more precisely when they are encountered again (Coico and Sunshine 2015). The B- and T-lymphocytes have numerous specialization possibilities before they have met any antigens, but when encountering the first antigens, they specialize to attack against them, and will not respond to any other antigens (Coico and Sunshine 2015). B-lymphocytes release

antibodies to the blood circulation that immobilize the pathogens, and T-lymphocytes produce cytokines that coordinate the immune response by controlling the functions of the defense mechanisms (Coico and Sunshine 2015).

Normally the immune cells can recognize the difference in between the pathogens and the human cells and attack only against the foreign material (Coico and Sunshine 2015). Sometimes this recognition is disturbed and the immune system attacks something other than a pathogen (Coico and Sunshine 2015). The immune reactions without the presence of a pathogen are called hypersensitivity or autoimmune reactions and they are ordinary immune reactions, but usually with a higher intensity and greater response than with the presence of a pathogen (Coico and Sunshine 2015). In the normal state human immunity system can recognize individual errors in the system and correct them before they become a problem, but sometimes the errors are too grand for the correction, and immune-mediated diseases develop (Coico and Sunshine 2015).

The hypersensitivity reactions can be divided into four different categories I-IV with the Coombs-Gell classification (Rajan 2003). Class I covers the immunoglobulin E (IgE) mediated hypersensitivity reactions, that are usually called allergies (Rajan 2003). In class II are all the humoral, cytolytic or cytotoxic reactions where immunoglobulin M (IgM) or immunoglobulin G (IgG) accidentally bind onto a human cell surface receptor and thus trigger an immune reaction that usually kills the host cell (Rajan 2003). Class III consists of complex immune reactions where IgM or IgG form complexes with their antigens, and these complexes gather in blood circulation or in tissue, accumulate the granulocytes to the area, and thus release lytic enzymes to the area (Rajan 2003). In class IV are the cell-mediated, delayed-type hypersensitivity (DTH) reactions that have no antibodies, but T-cell dependent CD4- and TH1-cells that release macrophage-activating cytokines to the area (Rajan 2003).

Immune-mediated diseases such as allergies, asthma and type 1 diabetes are nowadays a rapidly growing problem, especially in urban areas (Grönroos et. al. 2019, Barni et. al. 2020, Roslund et. al. 2021). They affect negatively to the lives of

those fallen ill, because the consequences of these diseases can be very severe (Sakaguchi et. al. 2008). What is different in immune-mediated diseases compared to other immunological diseases is their origin: in autoimmune diseases the persons own immune system attacks itself, when in other hypersensitivity disorders there usually is either mechanic, metabolic, toxicologic, pharmaceutical or functional disorder (Anvari et. al. 2019). Immune-mediated diseases are not contagious, but they cause a significant risk for people and their costs for health care seem to be massively increasing (Roslund et. al. 2021).

1.5 Effects of the diversity of the intestinal microbiota to human immunity

Intestines are considered as the largest immune organ a human being has, and the intestinal microbiota has a major role in its functions (Zou et. al. 2021). Immune homeostasis would not be possible to achieve and maintain without the highly regulated interaction of the intestinal microbiota and its host (Samuelson et. al. 2015). The intestinal microbiota and the pulmonary immunity have a recently confirmed, but forever on-going relationship that is crucial for the survival of both its parties (McAleer and Kolls 2018). With animal studies it has been proved that the intestinal microbiotas of the sick animals differ from the intestinal microbiotas of the healthy individuals (Tannock 2017).

It is commonly thought that a pathogen is the one making a person ill (DeSalle and Perkins 2015). Surprisingly this not always the case: sometimes the real reason for a human falling ill is the imbalance in the normal human microbiota, a phenomenon called dysbiosis (DeSalle and Perkins 2015). This dysbiosis can be caused by a pathogen, but it can also be caused by numerous other factors (DeSalle and Perkins 2015, Tannock 2017). Therefore, it would be more beneficial to examine the ecological balance of the intestinal microbiota, than an individual microbe and its possibility to act as a pathogen (DeSalle and Perkins 2015).

The short-chain fatty acids (SCFA's) are the main intestinal microbiota metabolites that have a crucial function in intestinal microbiota homeostasis (Maslowski et. al.

2009). High levels of the SCFA´s are expected to improve the pathological outcomes for example in respiratory infections such as influenza, respiratory syncytial virus, or the newly spread SARS-Cov2 (Corrêa-Olivieira et. al. 2016, Sencio et. al. 2022). A study done in SARS-CoV2 infected male hamsters suggested that during the infection the taxon of deleterious microbiota increases while the taxa of the SCFA producers decreases (Sencio et. al. 2022). Alteration mechanisms such as tissue damage and reduced anti-viral response were observed (Sencio et. al. 2022). Therefore, it can be deduced that the diversity of the intestinal microbiota has a direct effect on whether a human catches the respiratory virus or not, and how they recover from it (Sencio et. al. 2022).

The "gut-to-lung axis" is a relatively recently discovered connection in between the intestinal microbiota and the possibility to get respiratory infections (Sencio et. al. 2022). More instrumental tools are needed to fully understand its functions, but it is already certain that the intestinal microbiota has a crucial role in immunity and in recovering from infections (Sencio et. al. 2022).

The composition of the intestinal microbiota affects directly the intestinal epithelial barrier, and therefore has a significant role in whether the pathogens enter the blood circulation or other organs or not (Sencio et. al. 2022). For example, the disruption of the intestinal microbiota homeostasis can weaken the intestinal epithelial barrier and increase the portal influx of bacteria or their metabolic products into the liver (Sencio et. al. 2022). This event is most likely to cause an inflammation or even an injury in the liver, or indirectly have effects on serum lipid, lipoprotein, cholesterol, and triglyceride levels due to the changes in liver functions (Sencio et. al. 2022).

1.6 The biodiversity hypothesis

According to the biodiversity hypothesis the autoimmune diseases have become more common due to too hygienic, modern living environment (Haahtela 2019). The biodiversity hypothesis was first called hygiene hypothesis, but the name was changed due to the idea of the global biodiversity decline having a massive impact

on this phenomenon as well (Grönroos et. al. 2019). Three types of evidence have yet been found to support this hypothesis: in many immune-mediated diseases the personal microbiota of an ill person is reduced, the level of urbanization and other characteristics of the environment have a connection with the prevalence of immune-mediated diseases, and the diversity of the environmental microbiota has an association with this kind of diseases (Hanski et. al. 2012, Kondrashova et. al. 2005, Ege et. al. 2012).

This phenomenon is most likely caused by the biodiversity loss in urban environments and the urban lifestyle including processed food, use of antibiotics and too high hygiene levels (Roslund et. al. 2021, Noverr et. al. 2008). The highest risk for developing an immune-mediated disease is at childhood: if the exposure to diverse microbiota is inadequate then, the possibility of future health problems gets higher (Kondrashova et. al. 2012). Also, childhood infections, or the complete lack of them, have a huge impact on the risk of developing an immune-mediated disease (Kondrashova et. al. 2012). In cities the microbial biodiversity decreases mostly due to air pollutants such as polycyclic aromatic hydrocarbons (PAH's), and this has a direct impact on human microbiota, especially to those microbes accountable for immune defense (Roslund et. al. 2021).

The connection between high levels of PAH compounds, air pollutants in urban environments and the biodiversity loss in human microbiota requires more research, since with current information it cannot be fully proved (Roslund et. al. 2021). A recent study with daycare children exposed to increased levels of safe and diverse microbiota showed that the commensal microbiota of children living in different environments (traditional city daycare, nature-oriented daycare and a daycare going through a biodiversity intervention) varied in between the different groups (Roslund et. al. 2020). Most of the differences in between the different child daycare groups were observed in alpha-, beta and gammaproteobacteria (Roslund et. al. 2020). Especially the diversity of the skin gammaproteobacteria may have a significant connection with the functions of the human immune system, since it affects the level of TGF-β1 cytokine in blood serum, which has a direct effect on the

number of T-cells in blood circulation (Roberts and Sporn 1990, Roslund et. al. 2020). Therefore, it can be deduced that the immune systems of the children living in different environments function in different ways (Roslund et. al. 2020). During the 28-day experiment significant differences towards increasing diversity of skin- and intestinal microbiota were observed in the groups of daycare children living in different environments (Roslund et. al. 2020). The two-year follow-up research showed that these changes in the diversity of the skin- and intestinal microbiota were rather permanent (Roslund et. al. 2020). Similar kind of studies have not yet been made in adults (Roslund et. al. 2021).

Some of the peoples in modern world still live like the ancient hunter-gatherers, and when mapping their intestinal microbiota, the researchers found that the taxon *Treponema* has completely disappeared from the urban people's intestines (Tannock 2017). The *Treponema* bacteria decompose certain polysaccharides that are present in some plant roots, which are not a part of modern human's diet (Tannock 2017). When comparing the intestinal microbiotas of humans and apes, the number of different species inside the intestines is lower for humans (Tannock 2017). On the other hand, people moving into urban environments has exposed them to whole new, urban microbiota, but this exposure does not significantly change the diversity of human intestinal microbiota (DeSalle and Perkins 2015).

When comparing the intestinal microbiota composition of the hunter-gatherer Hadza-tribe in Tanzania to 16 other, modern countries human population, it has become clear that the feature that differentiates industrialized and traditional populations are the microbial taxa that varies the most in between different seasons (Smits et. al. 2017). Therefore, it can be deduced that the human intestinal microbiota changes due to the modernization of the environment (Smits et. al. 2017).

1.7 Altering the human microbiota

Improving the living environment for the human intestinal microbiota has not been in the center of attention of the researchers very long (Tannock 2017). Usually, the

altering process for human intestinal microbiota covers only the changes in the nutrition and for example adding prebiotics and probiotics to their diet (Tannock 2017). In a best possible situation, the diverse nutrition, especially one with high amounts of starch, cellulose, and hemicellulose, feeds the intestinal microbiota like fertilizer feeds the plants (Tannock 2017).

The more diverse the human microbiota is, the more beneficial impact it has on human health, and the better it can stand the changing environment (Grönroos et. al. 2019). A safe exposure to materials like soil or plants with high levels of naturally diverse microbiota immediately increases the diversity of human microbiota (Grönroos et. al. 2019). The contact with naturally diverse microbial communities is probably very beneficial for the commensal microbiota in humans, but more detailed research is needed to prove this completely (Nielsen et. al. 2020).

The composition of the human intestinal microbiota also alters itself (Clemente et. al. 2012). The microbiota that has no role gets removed from the intestines, as well as the ones that have too much competition inside their ecological niche (Tannock 2017). The natural selection inside the intestines can happen in different ways: the selection can favor similar microbial species up to a certain point, it can limit the number of species according to the limitations of the resources, or it might choose the conserved microbial species by the impact they have on the intestinal microenvironment (Tannock 2017).

In an experiment done with two volunteers rubbing their hands with 16 different soil, plant, and moss materials, it was discovered that the skin microbiota changed immediately and at least temporarily (Grönroos et. al. 2019). The diversity of *Acidobacteria*, *Actinobacteria, Bacteroidota, Proteobacteria* and *Alpha- Beta and Gammaproteobacteria* increased in the skin swabs (Grönroos et. al. 2019).

The modern medicine focuses on defending human bodies from the pathogens with drugs (DeSalle and Perkins 2015). This way of thinking is a bit old-fashioned, since nowadays it has become clear that for example the antibiotic resistance is a huge threat for human existence, and this way of treating pathogens is only speeding this phenomenon up (DeSalle and Perkins 2015). The drugs, such as antibiotics, kill also

the beneficial human microbiota and expose people to even more dangerous diseases, so the treating against pathogens should focus more on improving the human defense mechanisms instead of killing the pathogens (DeSalle and Perkins 2015).

1.8 Aims, details and hypotheses of this experiment

The ImmunoGarden study investigates the connection of safe and diverse exposure to intestinal microbes (Nature-Based Solutions 2021). This study is a part of Nature-Based Solutions -research entirety that has a goal of finding and developing nature-based solutions for health problems (Nature-Based Solutions 2021).

The aim of this pro gradu dissertation was to find out if persons intestinal microbiota richness and diversity change along with exposure to a safe and diverse plant and soil microbiota. Examining the connections in between the human intestinal microbiota composition, its alteration methods and human immune system is very important, to collect proof of the biodiversity hypothesis and develop solutions in preventing and curing the immune-mediated diseases.

The stool samples were collected from voluntary people who live in urban environments and were willing to practice urban indoor farming in their apartment or balcony. This urban indoor farming lasted for three months, and three samples were collected: a baseline sample before starting the experiment, one sample after one month of urban indoor farming, and the last one after three months of urban indoor farming.

Two different, stable seedbeds were used and randomly divided for the research subjects. The stability of the seedbed was considered as a vital safety issue since the microbiological consistency had to be ensured to stay healthy during the whole experiment.

The alpha- and beta diversities were examined from the bacterial communities in the stool samples. Overall richness, Shannon's diversity indexes and Faith's

Phylogenetic Diversities were calculated to address the differences in the alpha diversity, and Bray-Curtis's metrics were measured and compared with PERMANOVA statistical test to examine the beta diversity. A more detailed inspection was also made for the five most abundant phyla in the human intestinal microbiota, and the differences in them were tested with repeated measures of ANOVA and paired samples t-test.

The hypothesis for this study was that the microbial richness and diversity of the human intestinal microbiota increase in both research groups because of this experiment, but only in between the first two time points. The increase in the microbial richness and diversity was expected to be greater in the enriched group than in the control group. The worldwide COVID19-pandemic began after one month of this experiment, and the season changed from winter to spring, so it was expected that the microbial richness and the microbial diversity decrease in between the last two time points in both research groups due to the rapidly increased levels of hygiene and seasonal variation.

2 MATERIALS AND METHODS

2.1 Experimental design

This experiment was designed by the ADELE research group in 2018-2019. The samples were collected from the voluntary people in February, March, and May 2020. I joined the group to make the rest of the analysis in spring 2021.

The research subjects (n = 32, 8 males and 24 females, ages 31–74) were chosen for this indoor farming research according to voluntarity. To get accepted as a research subject for this study, a person had to be over 18 years old, live in urban environment, and not have a regular contact with nature via their work or hobbies. People with immune deficiencies, immunosuppressive medications, diabetes, memory disorders, acute depression, psychosis, or cancer were executed and did not qualify as research

subjects. If a person had had at least three severe infections within a year, they could not participate in this experiment either. The research subjects received a full medical examination before starting the experiment. The regional ethics committee of Tampere University hospital evaluated the research plan and delivered a favorable statement with the permit number R19077.

The research subjects were divided into two different subgroups, 15 persons to group A and 17 persons to group B. Each group was given a different seedbed for indoor farming in urban environment. The first research group (group A, control group) got some high-quality compost soil as a seedbed (seedbed A). The second research group (group B, enriched group) received a seedbed enriched with the diverse bacterial dust developed in ADELE-research group (seedbed B), a seedbed that includes 4–9 x 10^9 bacteria in 1 g of seedbed (Nurminen et. al. 2018). The bacterial dust was manufactured by combining different forest- and compost soil materials along with some plant debris, horticultural peat, sludge, tree bark mulch, leaf litter and animal dung (Nurminen et. al. 2018).

The experiment was double-blinded, so neither the researchers or the research subjects knew which research subject received seedbed A and which research subjects received seedbed B. The division for the two groups was performed by computer simple randomization method, and the program was given instructions to try to make the groups as homogenous as possible in terms of research subjects' sex and age.

The research subjects also received plant boxes and a package of plant seeds of the season (herbs, peas, carrots, radish, salads, tomatoes, potatoes) to be implanted into the received seedbed. The aims and details of this experiment can be found in Appendix 1.

The research subjects took care of their plants by watering, uprooting, and harvesting them, regularly for 5–10 minutes a day with their bare hands. They received education on how to take care of plants in urban environment before the beginning of the study. The letter that was sent to all research subjects before starting the experiment can be

found in Appendix 2, and the instructions for the urban indoor farming are presented in Appendix 3.

Interviews were also made to the research subjects on every time point. They were asked about their contact with nature or animals, their diet, their drug intake, if they had fallen ill (especially if they had a stomach flu), and other questions about their recent behavior that might affect the results of this experiment.

2.2 Sample collection and microbial analyses

Stool samples were collected from the research subjects before starting the experiment (0 months, the baseline sample; February 2020), and then one month (1 month; March 2020) and three months (3 months; May 2020) after daily urban indoor farming. The research subjects took the samples themselves and stored them in their own freezer (-20 ˚C) until handing them to the researchers. After that the stool samples were stored in -80˚C until the DNA isolation. The instructions about stool sample collection can be found in Appendix 3.

The DNA from the samples was isolated from the stool and seedbed samples using Qiagen PowerSoil -isolation kit (MoBio Laboratories, Inc., Carlsbad, CA, USA) and the manufacturer's protocol. A Qiagen vacuum was used to get the samples through the filter columns. Distilled water was used as negative control in each round of isolation, increasing the total amount of further analyzed stool samples to 103. The full sample sheet can be found in Appendix 4.

After the isolation the concentration of the DNA was measured using Picogreen method and PerkinElmer Wallac Victor3 1420 Multilabel Counter -device, to figure out whether the samples needed diluting. At the same time the success of the isolation was evaluated when measuring the negative control samples with Picogreen as well. The samples were diluted to distilled water to reach the target concentration of approximately 0.4 µg/ml for PCR.

Then the PCR was run with SimpliAmp Thermal Cycler to multiply the V4 regions of the 16S rRNA sequences in the samples with the 515F and 806R primers (60 base pairs) for PCR reactions, to separate only the bacterial 16S rRNA from the samples (Caporaso et. al. 2012). The volume of the samples in PCR was the total of 50 µl and the cover of the lid was warmed to 105˚C. Three replicates of each stool sample were run with the PCR, and the program consisted of 25 cycles. The details of the PCR program can be found at the table 1.

Table 1: The PCR program used to multiply the 16S rRNA sequences in the samples.

	Temperature (˚C)	Time (s)	Stage
1.	98	300	1
2.	94	60	
3.	50	10	2, repeated 25 times
4.	72	60	
5.	72	600	3
6.	4	Stored until next morning	

When the PCR was completed, the purity of the samples was checked with 3% agarose gel electrophoresis and the gels were imaged with BioRad. From the samples the bands of only the targeted size were observed, and the negative controls remained negative.

After this being done, and the quality of the PCR samples was accepted, the samples were collected into two separate 96-plates, stool samples to one, and the negative controls to another. The samples were then sent to FIMM Laboratory in Meilahti, Helsinki, to get further analysed with Illumina sequencing.

In the Illumina sequencing the index PCR was done in a volume of 20 µl containing 1µl of the amplified template product, 10 µl of 2x Phusion High-Fidelity PCR Master Mix, 0.375 µM of index primer 1 and 0.375 µM of index primer 2. The reaction mix was brought to a final volume with distilled water. The details of the index PCR for Illumina can be found at the table 2.

Table 2: The PCR program used to multiply the 16S rRNA sequences for Illumina sequencing.

	Temperature (˚C)	Time (s)	Stage
1.	98	30	1
2.	98	10	2, repeated 8 times
3.	65	30	
5.	72	20	3
6.	72	5	Final extension

After PCR samples were measured with LabChip GX Touch HT DNA High Sensitivity Reagent Kit (Perkin Elmer, Waltham, MA, USA) to check that the PCR had worked, and the product size was correct. Samples were pooled together in equal volumes and purified with Agencourt AMPure XP beads (Beckman Coulter, Brea, CA, USA) twice, using 0.8X volume of beads compared to the sample pool volume. Ready amplicon library was diluted (1:10 or more if needed) and quantitated with Agilent 2100 Bioanalyzer High Sensitivity DNA Analysis Kit (Agilent Genomics, Santa Clara, CA, USA). Pool was sequenced with Illumina MiSeq System using Illumina MiSeq Reagent Kit v3 600 cycles kit (Illumina, San Diego, CA, USA). The Illumina sequencing process was performed in paired end method.

2.3 Illumina sequencing analyses in QIIME 2

The results of Illumina sequencing were analysed as amplicon sequence variants (ASV's) in QIIME 2 (version 2022.2) and UNIX environments. Other than 16S rRNA sequences were removed from the analysis in QIIME 2 demux-script, so they would not interfere with the results (Estaki et. al. 2020). Samples with less than 24 000 sequences were excluded from the analysis, and the analysed sequences were trimmed from 240 (forward) or 220 (reverse) base pairs to cut off the primers and avoid noise in the further analysis (Estaki et. al. 2020).

The sequences with frequency lower than 10 were removed from each sample in QIIME 2, making the lowest accepted frequency 41. DADA2-script was used to

denoise the sequencing data from chimeras and to remove the negative controls from the further analysis, and to normalize the data with rarefaction and sampling depth control (Estaki et. al. 2020). Also, the *Mitochondria, Eukaryotes, Chloroplasts* and *Archaea* were removed with the DADA2-script to avoid noise in the further analysis, since they often seem like bacterial species in the comparisons. The sampling was rarefied with setting the sampling depth to 7 600 to normalise the data and to make it more meaningful to compare the sequencing results, as the different samples have unequal amounts of ASV reads (Estaki et. al. 2020). At this point, also the sample with the frequency of 41 sequences was removed. The denoised and normalized sequencing data was then compared to SILVA reference database to identify the bacterial taxonomy in the samples (Pruesse et. al. 2007).

The research subject KV05 had to be excluded from the result analysis because something had gone wrong in the Illumina sequencing, and only 182 sequences were observed. Also, the research subject KV15 had to be excluded from the result analysis. They had mixed up the labels of the stool sample containers and it was not clear which sample was which.

2.4 Statistical analyses

Kruskal-Wallis's test is a statistical test that determines whether there is a statistical difference in between the medians of three or more groups of data, that is not normalized (Kruskal and Wallis, 1952). It was chosen to measure the statistical difference in this data set because the ASV calculation is most likely uneven.

QIIME 2 was used to draw the more detailed diagrams and to perform the Kruskal-Wallis's test for the three different metrics of alpha diversity: observed numbers of ASV's, Shannon's diversity index values and Faith's Phylogenetic diversity metrics. The observed numbers of ASV's provide information of the overall richness in the samples. Shannon's diversity index is a weighted, geometric mean of the overall richness that is less sensitive to the occurrence of rare ASV's in the sample as the

observed number of ASV's (Shannon 1948). Shannon's diversity index values were calculated in QIIME 2 using the formula

$$-\Sigma p_i ln\ p_i \quad (1)$$

where the p_i is the proportion of each ASV (Shannon 1948). The Faith's Phylogenetic diversity metrics estimate the biodiversity of a sample by comparing it to the information about the branch lengths in the phylogenetic tree (Faith 1992).

The beta diversity among the stool samples was approached by Principal Coordinates analysis, viewed by ordination using multi-dimensional scaling. The dissimilarity in between two samples was measured as Bray-Curtis's distance and visualized as a map of non-Euclidean distances (Jolliffe and Cadima 2016).

A permutational multivariate analysis of variance (PERMANOVA) is a non-parametric statistical test that is a geometric partition of variation across the data, defined with one or more specific factors (Anderson 2017). That was performed as a statistical analysis for the beta diversity using the Bray-Curtis distances, to measure the dissimilarities in the composition of the different ASV's in the stool samples (Anderson 2017).

A repeated measures of analysis of variance (ANOVA) statistical test was performed in SPSS Statistics to test the statistical significance of the differences in the abundances of the five most abundant phyla on the human intestinal microbiota, in between the three time points within each research group. ANOVA is a test that compares the means of three or more different groups, in which the same subjects are present in each of the groups (Schober and Wetter 2018). A paired samples t-test was performed to compare the two different groups in each of the three time points. The paired samples t-test is used to measure the statistical difference in between the means of two groups of samples where each of the samples can be paired with an observation in the means of another group (Kim 2015). Simplified diagrams and further analyses were made in Microsoft Excel and SPSS Statistics.

3 RESULTS

In this experiment the bacterial 16S rRNA was isolated from the stool samples, multiplied in PCR, and sequenced with Illumina to see if the urban indoor farming influenced the human intestinal microbiota richness and diversity or not.

3.1 Alpha and beta diversity in the stool samples

3.1.1 Alpha diversity: observed numbers of ASV's

The observed numbers of ASV's were calculated in QIIME 2 to measure the overall bacterial richness in the stool samples (Figure 1). The temporal pattern seen in the Figure 1 is similar in between the control group and the enriched group (Figure 1). The observed numbers of ASV's start to increase for both research groups, but then decrease in between the 1 month and 3 months time points (Figure 1).

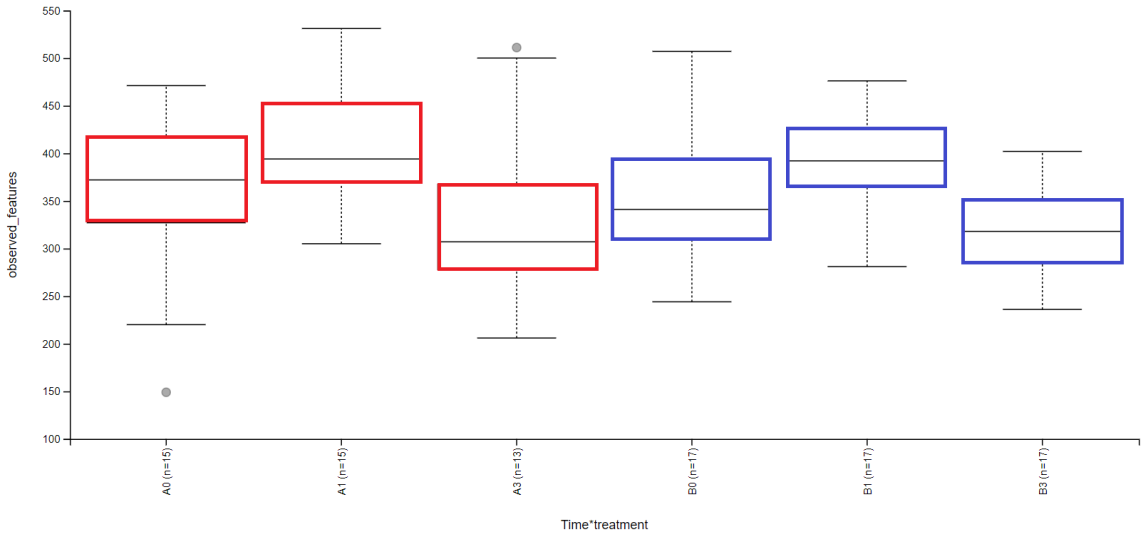

Figure 1: The temporal change in observed features for the control group (A, red) and the enriched group (B, blue) in three different time points. The boxes show means of the values in the middle, and the first and third quartiles of the values in the edges. The whiskers show the minimum and maximum values in each group. A0-A3 are the average amounts of observed features in control group, in time points 0 months (A0), 1 month (A1) and 3 months (A3), and B0-B3 are the average amounts of observed features in the enriched group, in time points 0 months (B0), 1 month (B1) and 3 months (B3). Observed features are the total numbers of different bacterial species in the samples.

The statistical significances of the differences in the observed numbers of ASV's were calculated in QIIME 2 with the Kruskal-Wallis's test (Table 3; Appendix 5, 1). From the p-values in the Kruskal-Wallis's test it can be discovered that the decrease in the overall richness in between 1 month and 3 months time points is statistically significant for both groups ($p<0.05$, Table 3; Appendix 5, 1), but there's no statistically significant difference in between the two research groups ($p>0.05$ in all time points, Table 3; Appendix 5, 1).

Table 3: A Kruskal-Wallis's statistical test to see if there were any statistically significant differences in the observed numbers of ASV's in between the different time points or treatment groups. A0-A3 are the numbers of observed ASV's in control group, in time points 0 months (A0), 1 month (A1) and 3 months (A3), and B0-B3 are the average numbers of observed ASV's in the enriched group, in time points 0 months (B0), 1 month (B1) and 3 months (B3).

Observed	Group 1	Group 2	H	p-value	q-value
Control group	A0 (n=15)	A1 (n=15)	1.39898	0.23690	0.32304
	A0 (n=15)	A3 (n=13)	1.49100	0.22206	0.32304
	A1 (n=15)	A3 (n=13)	5.96564	0.01459	0.07294
Enriched group	B0 (n=17)	B1 (n=17)	3.26990	0.07056	0.15120
	B0 (n=17)	B3 (n=17)	2.40274	0.12112	0.22711
	B1 (n=17)	B3 (n=17)	10.59446	0.00113	0.00851
Comparison for the two groups	A0 (n=15)	B0 (n=17)	0.32097	0.57102	0.61181
	A1 (n=15)	B1 (n=17)	0.43696	0.50859	0.58684
	A3 (n=13)	B3 (n=17)	0.01095	0.91666	0.91666

3.1.2 Alpha diversity: Shannon's diversity index

The overall bacterial richness and evenness was measured in QIIME 2 by calculating the Shannon's diversity indexes for the stool samples (Formula 1, Figure 2). From the Figure 2 it can be seen that for the control group the Shannon's diversity index values decrease throughout the experiment, but for the enriched group the Shannon's diversity index values start to increase, but then decrease in between the last two time points (Figure 2).

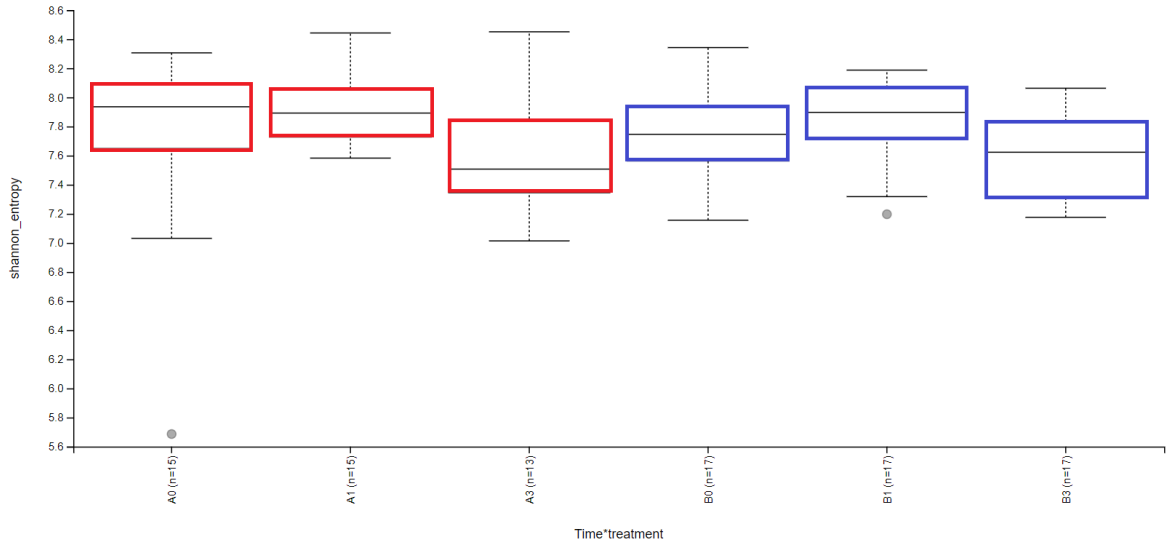

Figure 2: The temporal change in Shannon's diversity indexes for the control group (A, red) and the enriched group (B, blue) in three different time points. The boxes show means of the values in the middle, and the first and third quartiles of the values in the edges. The whiskers show the minimum and maximum values in each group. A0-A3 are the average amounts of observed features in control group, in time points 0 months (A0), 1 month (A1) and 3 months (A3), and B0-B3 are the average amounts of observed features in the enriched group, in time points 0 months (B0), 1 month (B1) and 3 months (B3).

The statistical significances of the differences in Shannon's diversity indexes were calculated in QIIME 2 with the Kruskal-Wallis's test (Table 4; Appendix 5, 2). From the p-values in the Kruskal-Wallis's test it can be discovered that the decrease in the Shannon's diversity index values in between 1 month and 3 months time points is statistically significant for both groups ($p<0.05$, Table 4; Appendix 5, 2), but there's no statistically significant difference in between the two research groups, even though the temporal patterns in the Figure 2 seem different ($p>0.05$ in all time points, Table 4; Appendix 5, 2).

Table 4: A Kruskal-Wallis's statistical test to see if there were any statistically significant differences in the observed numbers of Shannon's diversity indexes in between the different time points or treatment groups. A0-A3 are the values of Shannon's diversity indexes in control group, in time points 0 months (A0), 1 month (A1) and 3 months (A3), and B0-B3 are the values of Shannon's diversity indexes in the enriched group, in time points 0 months (B0), 1 month (B1) and 3 months (B3).

Shannon	Group 1	Group 2	H	p-value	q-value
Control group	A0 (n=15)	A1 (n=15)	0.03484	0.85193	0.98215
	A0 (n=15)	A3 (n=13)	1.72361	0.18923	0.38597
	A1 (n=15)	A3 (n=13)	4.58833	0.03219	0.16095
Enriched group	B0 (n=17)	B1 (n=17)	1.03243	0.30959	0.46438
	B0 (n=17)	B3 (n=17)	2.34928	0.12534	0.31335
	B1 (n=17)	B3 (n=17)	8.07464	0.00449	0.03677
Comparison for the two groups	A0 (n=15)	B0 (n=17)	0.65918	0.41685	0.56843
	A1 (n=15)	B1 (n=17)	0.01747	0.89485	0.98215
	A3 (n=13)	B3 (n=17)	0.01095	0.91667	0.98215

3.1.3 Alpha diversity: Faith's Phylogenetic diversity

The estimates of the bacterial diversity within a certain sample were calculated in QIIME 2 as the Faith's Phylogenetic diversity metrics, which describe the sums of lengths of all the branches in a phylogenetic tree that are members of the minimum spanning path (Figure 3). From the Figure 3 it can be seen that the temporal patterns for both research groups seem rather identical and are also alike the patterns for the observed numbers of ASV's (Figures 1 and 3). For both research groups the Faith's Phylogenetic diversity metrics start to increase in the beginning, but then decrease towards the end of the experiment (Figure 3).

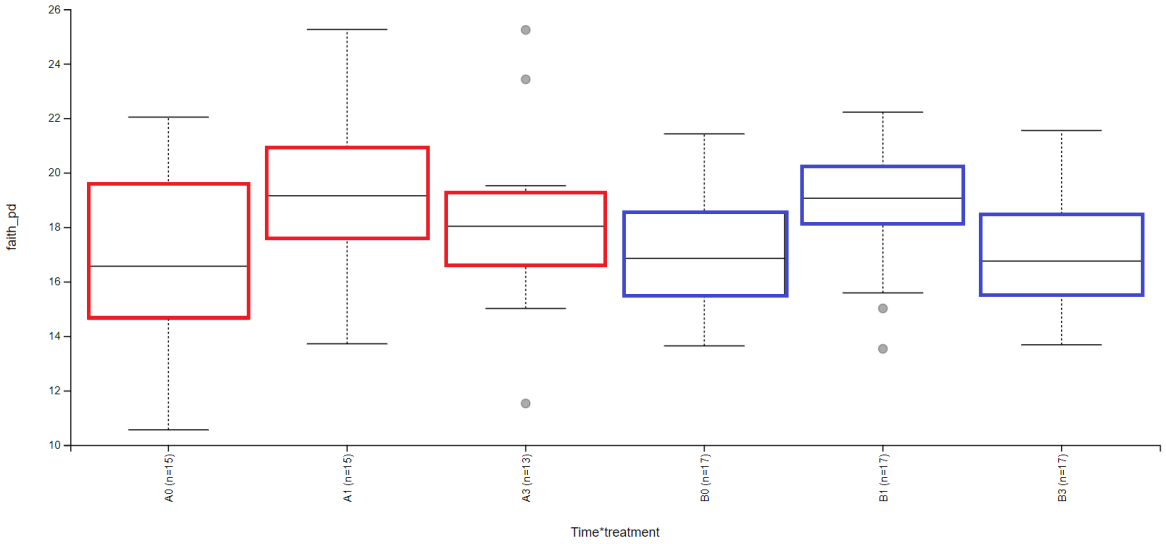

Figure 3: The temporal change in Faith's Phylogenetic diversity (faith_pd) for the control group (A, red) and the enriched group (B, blue) in three different time points. The boxes show means of the values in the middle, and the first and third quartiles of the values in the edges. The whiskers show the minimum and maximum values in each group. A0-A3 are the average amounts of observed features in control group, in time points 0 months (A0), 1 month (A1) and 3 months (A3), and B0-B3 are the average amounts of observed features in the enriched group, in time points 0 months (B0), 1 month (B1) and 3 months (B3).

The statistical significances of the differences in Faith's Phylogenetic diversity metrics were calculated in QIIME 2 with the Kruskal-Wallis's test (Table 5; Appendix 5, 3). From the p-values in the Kruskal-Wallis's test it can be discovered that the increase in the Faith's Phylogenetic diversity metrics in between the baseline sample and the 1 month sample is statistically significant for both groups, and also the decrease in between 1 month and 3 months time points is statistically significant for the enriched group ($p<0.05$, Table 5; Appendix 5, 3), but there's no statistically significant difference in between the two research groups ($p>0.05$ in all time points, Table 5; Appendix 5, 3).

Table 5: A Kruskal-Wallis's statistical test to see if there were any statistically significant differences in the observed numbers of Faith's Phylogenetic diversity in between the different time points or treatment groups. A0-A3 are the average Faith's Phylogenetic diversities in control group, in time points 0 months (A0), 1 month (A1) and 3 months (A3), and B0-B3 are the average Faith's Phylogenetic diversities in the enriched group, in time points 0 months (B0), 1 month (B1) and 3 months (B3).

Faith PD	Group 1	Group 2	H	p-value	q-value
Control group	A0 (n=15)	A1 (n=15)	3.88172	0.04881	0.14644
	A0 (n=15)	A3 (n=13)	0.57772	0.44721	0.60983
	A1 (n=15)	A3 (n=13)	1.84668	0.17417	0.37322
Enriched group	B0 (n=17)	B1 (n=17)	4.63421	0.03134	0.11753
	B0 (n=17)	B3 (n=17)	0.00267	0.95880	0.98494
	B1 (n=17)	B3 (n=17)	5.56668	0.01831	0.09153
Comparison for the two groups	A0 (n=15)	B0 (n=17)	0.00891	0.92479	0.98494
	A1 (n=15)	B1 (n=17)	0.12870	0.71979	0.89973
	A3 (n=13)	B3 (n=17)	0.88673	0.34636	0.57727

3.1.4 Beta diversity: Bray-Curtis's metrics

The beta diversity in the stool samples was examined in QIIME 2 using the Bray-Curtis's metric. A Principal Coordinates Analysis (PCoA) was performed to the Bray-Curtis's metrics as distances in between two samples. The dimensionality was reduced to two axes to be able to present the figure in 2D form.

Three different distance matrixes were drawn to see if there were any visible trends in the beta diversity of the stool samples. When looking at the distance matrix of the two research groups divided into three different time points, the data seems very scattered (Figure 4 a). When the colors in the picture were changed to describe the groups separately (Figure 4 b) or the time points separately (Figure 4 c), no clear clustering was visible either.

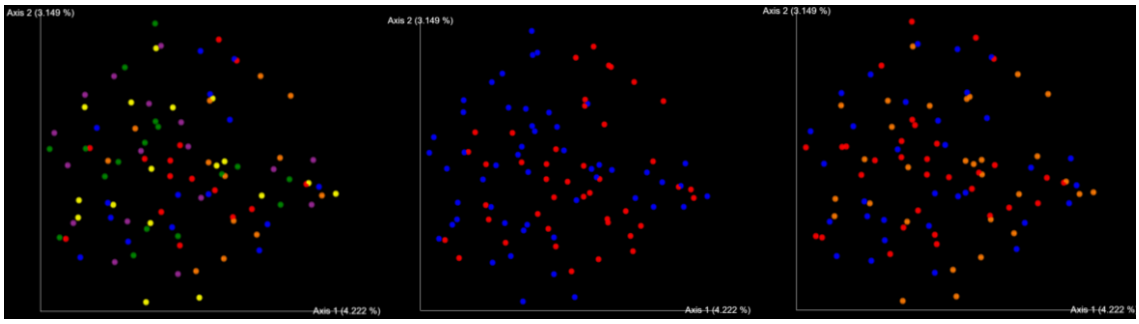

Figure 4: The Principal Coordinates Analysis of the stool samples using Bray-Curtis's metrics, separating the groups by colors in a) two different groups and three different time points (red = control group, 0 months; blue = control group, 1 month; orange = control group, 3 months; green = enriched group, 0 months; purple = enriched group, 1 month; yellow = enriched group, 3 months), b) separating the control group (red) and the enriched group (blue), and c) separating the three different time points (red = 0 months, blue = 1 month, orange = 3 months).

A PERMANOVA statistical test was performed to point out the statistical differences in between the samples (Table 6; Appendix 5, 4). According to the PERMANOVA test results the difference in between the two groups is statistically significant in the 3 months time point (Table 6; Appendix 5, 4).

Table 6: A PERMANOVA test was performed on the beta diversity of the stool samples in Bray-Curtis's metric. A0-A3 are the Bray-Curtis's metrics in control group, in time points 0 months (A0), 1 month (A1) and 3 months (A3), and B0-B3 are the Bray-Curtis's metrics in the enriched group, in time points 0 months (B0), 1 month (B1) and 3 months (B3).

	Group 1	Group 2	Sample size	Permutations	pseudo-F	p-value	q-value
Control group	A0	A1	30	4999	0.694083	0.9996	0.9996
	A0	A3	28	4999	1.039167	0.3144	0.4716
	A1	A3	28	4999	0.952118	0.6342	0.864818
Enriched group	B0	B1	34	4999	0.830503	0.947	0.9996
	B0	B3	34	4999	0.919419	0.7626	0.95325
	B1	B3	34	4999	0.79342	0.9836	0.9996
Time point	A0	B0	32	4999	1.095008	0.1148	0.287
	A1	B1	32	4999	1.038372	0.2834	0.4716
	A3	B3	30	4999	1.186739	0.0158	0.079

3.2 The inspection of the most abundant phyla in the intestinal microbiota

The five most abundant phyla in the human intestinal microbiota are *Firmicutes, Bacteroidota, Proteobacteria, Actinobacteria* and *Verrucomicrobia* (Tannock 2017). A

more detailed inspection was made to these phyla to see that there were no clear trends of changes in them during the experiment (Figure 5; Appendix 6).

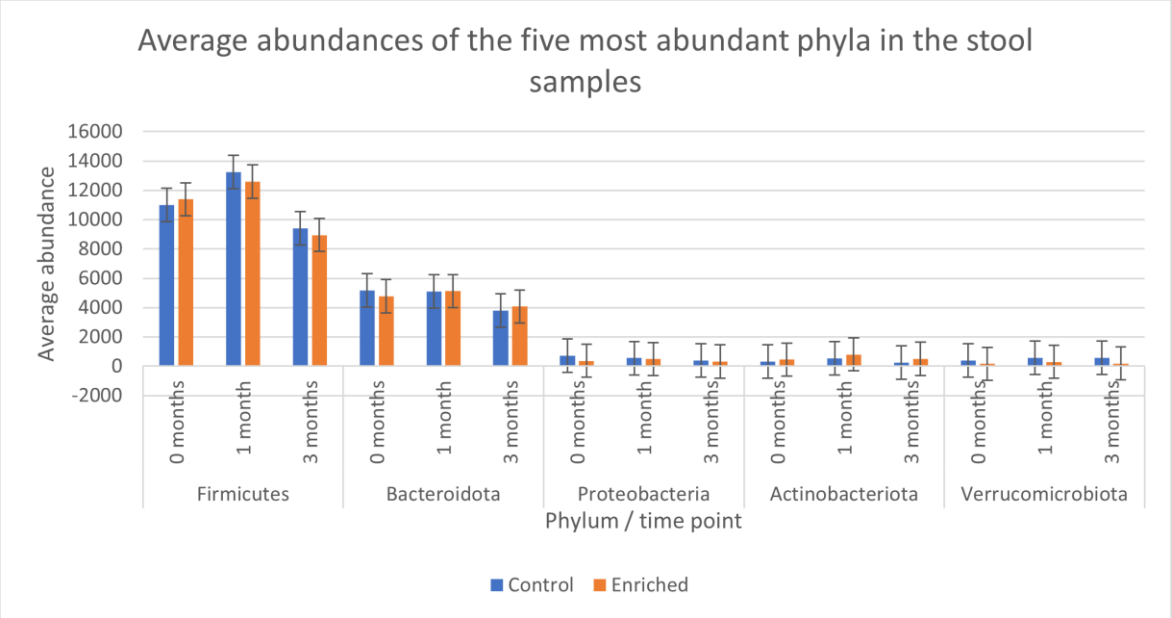

Figure 5: The average abundances of the five most abundant phyla in the human intestinal microbiota in the three different time points as means +/- standard errors.

The statistical significancy of the differences in the five most abundant phyla within each research group in the three different time points was tested with repeated measures of ANOVA in SPSS Statistics (Table 7; Appendix 5, 5-6). The test showed a statistically significant decrease in between the 1 month and 3 months samples in both groups for *Firmicutes*, and for the enriched group in *Bacteroidota*, *Proteobacteria* and *Actinobacteriota* ($p<0.05$, Table 7; Appendix 5, 5-6). Also, the overall decrease in abundances in between the baseline sample (0 months) and the final sample (3 months) was statistically significant in the enriched group for *Firmicutes*, and in the control group for *Bacteroidota* ($p<0.05$, Table 7; Appendix 5, 5-6).

Table 7: The p-values of the repeated measures of ANOVA statistical test for the five most abundant phyla in the human intestinal microbiota, testing the differences in between the three time points.

Phylum		Firmicutes		Bacteroidota		Proteobacteria		Actinobacteriota		Verrucomicrobiota	
Group											
Time point 1	Time point 2	Control	Enriched	Control	Enriched	Control	Enriched	Control	Enriched	Control	Enriched
0	1	0.096	0.16	0.901	0.484	0.563	0.207	0.043	0.019	0.221	0.116
0	3	0.268	0.023	0.05	0.129	0.237	0.554	0.602	0.623	0.3	0.768
1	3	0.008	0.001	0.082	0.032	0.302	0.041	0.067	0.009	0.94	0.092

The statistical significancy of the differences in between the two groups in the three different time points was tested with paired samples t-test (Table 8; Appendix 5, 7). The t-test showed a statistically significant difference only in the phylum *Actinobacteriota* in between the 1 month and 3 months time points, and in overall change in between the 0 months and 3 months time points, and in the phylum *Verrucomicrobiota* in between the 1 months and 3 months time points ($p<0.05$, Table 8; Appendix 5, 7).

Table 8: The p-values of the paired samples t-test for the five most abundant phyla, testing the differences in between the control group and the enriched group in the three different time points.

Time point 1	Time point 2	Firmicutes	Bacteroidota	Proteobacteria	Actinobacteriota	Verrucomicrobiota
0	1	0.236	0.229	0.092	0.072	0.035
0	3	0.228	0.426	0.389	0.006	0.089
1	3	0.315	0.495	0.222	0.036	0.006

4 DISCUSSION

4.1 Richness and diversity of the stool samples

According to the results of this experiment, the urban indoor farming did not change the alpha diversity of the intestinal microbiotas of the research subjects. The figures drawn of the observed numbers of ASV's, Shannon's diversity indexes and Faith's Phylogenetic diversity metrics were pretty much alike for both of the groups, despite the Shannon's diversity index values, where a small difference was observed (Figures 1-3). There were no statistical differences in any of the alpha diversity metrics in between the control group and the enriched group ($p>0.05$ in all the metrics and in all the time points, Tables 3-5). However, there were statistical differences in between the 1 month and 3 months time points in all the metrics within each group ($p<0.05$ in all the metrics in between the 1 month and 3 months time points, Tables 3-5). Since this change happens in all the metrics and in both

research groups, a conclusion can be drawn that the change was not related to this experiment.

The most likely reason for this statistically significant difference is the beginning of the COVID19 pandemic in between the 1 month and 3 months time points. The research subjects were interviewed at each time point, and they all told that they started using hand disinfectant after the 1 month time point. They also started isolating themselves and washing their hands with soap more frequently. This increase in their personal hygiene levels is plenty enough to change their intestinal microbiotas, and make them less diverse, since the hand hygiene has a straight effect on the intestinal microbiota (Roberts and Sporn 1990, Roslund et. al. 2020).

The COVID-19 pandemic affected the human intestinal microbiota in many ways, and it will most likely have effects on human health that last for multiple decades to come (Finlay et. al. 2020). The SARS-CoV2 -virus directly affected the microbial balance on humans, but the thing that mostly affected both infected and uninfected people were the pandemic control measures (Finlay et. al. 2020). With isolation, dietary changes and travel barriers, people did not get into contact with as diverse microbiota as they do in normal living conditions, and this previously ordinary diversity in the human microbiome might be lost for good (Finlay et. al. 2020). On the contrary, for some urban people the contact with nature might have also increased during the pandemic, because wandering in nature might have been the only activity allowed (Finlay et. al. 2020).

When looking at the temporal patterns in the figures of the alpha diversity metrics (Figures 1-3), the patterns of the Shannon's diversity index stand out. The Shannon's diversity index was the only alpha diversity metric, where the temporal patterns were not alike for the two groups (Figure 2). For the control group, the Shannon's diversity index values decrease throughout the experiment, but for the enriched group they start to increase in the beginning (Figure 2). This would have been a promising result if it had been statistically significant ($p>0.05$, Table 4). Still, the Kruskal-Wallis's test showed no statistical difference in the changes of the Shannon's diversity index, and therefore this result is coincidental.

The beta diversity of the stool samples was measured as Bray-Curtis distances, visualized with Principal Coordinates analysis (Figure 4) and tested with PERMANOVA to see that there was a statistically significant difference in between the two research groups in the 3 months time point (Table 6). This finding suggests that there would be a shift in the centroids of the samples in one of the groups at the end of this experiment. However, this finding should be taken with caution when making conclusions about the data, since the control group and the enriched group were small, and not of the same size (15 persons in the control group, 17 persons in the enriched group). The PERMANOVA test assumes that the group sizes are equal, and this assumption can have an impact on the result (Anderson et. al. 2017). However, it is possible that there was a statistically significant difference in between the two groups, but the group sizes are too small to draw a solid conclusion about this result.

4.2 The inspection of the five most abundant phyla in the human intestinal microbiota

When looking at the inspection of the five most abundant phyla in the human intestinal microbiota, no obvious trends were observed either. The overall decrease (from the baseline sample to the final sample) in the abundance of *Firmicutes* was statistically significant for the enriched group, and the overall decrease in the abundance of *Bacteroidota* was statistically significant for the control group (Figure 5, Table 7). In between the 1 month and 3 months samples more statistically significant differences were observed, but these differences happen most likely due to the increase in the hygiene levels (Figure 5, Table 7).

It has been discovered that the people suffering from obesity and type 2 diabetes tend to have higher amounts of *Firmicutes* and lower amounts of *Bacteroidota* in their intestinal microbiome than people within the normal body weight range (Ley et. al. 2006). Other studies also suggest that when person's calorie intake is limited for a year, the abundance of *Bacteroidota* increases, and the abundance of *Firmicutes* decreases, along with person's weight loss (de Wit et. al. 2012, Hildebrandt et. al.

2009). In this experiment, the abundance of *Firmicutes* decreased statistically significantly in both groups in between the last two time points (Figure 4, Table 7), but the overall decrease (the decrease in between the baseline sample and the final sample) was statistically significant only for the enriched group (Table 7). If this decrease was a result of this experiment, a deduction could have been made that the enriched seedbed would have decreased the risk of type 2 diabetes. But the decrease happened most likely due to the increased use of hand disinfectant, and therefore this kind of deduction cannot be made with this data set.

Increased adiposity and development of systemic inflammation are reasons of chronic exposure to lipopolysaccharides from Gram-negative bacteria, and *Bacteroidota* are the main phylum of Gram-negatives (Cani et. al. 2007, Zhang et. al. 2009). For the control group the repeated measures of ANOVA test showed a statistically significant overall decrease in the phylum *Bacteroidota* (Table 7). The decrease in between the 1 month and 3 months samples was also statistically significant in *Bacteroidota* for the enriched group (Table 7). If there was no increase in the hygiene measures in between the 1 month and 3 months samples, this kind of change could suggest that the action of practicing urban indoor farming could reduce the abundance of *Bacteroidota*. However, this change is also a result of the increased use of hand disinfectant, and therefore it cannot be thought as a reliable outcome.

The control group and the enriched group differ from each other in abundances only in the phyla *Actinobacteriota* (overall change) and *Verrucomicrobiota* (from 1 month sample to 3 months sample) ($p<0.05$, Table 8). The abundances are still rather low, and the groups so small that this statistically significant difference must be taken with caution. The odds are that in numbers this low the significance in the changes is coincidence.

4.3 The results of this experiment compared to the literature

Human intestinal microbiota has some seasonal variation, and the number of species is expected to change along with the seasons, especially in countries where the four seasons differ drastically from each other (Koliada et. al. 2020). In Finland the weather and thus the environmental conditions vary hugely in between the four seasons, and it directly affects the human intestinal microbiota. In research done in Hutterites, a hunter-gatherer tribe with a rather stable diet among the individuals and throughout the year, a significant decrease in human intestinal microbiota richness happened from spring to summer, and then again, a significant increase from summer to winter (Davenport et. al. 2014). This is a bit against the first initial thought, because in the areas with four different seasons, the microbial transfer from outdoors to inside is blocked by snow during winter (Hui et. al. 2019). Still, the seasonal variation is most probably caused by the change in diet (Davenport et. al. 2014). Therefore, it can be stated that in this experiment, where the first two samples were taken in winter and the last one in spring, the seasonal variation affects the results by decreasing the intestinal microbiota diversity in between the last two time points.

The intestinal microbiota composition changed significantly due to an exposure for diverse natural microbiota in a study done with infants in Edmonton (Nielsen et. al. 2020). 355 children under 4 months age were exposed to different natural environments within 500 m or 1000 m distance from their urban homes (Nielsen et. al. 2020). Surprisingly, the results of that experiment showed low Shannon and Simpson diversity index values in stool samples for children that were not breast-fed and lived with household pets (Nielsen et. al. 2020). Once again, these results cannot be directly compared and generalized with the research done in adults, but this decrease in the diversity for infants' intestinal microbiota, even though they had interaction with animals and exposure with nature, slightly argues with the biodiversity hypothesis.

In an experiment done with day-care children, the microbial diversity changed rather permanently both in the skin- and stool samples of the children, in the

presence of the added diverse microbial sources (Roslund et. al. 2021). Therefore, it could be thought that the modification of the human intestinal microbiota is possible via skin exposure to diverse microbiota, at least in the early childhood (Roslund et. al. 2021). The early childhood actions and lifestyle determine the guidelines for a person's intestinal microbiota, and usually these guidelines are formed by the age of 3 years (Yatsunenko et. al. 2012). Therefore, the results of the experiments in children for over 3 years can be considered as somewhat comparable to the ones of adults (Yatsunenko et. al. 2012).

The risk of developing type 1 diabetes in early childhood decreased significantly in the presence of agricultural land cover (Nurminen et. al. 2021). The decrease of the risk occurred also for the children carrying the HLA-DQ gene allele that carries the susceptibility for developing type 1 diabetes (Nurminen et. al. 2021). This decrease was thought to happen due to the direct exposure for diverse microbiota of the agricultural land cover (Nurminen et. al. 2021). These results are very promising for the future studies trying to find prevention and treatment methods for immune-mediated diseases, and this finding suggests that the exposure to the diverse microbiota through soil has impact on human immune system.

4.4 Evaluating the experiment setting

A pilot study was made with 14 adults having skin exposure to the same bacterial dust as in this experiment, to test the safety of the exposure and to find out if the skin contacts with diverse microbiota had a possibility to change the composition of the intestinal microbiota (Nurminen et. al. 2018). The results showed that the intestinal microbiota diversity increased in the research group during the two weeks of the experiment, and the same kind of increase did not happen in the control group (Nurminen et. al. 2018). These results were really promising when designing this ImmunoGarden -experiment set-up, but the direct exposure to the diverse microbiota dust provided more changes to the microbiota, than the indirect exposure that happened through the seedbed.

For the personnel in the universities of Lahti and Tampere, a green wall experiment was performed to see if the indoor exposure to green walls changed their skin microbiota and affected the levels of their immune system cytokines (Soininen et. al. 2022). During the two weeks of the experiment, significant increases in the alpha diversities of *Gammaproteobacteria* and *Lactobacillius* were observed, and both mentioned phyla have been proved to have beneficial impacts on human health (Soininen et. al. 2022, Hanski et. al. 2012, Delanghe et. al. 2021). The green wall experiment consisted of only 14 research subjects, and still the results showed an effect besides the individual variation rate being high (Soininen et. al. 2022). Therefore, it was expected that the effects of urban indoor farming would have become visible in this ImmunoGarden -experiment with a small research subject group, but that did not happen.

The early childhood and especially the birth timing and method have a huge impact on the human intestinal microbiota, since the formation of the bacterial communities inside the gastrointestinal tract happens during that time (Keag et. al. 2018, Fehr et. al. 2020). The intestinal microbiota formed in the birth and early childhood lasts for a lifetime and is altered only with significant and long-lasting exposures to different intestinal microbes (Keag et. al. 2018, Fehr et. al. 2020). For example, the intestinal microbiota composition is hugely different for pre-term infants and their term counterparts, in result of the hospital stay, possible use of antibiotics, and most of all the organ immaturity at the time of birth (Arboleya et. al. 2011). The question of the research subjects' birth gestational age was not asked in the interviews, so the question if it affected the results of this experiment, remains unanswered.

4.5 The weaknesses of this experiment

With the sample size of only 30 people, the individual variation had too much impact on the research results, and therefore overall conclusions about urban indoor farming affecting the human intestinal microbiota richness and diversity cannot be drawn according to this sample set. For example, diet, interaction with animals, personal

history, and drug intake affect the human intestinal microbiota, and these factors were not controlled enough within this experiment setting.

The overall immune activity, and changes in digestion and nutrition intake tend to weaken at 70 years of age (Toshitaka et. al. 2016). At that age lifestyle also often changes due to for example retirement, and these changes have a straight impact on the intestinal microbiota (Toshitaka et. al. 2016). Therefore, people aged over 70 years should have been excluded from this experiment, but due to the lack of research subjects this was not done. Including the people over 70 years might change the outcome of this experiment.

The actions required to take care of the plants in urban environment might have an impact on the results. If the research subject recruitment had been more succesful, it would have been interesting to add another control group which didn't perform urban indoor farming at all. In theory the placebo effect might interfere with the immune system and affect the immune responses, even when that has not yet been scientifically proved (Hadamitzky et. al. 2018).

Originally the plan was to recruit 60 people for this experiment and include another control group which would have used a sand-based seedbed with lower microbial diversity, or did not practice urban indoor farming at all, but due to the lack of volunteers a decision was made to divide the research subjects only into two study groups. We did not want the only control group to be one that did not practice urban indoor farming, because the actions required to maintain the plantation probably influence the variables we were measuring. Therefore, in this updated experiment set-up the control group acted as a placebo-control as well.

4.6 The need of further research and the unanswered questions of this experiment

This ImmunoGarden urban indoor farming study was one of the firsts made in adults, and thus the experiment set-up was still searching for a suitable form. With adults the environmental and lifestyle-related factors have had more time to affect the intestinal microbiota, and the composition of the intestinal microbiota is more

likely to need more drastic changes to adapt for environmental changes (Roslund et. al. 2021).

One question arisen from this sample set and the analysis after sequencing is whether the results answer the research question or not, since the COVID-19 pandemic began during this experiment. At the time of collection of samples from the first time point (0 months) there was no clue that a worldwide pandemic would start in a couple of weeks, and a massive hysteria of isolation and hand- and surface disinfection would begin. The samples collected at the third (3 months) time points can't therefore be directly compared to the samples collected at the first or second time point, because it is clear that the disinfection has changed the intestinal microbiota. When looking at the results it also seems that the trend was that the intestinal microbiota of the research subjects began to increase after one month (1 month's sample) of urban indoor farming (Figures 1-3). The isolation and hand- and surface disinfection started around the time when the 1 month's samples were collected, and therefore the 3 months samples have significantly lower amounts of richness and diversity (Figures 1-3).

These Illumina sequencing results were analysed as amplicon sequence variants (ASV's) instead of the traditional operational taxonomic units (OTU's). The ASV's provide a more detailed and exact way of examining the sequences because they consider a differentiation of one base-pair to be a sign of two different species in the sample (Callahan et. al. 2017). This makes it difficult to compare our results into the previously made intestinal microbiota mappings, because the previous results are usually analysed as OTU's.

In the future it would be interesting to repeat this experiment with a larger number of research subjects to minimize the effects of individual variation. For more reliable results it would also be important to reduce the effect of individual and environmental factors such as diet, drug intake, and by selecting more similar research subjects into the experiment.

If this kind of experiment was repeated in the future, maybe sampling in two- or three-months intervals during for example two years would provide much more detailed and reliable information, and it would also reduce the risk of seasonal variability affecting the results.

5 CONCLUSIONS

According to the results of this experiment, it remains unclear if the three months of urban indoor farming affected the intestinal microbiotas of the research subjects or not. When examining the results, time had a role in changing the human intestinal microbiota, but the statistically different changes happen only due to the increased hygiene levels, not because of the experiment. There was a lot of variation in between individuals and the variation was not dependant on the research group each subject was in.

Further research is needed, without a worldwide pandemic beginning during it, to figure out if the urban indoor farming affects the human intestinal microbiota richness or diversity.

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APPENDIX 1. The aims of the ImmunoGarden study (by ADELE-research group, in Finnish).

1. Tutkimuksen tavoitteet ja merkitys

1.1. Tutkimuksen tausta

Nykyisin immuunijärjestelmän vakavista häiriöistä kuten allergioista, astmasta ja tyypin 1 diabeteksestä kärsii arviolta viidennes teollisuusmaiden väestöstä. Yksin EU:n alueella tämä tarkoittaa sataa miljoonaa ihmistä. Tästä aiheutuvat kustannukset on arvioitu vuositasolla yli sadaksi miljardiksi euroksi.

Osana laajempaa ADELE-tutkimuskokonaisuutta tässä *ImmunoGarden*-hankkeessa kehitetään prototyyppimateriaaleja ja tapoja, joilla ehkäistään immuunivälitteisten sairauksien syntyä. 1900-luvun alkupuoliskolla näiden häiriöiden yleisyys oli murto-osa nykyisestä. Nykykäsityksen mukaan keskeinen syy sairauksien yleistymiseen on hygieniatason nousu ja ympäristön biologisen monimuotoisuuden kaventuminen, mikä on vähentänyt immuunijärjestelmän luonnollista kehitystä edistävää altistusta mikrobeille ja niiden rakenteille. Toistuvaa luontoaltistusta pidetään keskeisenä tapana suojautua näiltä taudeilta; luontoaltistuksella tarkoitetaan metsän ja niittyjen monipuolista mikrobi- ja pieneliöyhteisöä. Nykyisin luontoaltistus on täytynyt hankkia luonnosta; ImmunoGarden-hanke tähtää siihen, että sen saa kuluttajatuotteista, joihin on lisätty immuuniterveyttä ylläpitävä ja immuunivälitteisiltä taudeilta suojaava biodiversiteettikomponentti. Materiaalien raaka-aineina hyödynnetään metsätaloudessa syntyviä sivuainevirtoja, jotka sisältävät luonnon hitaasti kasvavan, patogeenittömän mikrobilajiston.

ADELE – Immuunijärjestelmän häiriöt ja elinympäristö -hankkeessa (strateginen tutkimusavaus 2015–2017, ekosysteemihanke 2018–2021) on tutkittu biologisen monimuotoisuuden ja immuunijärjestelmän toiminnan välistä yhteyttä kahdenlaisin tutkimuksin. Seurantatutkimuksissa kartoitetaan ihmisen terveyttä ja terveyteen mahdollisesti vaikuttavaa mikrobistoa ihmisestä ja elinympäristöstä. Seurantatutkimukset voivat paljastaa terveyden ja biologisen monimuotoisuuden välisiä assosiaatioita, mutta ne eivät osoita syy-seuraussuhdetta (esim. Parajuli ym. tulossa). Syy-seuraussuhteen osoittamiseksi tarvitaan altistuskokeita, joissa osa tutkittavista altistuu biologiselle monimuotoisuudelle normaalia enemmän ja osa jatkaa elämäänsä normaalisti. Nämä altistuskokeet on hyvä toteuttaa kaupunkiympäristössä, jossa tavanomainen altistus on vähäisempää kuin maaseudulla (Hanski ym. 2012; Parajuli ym. 2018, Hui ym. 2019). Pyrkimyksenä on tuoda luonnon monipuolinen mikrobisto kaupunkiympäristöön ja kehittää tapoja, joilla ehkäistään immuunipuolustuksen häiriöiden syntyä.

2.2. Intervention kulku ja päämäärät

2.2.1. Interventioryhmät ja intervention toteutus

Tutkimuksessa vapaaehtoiset, kaupunkiympäristöstä rekrytoitavat henkilöt harrastavat viljelyä kasvatuslaatikoissa. Tutkittavat satunnaistetaan kolmeen 20 henkilön ryhmään (yhteensä 60 vapaaehtoista), joista kukin ryhmä käyttää viljelyssä erilaista kasvualustaa. Tutkittavat eivät tiedä käyttämänsä kasvualustan laatua.

2.2.2. Altistusmateriaalien valinta

Ensimmäinen vapaaehtoisten tutkittavien ryhmä käyttää viljelyssä kasvualustaa, joka on laadultaan heikkoa hiekkaa, turvetta ja ravinteita sisältävää kylvömultaa. Toisen ryhmän käyttämä kasvualusta on laadukkaampaa kompostiseosta sisältävää multaa. Kolmas ryhmä käyttää ImmunoGarden- ja ADELE-hankkeissa kehitettyä mikrobistoltaan monimuotoista kasvualustaa.

Tämän kolmannen kasvualustan materiaalit on valittu siten, että bakteeriyhteisö vastaa metsämaan bakteeriyhteisöä. Metsämaassa on tyypillisesti 4–9 miljardia bakteeria grammassa maata. Metsämaa sisältää erittäin monimuotoisen mikrobiyhteisön, josta emme ole löytäneet ihmisperäisiä mikrobeja. Kasvualusta on samaa maa- ja kasviainesta, jota käytettiin aiemmassa aikuisaltistustutkimuksessa (Nurminen ym. 2018).

2.2.3. Altistusmateriaalin toimittaminen ja käyttö

Tutkimukseen osallistuville jaetaan kasvualustat, erilaisia siemeniä/taimia (ajankohdasta riippuen esim. yrtit, herne, porkkana, retiisi, salaatti, tomaatti, siemenperunat) ja kotiviljelyyn (esim. parvekkeelle) sopivat viljelylaatikot. Tutkittavat perehdytetään kaupunkiviljelyyn.

Kokeen alussa tutkittavat täyttävät saamansa viljelylaatikot kasvualustalla ja istuttavat siihen taimia sekä versotettavia siemeniä. Viljelmää huolletaan päivittäin tarkistamalla kasvualustan kosteus ja harventamalla kasvustoa lajikohtaisten ohjeiden mukaisesti. Sadonkorjuun jälkeen viljelmä kunnostetaan poistamalla vanhat kasvinosat, lisäämällä kasvualustaa ja kylvämällä uudet siemenet. Viljelmän ylläpitäminen vie päivittäin aikaa 5–10 minuuttia.

Keskeistä tutkimuksen turvallisuuden kannalta on, että valitut maamateriaalit ovat interventioaika huomioon ottaen stabiileja. Tällaisten stabiilien maamateriaalien (esim.

monet humukset) mikrobiologinen koostumus säilyy samankaltaisena pitkiä aikoja. Esimerkiksi kaupalliset multavalmisteet ovat tällä tavoin määritellen stabiileja ja niiden mikrobikoostumus ei merkittävästi muutu kokeen aikana.

Ulostenäytteet

Tutkittavat keräävät ulostenäytteet ennen sekä yhden ja kahden kuukauden interventiojakson eli kotiviljelyn jälkeen heille annettujen ohjeiden mukaisesti. Näytteille 16S RNA eristys ja Illumina-sekvensointi.

APPENDIX 2. The newssheet of the research (in Finnish).

TIEDOTE TUTKIMUKSESTA 13.6.2019

Tutkimus – ImmunoGarden, kaupunkiviljelyn terveysvaikutukset

Pyydämme Teitä osallistumaan ImmunoGarden-tutkimukseen, jossa tutkitaan kaupunkiviljelyn vaikutuksia ihmisen terveyteen. Tavoitteena on kehittää tapoja immuunivälitteisten sairauksien, kuten allergioiden, astman ja tyypin 1 diabeteksen, ehkäisyyn. ImmunoGarden-tutkimukseen sisältyy osatutkimus, joka toteutetaan vapaaehtoisten täysi-ikäisten henkilöiden keskuudessa.

Perehdyttyänne rauhassa tähän tiedotteeseen teille järjestetään mahdollisuus esittää kysymyksiä tutkimuksesta. Jos päätätte osallistua tutkimukseen, teiltä pyydetään suostumus tutkimukseen osallistumisesta ja henkilötietojenne käsittelystä osana tätä tutkimusta.

Tutkimuksen tarkoitus

Tämän tutkimuksen tarkoituksena on selvittää mahdollisuuksia parantaa elimistön puolustusjärjestelmän (immuunijärjestelmän) toimintaa kaupunkiympäristössä. Tämän osatutkimuksen tavoitteena on selvittää, miten erilaisten kotiviljelyssä käytettävien kasvualustojen kautta saatava pitkäkestoinen maa- ja kasvialtistus vaikuttaa ihmisten mikrobistoon ja puolustusjärjestelmän toimintaan.

Tampereen yliopistollisen sairaalan erityisvastuualueen alueellinen eettinen toimikunta on arvioinut tutkimussuunnitelman ja antanut siitä puoltavan lausunnon.

Tutkimuksen toteuttaja

Tämän tutkimuksen toteuttavat Helsingin ja Tampereen yliopistot. Tutkimuksen rekisterinpitäjiä ovat Helsingin ja Tampereen yliopistot, jotka vastaavat tutkimuksen yhteydessä tapahtuvan henkilötietojen käsittelyn lainmukaisuudesta.

Tutkimuksen kulku

Tutkimukseen pyydetään osallistujiksi henkilöitä, jotka haluavat osallistua tutkimukseen ja jotka soveltuvat siihen (ks. poissulkukriteerit). Jos suostutte tutkimukseen, teidät haastatellaan terveydentilan kartoittamiseksi. Näytteenottoon koulutettu henkilö ottaa teiltä verinäytteet, joista määritetään täydellinen verenkuva sekä tetanusvasta-aineet (jäykkäkouristusrokotuksen aikaansaamat vasta-aineet). Määritysten tulokset tulkitsee luottamuksellisina tutkimuksen vastuulääkäri. Lisäksi täytätte kyselylomakkeen, jossa kysytään asumishistoriasta,

kotieläimistä, ulkoiluharrastuksista, terveydentilasta ja elintavoista. Kaikkien näiden perusteella selvitetään, sovellutteko osallistumaan tutkimukseen. Teille kerrotaan, jos teidät suljetaan pois tutkimuksesta. Ette voi osallistua tutkimukseen, jos teillä on immuunijärjestelmän toimintaan vaikuttava sairaus tai lääkitys, lääkärin toteama muistisairaus tai mielenterveysongelma, olette sairastanut useita sairaalahoitoa vaatineita infektioita viimeisen viiden vuoden aikana, olette saanut syöpä-diagnoosin viimeisen kahden vuoden aikana tai olette parhaillaan syöpähoidoissa. Ette voi osallistua, jos teillä on diabetes, ihottuma tai ihon haavaumia käsissä, olette vajaavaltainen, tai jos teillä on normaaliarvoista poikkeava tulos verenkuvassa tai negatiiviset tetanusvasta-aineet. Ette voi myöskään osallistua tutkimukseen, jos tupakoitte päivittäin, teillä on lemmikkieläimiä, asutte viljelyä harjoittavassa perheessä tai yhteisössä tai olette alle 18-vuotias.

Kyselyvastausten ja verikokeen perusteella tutkimukseen valitaan kolme kahdenkymmenen henkilön ryhmää. Ryhmät muodostetaan siten, että ne ovat mahdollisimman vertailukelpoisia iän, sukupuolen ym. taustatekijöiden suhteen. Tutkimuksen aikana kaikki ryhmät harrastavat aktiivisesti kotiviljelyä mutta käyttävät erilaisia kasvualustoja. Ette tiedä käyttämänne kasvualustan laatua. Yksi kasvualustoista on kehitetty Helsingin yliopistossa ja kaksi muuta ovat yleisesti myynnissä olevia valmisteita. Teille toimitetaan kotiin kasvualustat, viljelylaatikot ja -ohjeita sekä siemeniä/taimia, ja teidät perehdytetään kotiviljelyyn. Kokeen alussa täytätte saamanne viljelylaatikon kasvualustalla ja istutatte siihen taimia sekä versotettavia siemeniä. Huollatte viljelmää päivittäin tarkistamalla kasvualustan kosteuden ja harventamalla kasvustoa lajikohtaisten ohjeiden mukaisesti. Sadonkorjuun jälkeen kunnostatte viljelmän poistamalla vanhat kasvinosat, lisäämällä kasvualustaa ja kylvämällä uudet siemenet.
Viljelmän ylläpitäminen vie päivittäin aikaa 5–10 minuuttia.

Tutkimuksessa kerätään verinäytteet, ihon sivelynäytteet sekä sylki- ja ulostenäytteet. Verinäytteistä tutkitaan immuunipuolustuksen säätely- ja toimintakykyä määrittämällä veren valkosolujen geenien ilmentyminen ja valkosolujen erittämät tulehdusmerkkiaineet. Sylki-, iho- ja ulostenäytteistä tutkitaan mikrobiyhteisö. Näytteenottoon koulutettu henkilö ottaa veri- ja ihonäytteet ennen koetta, yhden kuukauden päästä ja kolmen kuukauden päästä. Näytteet otetaan osallistujien kotona tai yliopiston tiloissa. Ihon sivelynäyte otetaan pumpulipuikolla kädestä/käsivarresta. Teitä pyydetään ottamaan samoina ajankohtina itse sylki- ja ulostenäytteet annettujen ohjeiden mukaisesti. Sylkinäyte otetaan näytetikkuja suussa pitämällä ja ulostenäyte pieneen näytteenottoputkeen. Teidät haastatellaan näytteenottojen yhteydessä (esim. terveydentila, subjektiiviset kokemukset).

Tutkimukseen liittyvät hyödyt ja riskit

On mahdollista, että tähän tutkimukseen osallistumisesta ei ole teille hyötyä. Tutkimuksen avulla pyritään kuitenkin selvittämään mahdollisuuksia parantaa elimistön puolustusjärjestelmän toimintaa.
Tutkimuksessa tehtävistä verikokeista selviää hemoglobiinitaso (mahdollinen anemia), muu verenkuva ja tetanusvasta-aineet. Jos tetanusvasta-aineita ei ole veressä, suositellaan tetanusrokotetta, joka suojaa jäykkäkouristukselta. Jos verenkuva poikkeaa normaalista, suositellaan lääkärikäyntiä.

Altistuksen riskit ovat vähäiset. Kaksi tutkimuksessa käytettävää kasvualustaa ovat yleisesti myynnissä olevia multavalmisteita. Kolmannen, yliopistossa kehitetyn kasvualustan raaka-aineet ovat peräisin tunnetuilta suomalaisilta teollisilta valmistajilta ja vastaavat koostumukseltaan kukka- ja puutarhamultaa. Esitutkimuksissa on poissuljettu tutkittavien käsien haavaumat ja immuunivajavuustilat.

Tutkimukseen osallistumisesta aiheutuu vain vähäistä haittaa ajankäyttönne suhteen. Verinäytteitä antaessanne saatatte tuntea pientä epämukavuuden tunnetta.

Luottamuksellisuus, tietojen käsittely ja säilyttäminen

Henkilötietojanne käsitellään ylläkuvattua tieteellistä tutkimusta varten. Henkilötietojen käsittelyn perusteena on teidän nimenomainen suostumuksenne.
Teistä kerättyä tietoa ja tutkimustuloksia käsitellään luottamuksellisesti henkilötietojen käsittelyä koskevan lainsäädännön tavalla. Tutkimuksessa yksittäisen tutkimushenkilön nimi, henkilötunnus ja yhteystiedot korvataan yksilöllisellä tunnistekoodilla. Teidän tietonne ja teistä otetut näytteet
säilytetään tutkimusaineistossa koodattuna ja teihin viitataan niissä vain tunnistekoodilla. Tutkimusaineisto ja teidän tietonne sen osana analysoidaan koodattuina, jolloin yksittäinen henkilö ei ole niistä suoraan tunnistettavissa ilman erillistä koodiavainta. Tätä koodiavainta eli tietoa, jonka avulla yksittäisen tutkittavan tiedot ja hänen tutkimustuloksensa voidaan yhdistää toisiinsa, säilyttävät rajatut ja ennalta määritellyt tutkimusryhmän jäsenet. Näitä tietoja ei anneta tutkimuksen ulkopuolisille henkilöille. Lopulliset tutkimustulokset raportoidaan pääasiallisesti ryhmätasolla. Yksittäisen tutkittavan tunnistaminen ei ole mahdollista tutkimustulosten julkaisuista.

Tutkimuksessa teistä kerätään seuraavia tietoja: kysely- ja haastattelutiedot (esim. nimi, henkilötunnus, osoite, puhelinnumero, asumishistoria, kotieläimet, ulkoiluharrastukset, terveyden tila, elintavat). Tiedot kerätään teiltä itseltänne. Tutkimuksessa teitä koskevia tietoja ja teistä otettuja näytteitä käsittelee tätä tutkimusta toteuttava tutkimushenkilökunta. Kaikki tietojanne käsittelevät tahot ja henkilöt ovat salassapitovelvollisia.

Henkilötietojenne säilytys: Ei-sähköisessä muodossa olevia henkilötietojanne sisältäviä aineistoja säilytetään Helsingin yliopistossa lukituissa tiloissa, joihin on pääsy ainoastaan nimetyillä henkilöillä. Sähköisiä tutkimustietojanne säilytetään Helsingin ja Tampereen yliopistojen suojatuilla palvelimilla tiedostoissa, joihin vain tutkimusryhmällä on pääsy.
Teitä koskevien tietojen säilytyksestä vastaa tutkimuksen johtaja Aki Sinkkonen. Tietojenne säilytysaikaa sääntelee lainsäädäntö sekä hyvä kliininen tutkimustapa. Tutkimuksen yhteydessä kerättyjä näytteitä ja tietoja säilytetään Helsingin ja Tampereen yliopistoissa 15 vuotta, jonka jälkeen ne hävitetään.

Tieteelliseen tutkimukseen liittyy olennaisesti tutkimustulosten julkaiseminen kansainvälisissä tieteellisissä julkaisuissa.

Vapaaehtoisuus

Tutkimukseen osallistuminen on vapaaehtoista ja voitte kieltäytyä siitä tai keskeyttää tutkimuksen koska tahansa syytä ilmoittamatta ilman, että siitä koituu teille mitään haittaa. Tutkimuksesta kieltäytyminen tai sen keskeyttäminen ei vaikuta oikeuteenne saada terveydenhuollon palveluja. Voitte myös peruuttaa antamanne suostumuksen ilman perusteluita ilmoittamalla siitä tutkimushenkilökunnalle. Suostumuksen peruuttamisesta ei koidu teille mitään haittaa. Jos päätätte peruuttaa suostumuksenne, tai osallistumisenne tutkimukseen keskeytyy jostain muusta syystä, siihen mennessä kerättyjä tietoja voidaan käsitellä tässä tutkimuksessa, mikäli lainsäädäntö sen sallii tai sitä edellyttää.

Teillä on oikeus tarkastaa tietonne ja pyytää tietojenne oikaisemista tai täydentämistä esimerkiksi, jos havaitsette niissä virheen tai ne ovat puutteellisia tai epätarkkoja. Teillä on myös oikeus pyytää tietojenne poistamista tieteellisestä tutkimuksesta (”oikeus tulla unohdetuksi”) tai niiden käytön rajoittamista ilmoittamalla siitä tutkimushenkilökunnalle. Tieteellisen tutkimuksen yhteydessä näitä oikeuksia voidaan myös rajoittaa. Esimerkiksi lainsäädäntö voi velvoittaa rekisterinpitäjän säilyttämään tutkimustietonne tietyn määräajan rekisteröidyn oikeuksista riippumatta ja sallii poikkeukset rekisteröidyn oikeuksista, silloin kun se on välttämätöntä tieteellisten tutkimustulosten ja tutkittavien turvallisuuden varmistamiseksi.

Teillä on oikeus ottaa yhteyttä tietosuojavastaavaan:
Lotta Ylä-Sulkava, Helsingin yliopisto, tietosuoja@helsinki.fi tai Jukka Tuomela, Tampereen yliopisto, jukka.tuomela@tuni.fi.

Teillä on oikeus tehdä valitus valvontaviranomaiselle, jos katsotte, että henkilötietojenne käsittelyssä rikotaan EU:n yleistä tietosuoja-asetusta (EU) 2016/679. Suomessa valvontaviranomainen on tietosuojavaltuutettu.

Tietosuojavaltuutetun toimisto
Ratapihantie 9, 6. krs, 00520 Helsinki, PL 800, 00521 Helsinki
Puhelinvaihde: 029 566 6700
Sähköposti: tietosuoja@om.fi

Tutkittavien vakuutusturva

Jos tutkimuksen takia tehdystä toimenpiteestä aiheutuu teille henkilövahinko, voitte hakea korvausta. Tutkimus kuuluu Helsingin yliopiston potilasvakuutuksen piiriin. Vakuutus kattaa tutkimustoimintaa harjoittavan henkilöstön tutkimukset myös terveille henkilöille. Muusta syystä kuin tutkimuslääkkeestä aiheutuneista henkilövahingoista haetaan korvausta tutkimuskeskuksen potilasvakuutuksesta. Se korvaa potilasvahinkolain mukaisesti terveyden- ja sairaanhoidon yhteydessä aiheutuneita henkilövahinkoja laissa tarkemmin säädellyin edellytyksin. Potilasvakuutuskeskus huolehtii potilasvahinkojen korvauskäsittelystä.

Tutkimuksen kustannukset ja taloudelliset selvitykset

Tutkimukseen osallistumisesta ei makseta palkkiota. Tutkimuskäynnit ovat teille maksuttomia. Tutkimuksen rahoituksesta vastaavat Business Finland (ent. TEKES) ja Helsingin yliopisto. Tutkijoille ei makseta erillistä korvausta tutkimuksen toteuttamisesta. Helsingin yliopisto on anonut patenttia (nro 20175196) tutkijoiden Aki Sinkkosen, Mira Grönroosin ja Marja Roslundin tekemälle keksinnölle, jolle tämä tutkimus perustuu.

Tutkimustuloksista tiedottaminen

Teille lähetetään tiedot veren hemoglobiini- ja tetanusvasta-ainetasoista sekä muusta verenkuvasta. Tutkimuksesta tehtävät julkaisut ja niiden suomenkieliset tiivistelmät lisätään tutkimusryhmän internetsivuille (https://www.helsinki.fi/fi/tutkimusryhmat/luontopohjaiset-ratkaisut).

Lisätiedot ja tutkijoiden yhteystiedot

Mahdollisia kysymyksiä tutkimuksesta pyydämme teitä esittämään FM, tohtorikoulutettava Marja Roslundille, Helsingin yliopisto, puhelin 050 3118024, marja.roslund@helsinki.fi.

Dosentti Aki Sinkkonen, tutkimuksen johtaja
Helsingin yliopisto
Bio- ja ympäristötieteellinen tiedekunta
Ekosysteemit ja ympäristö -tutkimusohjelma
Niemenkatu 73, 15140 Lahti

Professori Heikki Hyöty, tutkimuksen vastuulääkäri
Tampereen yliopisto
Lääketieteen ja terveysteknologian tiedekunta
Arvo Ylpön katu 34, 33014 Tampere

APPENDIX 3. The instructions for the research subjects about the urban indoor farming, and the sample collection (in Finnish).

Kotiviljelyohjeita

Aluksi

Viljelylaatikko puhdistetaan ja sen pohjalle laitetaan tasainen kerros lekasoraa, joka on omassa pussissaan. Tämän jälkeen viljelylaatikko täytetään toisessa pussissa olevalla kasvualustalla ilmavasti noin viiden sentin päähän laatikon pinnasta paljain käsin levittämällä. Jäljelle mahdollisesti jäävä kasvualusta säästetään pussissa ja sitä voidaan lisätä viljelyn aikana uusia siemeniä kylvettäessä. Viljelylaatikko asetetaan esimerkiksi ikkunalaudalle tai pöydälle ikkunan läheisyyteen. Lamppu asennetaan valaisimeen, joka asetetaan lähelle viljelylaatikkoa.

Laatikon yhteen päähän istutetaan pala inkivääriä ja valkosipulinkynnet, toiseen päähän kylvetään retiisiä, jonka on tarkoitus tuottaa juurekkaita. Laatikon keskiosaa käytetään versotettavien siementen kylvämiseen alla olevan kaavion mukaisesti. Kun kylvö/istutus on suoritettu, multa kastellaan kauttaaltaan.

Inkivääri	Salaatti	Herne	Sinapinsiemen	Härkäpapu	Retiisi
Valkosipuli					

Viljelyn aikana

Kasvatusalustan kosteus tarkastetaan päivittäin työntämällä sormet syvälle multaan. Kosteus tarkistetaan viidestä eri kohdasta laatikkoa. Jos multa on syvemmältä kuivaa, viljelmää kastellaan runsaammin. Muutoin viljelmää suihkutetaan kevyesti päivittäin, etenkin siementen kylvö- ja itämisvaiheessa. Lisävaloa annetaan etenkin siementen idettyä eli niiden kasvuvaiheessa. Valoa voi pitää päällä aina kotona oltaessa. Kasveja tarkkaillaan päivittäin tuhohyönteisten varalta.

Kasveja voidaan korjata ravinnoksi monessa eri vaiheessa. Osaa voi kokeilla hyödyntää nopeasti ensimmäisten lehtien muodostuttua muutaman cm:n mittaisina (sinapinsiemen), osaa voi kasvattaa pidemmälle 10 cm:iin asti ja korjata versoina (herne, härkäpapu), osasta voi korjata satoa pienissä erissä ja jättää kasvin jatkamaan kasvuaan (valkosipuli, salaatti, herne, härkäpapu) ja osaa kasvatetaan niin pitkään, että maanalainen osa on valmis korjattavaksi (retiisi, inkivääri).

Jos sato korjataan siten, että koko kasvin verso/taimi tulee käytetyksi, poistetaan myös juuristo käsin hellävaraisesti nostamalla pyrkien varistelemaan juuriin tarttunut kasvualusta takaisin laatikkoon. Tilalle kylvetään seuraavat siemenerä. Jos siemenet loppuvat, ottamalla yhteyttä Mikaan niitä saa lisää.

Kylvö, kasvien hoito, kosteuden tarkistaminen, korjuu sekä vanhojen kasvinosien poistaminen korjuun jälkeen tapahtuu paljain käsin!

Herne

Laita herneet yöksi veteen likoamaan ja painele ne seuraavana päivänä multaan lähelle toisiaan.

Versot korjataan noin 10 cm pituisina joko kokonaisina tai saksimalla ensimmäisen lehtiparin yläpuolelta, jolloin verso jatkaa kasvuaan, ja käytetään tuoreina tai osana ruoanlaittoa.

Härkäpapu

Laita härkäpavut yöksi veteen likoamaan ja painele seuraavana päivänä multaan lähelle toisiaan siten, että ne peittyvät.

Versot korjataan noin 10 cm pituisina joko kokonaisina tai saksimalla ensimmäisen lehtiparin yläpuolelta, jolloin verso jatkaa kasvuaan, ja käytetään tuoreina tai osana ruoanlaittoa.

Valkosipuli

Jaa valkosipuli kynsiin, mutta älä kuori niitä, ja painele kynnet multaan vieri viereen siten, että kynnen kärki osoittaa ylöspäin.

Korjaa osa versosta sen ollessa noin 15 cm pituinen ja jätä kynsi multaan kasvamaan. Käytä verso ruohosipulin/kevätsipulin tavoin.

Sinapinsiemen

Kylvä sinapinsiemenet mullan pinnalle suhteellisen tiheästi, älä peitä.

Korjaa noin 5 cm:n pituisina ja käytä mausteisia versoja esimerkiksi wokkeihin.

Retiisi

Kylvä 5-10 siementä per 10 cm siksakkiin leveään riviin noin 1 cm syvyyteen.

Juureksia kasvatettaessa maaperän on pysyttävä kosteana, jotta vältetään kitkeröityminen. Pienten retiisien kehittyminen vie nopeimmillaan kolme viikkoa.

Salaatti

Kylvä siemenet muutaman sentin välein ja peitä kevyesti mullalla. Kastele multa ja pidä kosteana - salaatti kitkeröityy kuivassa alustassa!

Satoa voi alkaa korjaamaan, kun lehdet ovat n. 5 cm tai harventamalla tiheää kasvustoa. Satoa voi korjata napsimalla yksittäisiä lehtiä tai leikkaamalla koko ruusukkeen 2-3 cm päähän maanrajasta, jolloin loppuosa jatkaa kasvuaan. Uuden erän voi kylvää 2-3 viikon kuluttua.

Inkivääri

Laita pala likoamaan viileään veteen noin viideksi tunniksi. Istuta pala nystyrät ylöspäin kaivamalla palan mentävä kuoppa ja peittämällä pala mullalla. Kastele kasvualusta inkiväärin kohdalta läpikotaisin.

Kasvu alkaa muutamien viikkojen kuluttua ja sadonkorjuuseen menee kuukausia. Juuren koon voi tarkistaa kaivamalla multaa kasvin ympäriltä ja tunnustelemalla juuren kokoa - yleensä korjattavissa olevan inkiväärin lehtien koko on noin metrin luokkaa.

Jos sato on korjattavissa, koko kasvi voidaan nostaa juurineen kasvualustasta ja vihreät kasvinosat sekä juuret voidaan karsia esimerkiksi saksilla - vihreää osaa ei syödä.

Helsingin yliopisto
Niemenkatu 73, 15140 Lahti
Näytteenotto-ohjeet

versio 9.5.2019

ULOSTENÄYTTEEN KERÄÄMINEN KOTONA

Olette saaneet näytteenottosetin, josta löytyvät näytteenottovälineet. Täyttäkää lähetteeseen ulostenäytettä koskevat tiedot.

Ulostenäyte tulisi ottaa samana päivänä sylkinäytteen kanssa.

1. Kertakäyttölautanen asetetaan WC-istuimen pohjalle vesirajan yläpuolelle ennen ulostamista.

2. Ulostetta sisältävä kerta-käyttölautanen nostetaan lattialle näytteen keräämistä varten.

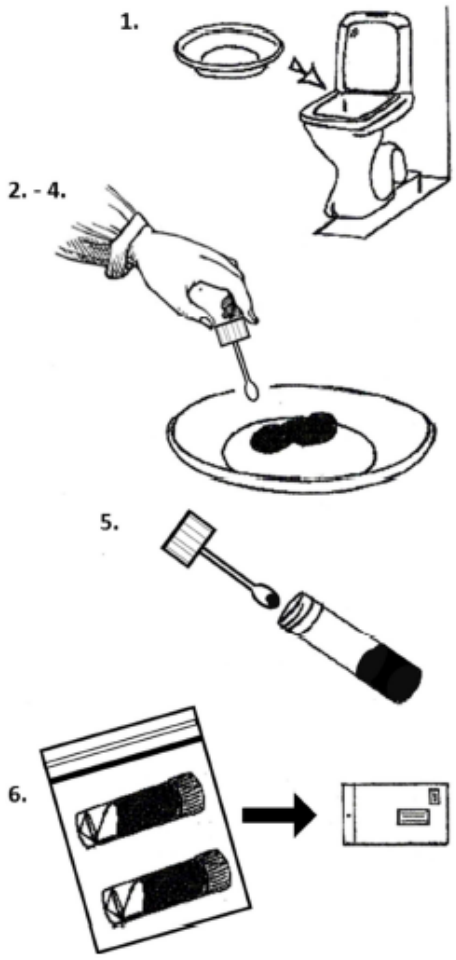
3. Näyteputki avataan ja putken sisällä oleva lusikka otetaan ulos.

4. Lusikka työnnetään ulosteeseen ja siihen pyritään ottamaan mahdollisimman paljon näytettä. Ulostetta tulee ottaa kahteen näyteputkeen samalla kerralla.

5. Lusikka laitetaan takaisin näyteputken sisään ja korkki ruuvataan tiukasti kiinni. Putkiin liimataan tarrat, joihin on kirjoitettu näytteenottopäivä-määrä. Putket pakataan Minigrip -pussiin (2 putkea).

6. Näytteet laitetaan näytekeräys-laatikkoon geelin päälle ja laatikko säilytetään pakastimessa (-20 °C). Sylkinäyte säilytetään samassa laatikossa.

7. Näytteisiin liittyvä lähete täytetään ja annetaan tutkimushenkilökunnalle jäisen näytelaatikon kanssa seuraavalla tutkimuskäynnillä.



APPENDIX 4. The sample sheet.

Group A

NGScode	Person ID	Sex	Age	Seedbed	Sampling date	Time point	Antibiotics	Probiotics	Stomach flu	NSAIDs	Hand disinfectant
5087	KV_05	F	36	A	13.2.2020	0	No	Yes	No	No	No
5149	KV_05	F	36	A	15.3.2020	1	No	Yes	No	No	No
5112	KV_05	F	36	A	25.5.2020	3	No	Yes	No	No	Yes
5136	KV_10	F	36	A	14.2.2020	0	No	No	No	No	No
5143	KV_10	F	36	A	15.3.2020	1	No	No	No	No	No
5116	KV_10	F	36	A	25.5.2020	3	No	No	No	No	Yes
5137	KV_11	F	50	A	18.2.2020	0	No	No	No	No	No
5146	KV_11	F	50	A	16.3.2020	1	No	No	No	No	No
5101	KV_11	F	50	A	25.5.2020	3	No	No	No	No	Yes
5092	KV_15	F	38	A	18.2.2020	0	No	No	No	No	No
5154	KV_15	F	38	A	16.3.2020	1	No	No	No	No	No
	KV_15	F	38	A		3					
	KV_15	F	38	A		3					
5074	KV_17	F	74	A	10.2.2020	0	No	No	No	No	No
5164	KV_17	F	74	A	8.3.2020	1	No	No	No	No	No
5125	KV_17	F	74	A	9.5.2020	3	No	No	No	No	Yes
5075	KV_18	F	31	A	13.2.2020	0	No	No	No	No	No
5165	KV_18	F	31	A	12.3.2020	1	No	No	No	No	No
5103	KV_18	F	31	A	24.5.2020	3	No	No	No	No	Yes
5077	KV_23	F	38	A	13.2.2020	0	No	Sometimes	No	No	No
5168	KV_23	F	38	A	10.3.2020	1	No	Sometimes	No	No	No
5127	KV_23	F	38	A	12.5.2020	3	No	Sometimes	No	No	Yes
5078	KV_24	M	38	A	13.2.2020	0	No	Sometimes	No	No	No
5169	KV_24	M	38	A	10.3.2020	1	No	Sometimes	No	No	No
5128	KV_24	M	38	A	12.5.2020	3	No	Sometimes	No	No	Yes
5081	KV_28	F	74	A	14.2.2020	0	No	No	No	No	No
5082	KV_28	F	74	A	11.3.2020	1	No	No	No	No	No
5131	KV_28	F	74	A	17.5.2020	3	No	No	No	No	Yes
5083	KV_29	F	56	A	11.2.2020	0	No	No	No	No	No
5172	KV_29	F	56	A	11.3.2020	1	No	No	No	No	No
5106	KV_29	F	56	A	15.5.2020	3	No	Sometimes	No	No	Yes
5094	KV_32	F	64	A	18.2.2020	0	No	No	No	No	No
5155	KV_32	F	64	A	15.3.2020	1	No	No	No	No	No
5107	KV_32	F	64	A	19.5.2020	3	No	Yes	No	No	Yes
5095	KV_33	F	38	A	20.2.2020	0	No	No	No	No	No
5156	KV_33	F	38	A	15.3.2020	1	No	No	Yes	Yes	No
5119	KV_33	F	38	A	24.5.2020	3	No	No	No	No	Yes
5096	KV_34	F	68	A	18.2.2020	0	**Yes**	No	No	No	No
5157	KV_34	F	68	A	15.3.2020	1	**Yes**	No	No	No	No
5108	KV_34	F	68	A	24.5.2020	3	**Yes**	No	No	No	Yes
5097	KV_35	M	70	A	18.2.2020	0	No	No	No	No	No
5158	KV_35	M	70	A	15.3.2020	1	No	No	No	No	No
5110	KV_35	M	70	A	24.5.2020	3	No	No	No	No	Yes
5098	KV_36	M	38	A	19.2.2020	0	**Yes**	No	No	No	No
5159	KV_36	M	38	A	15.3.2020	1	**Yes**	No	No	No	No
5120	KV_36	M	38	A	24.5.2020	3	**Yes**	No	No	No	Yes

Group B

5086	KV_02	M	67	B	13.2.2020	0	No	Sometimes	No	No	No
5148	KV_02	M	67	B	13.3.2020	1	No	Yes	No	No	No
5100	KV_02	M	67	B	24.5.2020	3	No	Sometimes	No	No	Yes
5072	KV_03	F	66	B	12.2.2020	0	No	No	No	No	No
5161	KV_03	F	66	B	10.3.2020	1	No	No	No	No	No
5122	KV_03	F	66	B	18.5.2020	3	No	No	No	No	Yes
5134	KV_04	F	37	B	20.2.2020	0	No	No	No	No	No
5141	KV_04	F	37	B	19.3.2020	1	No	No	No	No	No
5111	KV_04	F	37	B	24.5.2020	3	Yes	No	No	No	Yes
5088	KV_06	F	35	B	18.2.2020	0	No	No	No	No	No
5150	KV_06	F	35	B	16.3.2020	1	No	No	No	No	No
5113	KV_06	F	35	B	18.5.2020	3	No	No	No	No	Yes
5089	KV_07	M	43	B	18.2.2020	0	No	No	No	No	No
5151	KV_07	M	43	B	16.3.2020	1	No	No	No	No	No
5114	KV_07	M	43	B	18.5.2020	3	No	No	No	No	Yes
5135	KV_08	M	60	B	16.2.2020	0	No	No	No	No	No
5142	KV_08	M	60	B	16.3.2020	1	No	No	No	No	No
5115	KV_08	M	60	B	24.5.2020	3	No	No	No	No	Yes
5090	KV_12	F	66	B	18.2.2020	0	No	No	No	Yes	No
5152	KV_12	F	66	B	15.3.2020	1	No	No	No	Yes	No
5117	KV_12	F	66	B	24.5.2020	3	No	No	No	No	Yes
5073	KV_13	F	36	B	13.2.2020	0	No	No	No	No	No
5162	KV_13	F	36	B	10.3.2020	1	No	Sometimes	No	No	No
5123	KV_13	F	36	B	17.5.2020	3	No	No	No	No	Yes
5091	KV_14	F	39	B	13.1.2020	0	Yes	No	No	No	No
5153	KV_14	F	39	B	15.3.2020	1	Yes	No	No	No	No
5102	KV_14	F	39	B	13.5.2020	3	Yes	No	No	No	Yes
5138	KV_16	F	65	B	13.2.2020	0	No	No	No	No	No
5163	KV_16	F	65	B	10.3.2020	1	No	No	No	No	No
5124	KV_16	F	65	B	17.5.2020	3	No	No	No	No	Yes
5093	KV_19	F	45	B	13.2.2020	0	No	No	No	No	No
5166	KV_19	F	45	B	11.3.2020	1	No	No	No	No	No
5126	KV_19	F	45	B	19.5.2020	3	No	No	No	No	Yes
5139	KV_20	F	39	B	14.2.2020	0	No	No	No	No	No
5144	KV_20	F	39	B	17.3.2020	1	No	Sometimes	No	ometime	No
5104	KV_20	F	39	B	18.5.2020	3	No	No	No	No	Yes
5140	KV_21	M	39	B	16.2.2020	0	No	No	No	No	No
5145	KV_21	M	39	B	17.3.2020	1	No	No	No	No	No
5105	KV_21	M	39	B	18.5.2020	3	No	No	No	No	Yes
5076	KV_22	F	72	B	11.2.2020	0	No	No	No	No	No
5167	KV_22	F	72	B	8.3.2020	1	No	No	No	No	No
5118	KV_22	F	72	B	20.5.2020	3	Yes	No	No	No	Yes
5079	KV_26	F	31	B	12.2.2020	0	No	No	No	No	No
5170	KV_26	F	31	B	8.3.2020	1	No	No	No	No	No
5129	KV_26	F	31	B	17.5.2020	3	No	No	No	No	Yes
5080	KV_27	M	34	B	12.2.2020	0	No	No	No	No	No
5171	KV_27	M	34	B	8.3.2020	1	No	No	No	No	No
5130	KV_27	M	34	B	17.5.2020	3	No	No	No	No	Yes
5084	KV_31	F	66	B	15.2.2020	0	No	Yes	No	No	No
5173	KV_31	F	66	B	10.3.2020	1	No	Yes	No	No	No
5132	KV_31	F	66	B	17.5.2020	3	No	Yes	No	No	Yes
5085	H2O										
5099	H2O										
5109	H2O										
5121	H2O										
5133	H2O										
5147	H2O										
5160	H2O										
5174	H2O										

APPENDIX 5. The detailed tables and figures of the statistical analyses.

1. **The full Kruskal-Wallis's test result table comparing the observed numbers of the bacterial species in each of the research groups and in each of the time points.**

Group 1	Group 2	H	p-value	q-value
A0 (n=15)	A1 (n=15)	1.3989755011135796	0.2368951804519204	0.32303888243443696
A0 (n=15)	A3 (n=13)	1.4910017693150202	0.22206109472102842	0.32303888243443696
A0 (n=15)	B0 (n=17)	0.32097327379797463	0.5710233293256041	0.6118107099917186
A0 (n=15)	B1 (n=17)	0.5706191534186169	0.45001298617143903	0.5625162327142987
A0 (n=15)	B3 (n=17)	4.236427095127692	0.03956534796515055	0.09891336991287637
A1 (n=15)	A3 (n=13)	5.965640604260152	0.014587299924078161	0.0729364996203908
A1 (n=15)	B0 (n=17)	4.238758217887301	0.039511056183550365	0.09891336991287637
A1 (n=15)	B1 (n=17)	0.4369604067557853	0.5085931979173941	0.5868383052893009
A1 (n=15)	B3 (n=17)	12.074301286337791	0.0005112161920013403	0.007668242880020104
A3 (n=13)	B0 (n=17)	1.5246422077098227	0.2169183382641676	0.32303888243443696
A3 (n=13)	B1 (n=17)	4.646632916286567	0.03111452179921601	0.09891336991287637
A3 (n=13)	B3 (n=17)	0.010949742945350274	0.9166606645707759	0.9166606645707759
B0 (n=17)	B1 (n=17)	3.269896193771629	0.0705620884375683	0.15120447522336064
B0 (n=17)	B3 (n=17)	2.4027398245361806	0.1211229491271924	0.22710552961348576
B1 (n=17)	B3 (n=17)	10.594463667820094	0.0011342679808981218	0.008507009856735914

2. **The full Kruskal-Wallis's test result table comparing the Shannon's index values in each of the research groups and in each of the time points.**

Group 1	Group 2	H	p-value	q-value
A0 (n=15)	A1 (n=15)	0.03483870967741609	0.8519340379411444	0.9821463230683029
A0 (n=15)	A3 (n=13)	1.723607427055697	0.1892293514005241	0.3859738048530641
A0 (n=15)	B0 (n=17)	0.6591800356506212	0.4168495708329325	0.5684312329539989
A0 (n=15)	B1 (n=17)	0.0003565062388588558	0.9849357392442808	0.9849357392442808
A0 (n=15)	B3 (n=17)	3.494117647058829	0.06158721940935912	0.23095207278509672
A1 (n=15)	A3 (n=13)	4.588328912466835	0.032190384504586375	0.16095192252293186
A1 (n=15)	B0 (n=17)	1.6003565062388532	0.2058526959216342	0.3859738048530641
A1 (n=15)	B1 (n=17)	0.017468805704083934	0.8948501382276483	0.9821463230683029
A1 (n=15)	B3 (n=17)	7.914795008912648	0.004903213573130885	0.03677410179848164
A3 (n=13)	B0 (n=17)	1.4227120128448547	0.23295753439309175	0.3882625573218196
A3 (n=13)	B1 (n=17)	2.5962633192234676	0.10711599829412105	0.31335156512199397
A3 (n=13)	B3 (n=17)	0.010947306962492576	0.916669901530416	0.9821463230683029
B0 (n=17)	B1 (n=17)	1.0324270884824642	0.3095892953526004	0.46438394302890057
B0 (n=17)	B3 (n=17)	2.34928324270885	0.1253406260487976	0.31335156512199397
B1 (n=17)	B3 (n=17)	8.07464162135443	0.0044888984185371865	0.03677410179848164

3. **The full Kruskal-Wallis's test result table comparing the Faith's Phylogenetic diversity metrics in each of the research groups and in each of the time points.**

Group 1	Group 2	H	p-value	q-value
A0 (n=15)	A1 (n=15)	3.881720430107535	0.0488145118658309	0.1464435355974927
A0 (n=15)	A3 (n=13)	0.5777188328912501	0.44720785725606593	0.6098288962582717
A0 (n=15)	B0 (n=17)	0.008912655971471395	0.9247859634718736	0.9849357392442808
A0 (n=15)	B1 (n=17)	3.494117647058829	0.06158721940935912	0.1539680485233978
A0 (n=15)	B3 (n=17)	0.0003565062388588558	0.9849357392442808	0.9849357392442808
A1 (n=15)	A3 (n=13)	1.8466843501326338	0.1741695170165113	0.3732203936068099
A1 (n=15)	B0 (n=17)	5.932620320855619	0.01486312507129314	0.09152702213661383
A1 (n=15)	B1 (n=17)	0.12869875222817484	0.7197850505569157	0.8997313131961445
A1 (n=15)	B3 (n=17)	6.497326203208559	0.010803684532845347	0.09152702213661383
A3 (n=13)	B0 (n=17)	0.7360969201576495	0.39091404961745413	0.5863710744261812
A3 (n=13)	B1 (n=17)	1.6293971682965918	0.20178644891900693	0.378349591723138
A3 (n=13)	B3 (n=17)	0.8867318639614581	0.3463644810224633	0.5772741350374389
B0 (n=17)	B1 (n=17)	4.634206623826017	0.031340631788509245	0.11752736920690966
B0 (n=17)	B3 (n=17)	0.002669303015338187	0.9587953884932456	0.9849357392442808
B1 (n=17)	B3 (n=17)	5.566683143845779	0.018305404427322768	0.09152702213661383

4. **The full PERMANOVA test result table comparing the beta diversity of the bacterial species in each of the research groups and in each of the time points using Bray-Curtis's metric.**

Group 1	Group 2	Sample size	Permutations	pseudo-F	p-value	q-value
A0	A1	30	4999	0.6940831954595185	0.9996	0.9996
A0	A3	28	4999	1.0391669237777805	0.3144	0.4716
A0	B0	32	4999	1.0950078168993476	0.1148	0.287
A0	B1	32	4999	1.1077910962972959	0.073	0.2442
A0	B3	32	4999	1.104691005316111	0.0814	0.2442
A1	A3	28	4999	0.9521182145487357	0.6342	0.8648181818181818
A1	B0	32	4999	1.0602119110285773	0.2258	0.423375
A1	B1	32	4999	1.0383722040482721	0.2834	0.4716
A1	B3	32	4999	1.0696253844155375	0.1622	0.3475714285714286
A3	B0	30	4999	1.4073143203138845	0.0008	0.006
A3	B1	30	4999	1.4124241228057888	0.0004	0.006
A3	B3	30	4999	1.1867387623954813	0.0158	0.079
B0	B1	34	4999	0.8305028119746661	0.947	0.9996
B0	B3	34	4999	0.9194192705604267	0.7626	0.9532499999999999
B1	B3	34	4999	0.7934201175846153	0.9836	0.9996

5. **The full repeated measures of ANOVA table comparing the abundances of the five most abundant phyla in the stool samples in the control group in the three time points.**

Pairwise Comparisons

Measure	(I) TimePoint	(J) TimePoint	Mean Difference (I-J)	Std. Error	Sig.[b]	95% Confidence Interval for Difference[b] Lower Bound	Upper Bound
Firmicutes	1	2	-2234,769	1238,180	,096	-4932,532	462,994
		3	1612,308	1388,808	,268	-1413,645	4638,261
	2	1	2234,769	1238,180	,096	-462,994	4932,532
		3	3847,077*	1224,259	,008	1179,645	6514,509
	3	1	-1612,308	1388,808	,268	-4638,261	1413,645
		2	-3847,077*	1224,259	,008	-6514,509	-1179,645
Bacteroidota	1	2	73,923	581,184	,901	-1192,368	1340,215
		3	1384,000*	634,229	,050	2,133	2765,867
	2	1	-73,923	581,184	,901	-1340,215	1192,368
		3	1310,077	690,146	,082	-193,622	2813,776
	3	1	-1384,000*	634,229	,050	-2765,867	-2,133
		2	-1310,077	690,146	,082	-2813,776	193,622
Proteobacteria	1	2	154,462	259,593	,563	-411,143	720,066
		3	302,923	243,242	,237	-227,055	832,902
	2	1	-154,462	259,593	,563	-720,066	411,143
		3	148,462	137,706	,302	-151,574	448,497
	3	1	-302,923	243,242	,237	-832,902	227,055
		2	-148,462	137,706	,302	-448,497	151,574
Actinobacteriota	1	2	-223,308*	98,636	,043	-438,218	-8,398
		3	53,308	99,634	,602	-163,777	270,392
	2	1	223,308*	98,636	,043	8,398	438,218
		3	276,615	137,540	,067	-23,058	576,289
	3	1	-53,308	99,634	,602	-270,392	163,777
		2	-276,615	137,540	,067	-576,289	23,058
Verrucomicrobiota	1	2	-170,769	132,234	,221	-458,881	117,343
		3	-154,385	142,505	,300	-464,875	156,106
	2	1	170,769	132,234	,221	-117,343	458,881
		3	16,385	212,965	,940	-447,626	480,396
	3	1	154,385	142,505	,300	-156,106	464,875
		2	-16,385	212,965	,940	-480,396	447,626

Based on estimated marginal means

*. The mean difference is significant at the ,05 level.

b. Adjustment for multiple comparisons: Least Significant Difference (equivalent to no adjustments).

6. **The full repeated measures of ANOVA table comparing the abundances of the five most abundant phyla in the stool samples in the enriched group in the three time points.**

Pairwise Comparisons

Measure	(I) TimePoint	(J) TimePoint	Mean Difference (I-J)	Std. Error	Sig.[b]	95% Confidence Interval for Difference[b] Lower Bound	Upper Bound
Firmicutes	1	2	-1202,353	815,733	,160	-2931,630	526,924
		3	2449,941*	972,447	,023	388,446	4511,437
	2	1	1202,353	815,733	,160	-526,924	2931,630
		3	3652,294*	550,392	<,001	2485,514	4819,074
	3	1	-2449,941*	972,447	,023	-4511,437	-388,446
		2	-3652,294*	550,392	<,001	-4819,074	-2485,514
Bacteroidota	1	2	-346,471	483,890	,484	-1372,272	679,331
		3	699,353	436,492	,129	-225,969	1624,675
	2	1	346,471	483,890	,484	-679,331	1372,272
		3	1045,824*	444,174	,032	104,216	1987,431
	3	1	-699,353	436,492	,129	-1624,675	225,969
		2	-1045,824*	444,174	,032	-1987,431	-104,216
Proteobacteria	1	2	-128,588	97,807	,207	-335,930	78,753
		3	41,529	68,665	,554	-104,035	187,094
	2	1	128,588	97,807	,207	-78,753	335,930
		3	170,118*	76,625	,041	7,681	332,554
	3	1	-41,529	68,665	,554	-187,094	104,035
		2	-170,118*	76,625	,041	-332,554	-7,681
Actinobacteriota	1	2	-353,941*	135,395	,019	-640,966	-66,916
		3	-58,118	116,001	,623	-304,028	187,793
	2	1	353,941*	135,395	,019	66,916	640,966
		3	295,824*	98,827	,009	86,320	505,327
	3	1	58,118	116,001	,623	-187,793	304,028
		2	-295,824*	98,827	,009	-505,327	-86,320
Verrucomicrobiota	1	2	-120,000	72,162	,116	-272,976	32,976
		3	-16,176	53,854	,768	-130,343	97,990
	2	1	120,000	72,162	,116	-32,976	272,976
		3	103,824	57,996	,092	-19,121	226,769
	3	1	16,176	53,854	,768	-97,990	130,343
		2	-103,824	57,996	,092	-226,769	19,121

Based on estimated marginal means

*. The mean difference is significant at the ,05 level.

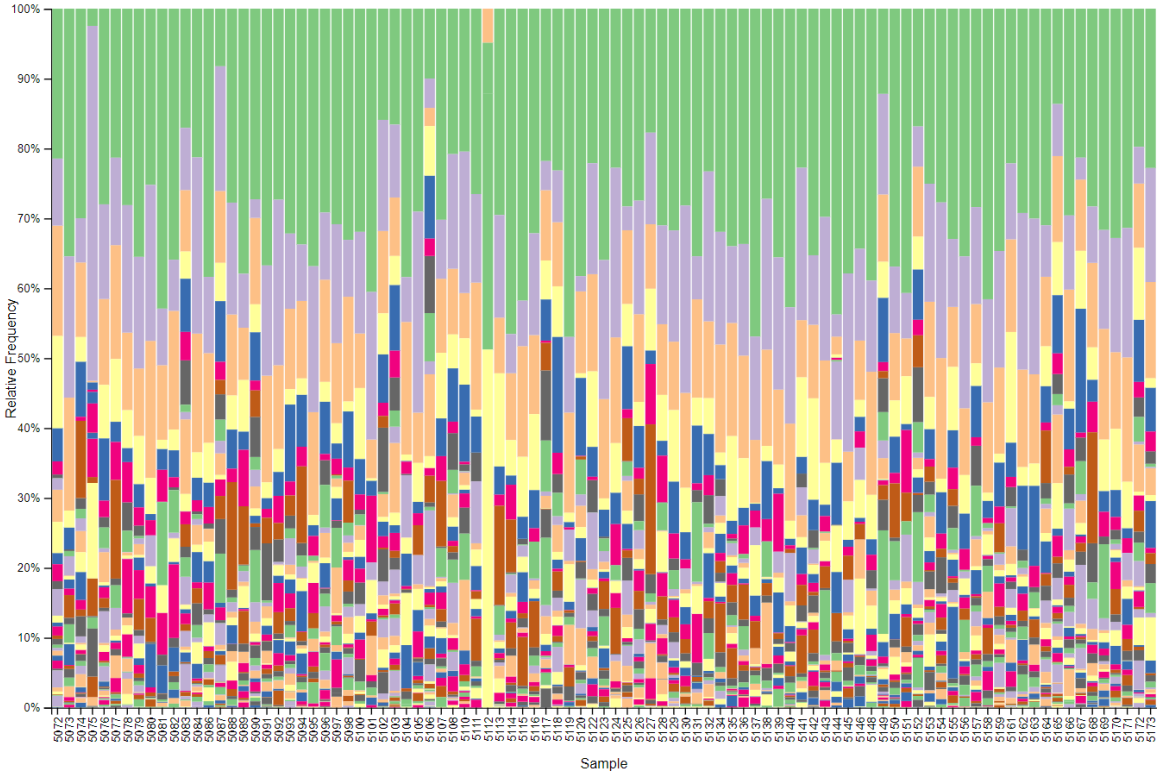
b. Adjustment for multiple comparisons: Least Significant Difference (equivalent to no adjustments).

7. **The full paired samples t-test table comparing the abundances of the five most abundant phyla in the stool samples in between the two research groups in each of the time points.**

Paired Samples Test

		Paired Differences					t	df	Significance	
					95% Confidence Interval of the Difference					
		Mean	Std. Deviation	Std. Error Mean	Lower	Upper			One-Sided p	Two-Sided p
Pair 1	A_Firmicutes_0 - B_Firmicutes_0	-771,15385	3745,73544	1038,88009	-3034,67912	1492,37143	-,742	12	,236	,472
Pair 2	A_Firmicutes_1 - B_Firmicutes_1	763,84615	3572,74264	990,90052	-1395,14062	2922,83292	,771	12	,228	,456
Pair 3	A_Firmicutes_3 - B_Firmicutes_3	646,30769	4721,85344	1309,60651	-2207,07978	3499,69517	,494	12	,315	,631
Pair 4	A_Bacteroidota_0 - B_Bacteroidota_0	465,53846	2186,76685	606,50000	-855,91152	1786,98845	,768	12	,229	,458
Pair 5	A_Bacteroidota_1 - B_Bacteroidota_1	-105,46154	2004,03916	555,82046	-1316,49028	1105,56720	-,190	12	,426	,853
Pair 6	A_Bacteroidota_3 - B_Bacteroidota_3	-8,69231	2474,30355	686,24833	-1503,89898	1486,51436	-,013	12	,495	,990
Pair 7	A_Proteobacteria_0 - B_Proteobacteria_0	410,76923	1052,93575	292,03183	-225,51347	1047,05194	1,407	12	,092	,185
Pair 8	A_Proteobacteria_1 - B_Proteobacteria_1	61,53846	768,03099	213,01347	-402,57802	525,65494	,289	12	,389	,778
Pair 9	A_Proteobacteria_3 - B_Proteobacteria_3	109,53846	499,07575	138,41871	-192,04999	411,12692	,791	12	,222	,444
Pair 10	A_Actinobacteriota_0 - B_Actinobacteriota_0	-190,5384615	438,7726472	121,6936367	-455,6861186	74,6091955	-1,566	12	,072	,143
Pair 11	A_Actinobacteriota_1 - B_Actinobacteriota_1	-380,1538462	470,1155614	130,3865971	-664,2418367	-96,0658557	-2,916	12	,006	,013
Pair 12	A_Actinobacteriota_3 - B_Actinobacteriota_3	-304,2307692	557,5993415	154,6502321	-641,1846790	32,7231405	-1,967	12	,036	,073
Pair 13	A_Verrucomicrobiota_0 - B_Verrucomicrobiota_0	254,23077	459,58118	127,46488	-23,49136	531,95290	1,995	12	,035	,069
Pair 14	A_Verrucomicrobiota_1 - B_Verrucomicrobiota_1	283,84615	714,73513	198,23186	-148,06396	715,75627	1,432	12	,089	,178
Pair 15	A_Verrucomicrobiota_3 - B_Verrucomicrobiota_3	396,30769	483,39759	134,07037	104,19345	688,42193	2,956	12	,006	,012

APPENDIX 6. The taxonomic bar plot of the phyla in all the stool samples.




d__Bacteria;p__Firmicutes;c__Clostridia;o__Lachnospirales;f__Lachnospiraceae
d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae
d__Bacteria;p__Firmicutes;c__Clostridia;o__Oscillospirales;f__Ruminococcaceae
d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Rikenellaceae
d__Bacteria;p__Firmicutes;c__Clostridia;o__Oscillospirales;f__Oscillospiraceae
d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Tannerellaceae
d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Prevotellaceae
d__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridia_UCG-014;f__Clostridia_UCG-014
d__Bacteria;p__Verrucomicrobiota;c__Verrucomicrobiae;o__Verrucomicrobiales;f__Akkermansiaceae
d__Bacteria;p__Actinobacteriota;c__Actinobacteria;o__Bifidobacteriales;f__Bifidobacteriaceae
d__Bacteria;p__Firmicutes;c__Clostridia;o__Christensenellales;f__Christensenellaceae
d__Bacteria;p__Firmicutes;c__Bacilli;o__Erysipelotrichales;f__Erysipelatoclostridiaceae
d__Bacteria;p__Firmicutes;c__Clostridia;o__Peptostreptococcales-Tissierellales;f__Peptostreptococcaceae
d__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Burkholderiales;f__Sutterellaceae
d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Barnesiellaceae
d__Bacteria;p__Firmicutes;c__Clostridia;o__Oscillospirales;f__[Eubacterium]_coprostanoligenes_group
d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Muribaculaceae
d__Bacteria;p__Firmicutes;c__Bacilli;o__RF39;f__RF39
d__Bacteria;p__Firmicutes;c__Negativicutes;o__Acidaminococcales;f__Acidaminococcaceae
d__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridiales;f__Clostridiaceae
d__Bacteria;p__Firmicutes;c__Negativicutes;o__Veillonellales-Selenomonadales;f__Veillonellaceae
d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Marinifilaceae
d__Bacteria;p__Firmicutes;c__Bacilli;o__Lactobacillales;f__Streptococcaceae
d__Bacteria;p__Firmicutes;c__Clostridia;o__Oscillospirales;f__Butyricicoccaceae
d__Bacteria;p__Firmicutes;c__Clostridia;o__Monoglobales;f__Monoglobaceae
d__Bacteria;p__Firmicutes;c__Clostridia;o__Oscillospirales;f__UCG-010
d__Bacteria;p__Firmicutes;c__Bacilli;o__Erysipelotrichales;f__Erysipelotrichaceae
d__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Enterobacterales;f__Enterobacteriaceae
d__Bacteria;p__Firmicutes;c__Clostridia;o__Peptostreptococcales-Tissierellales;f__Anaerovoracaceae
d__Bacteria;p__Actinobacteriota;c__Coriobacteriia;o__Coriobacteriales;f__Coriobacteriales_Incertae_Sedis
d__Bacteria;p__Desulfobacterota;c__Desulfovibrionia;o__Desulfovibrionales;f__Desulfovibrionaceae
d__Bacteria;p__Actinobacteriota;c__Coriobacteriia;o__Coriobacteriales;f__Eggerthellaceae
d__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Pasteurellales;f__Pasteurellaceae
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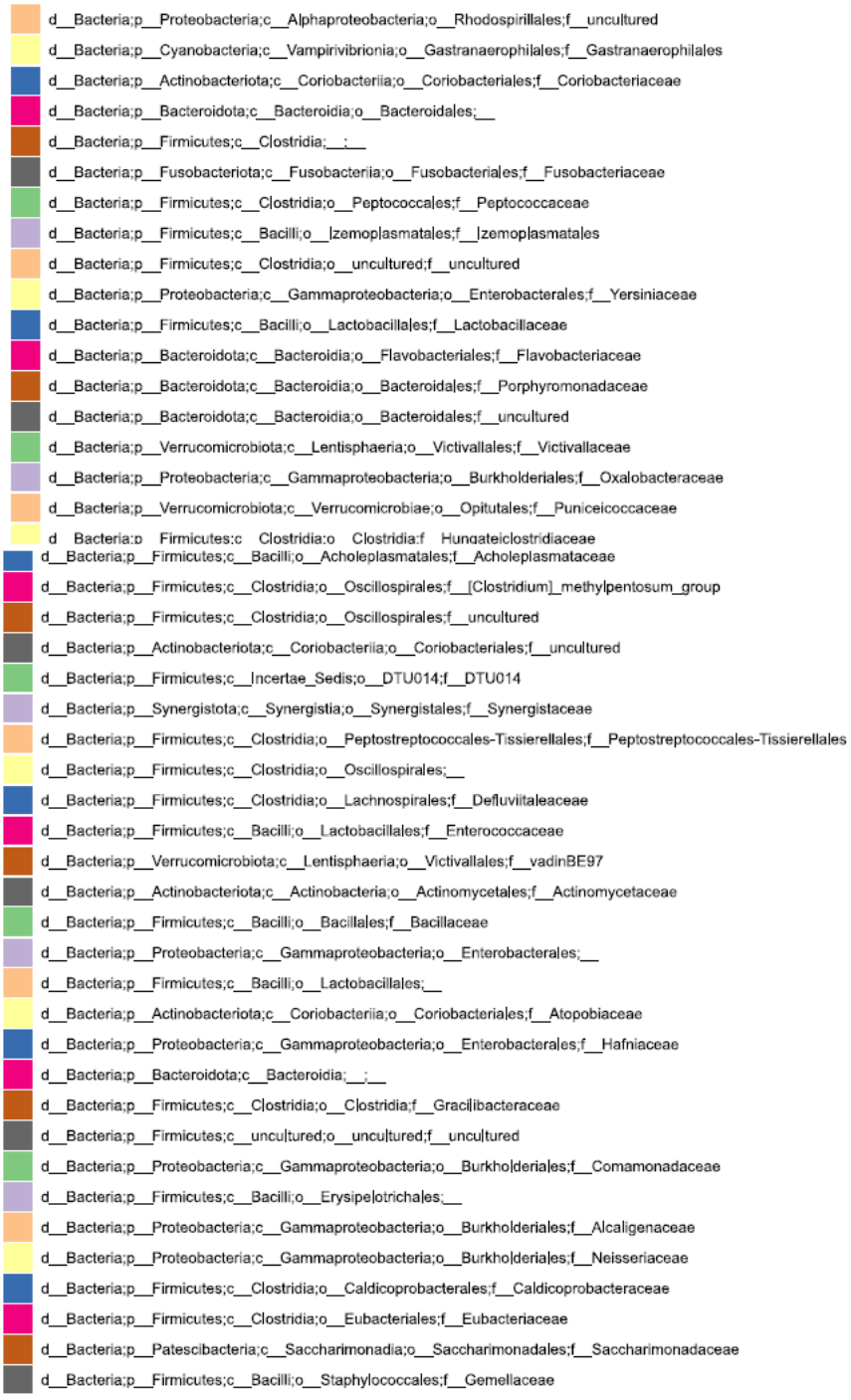
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d__Bacteria;p__Cyanobacteria;c__Vampirivibrionia;o__Gastranaerophilales;f__Gastranaerophilales
d__Bacteria;p__Actinobacteriota;c__Coriobacteriia;o__Coriobacteriales;f__Coriobacteriaceae
d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;__
d__Bacteria;p__Firmicutes;c__Clostridia;__;__
d__Bacteria;p__Fusobacteriota;c__Fusobacteriia;o__Fusobacteriales;f__Fusobacteriaceae
d__Bacteria;p__Firmicutes;c__Clostridia;o__Peptococcales;f__Peptococcaceae
d__Bacteria;p__Firmicutes;c__Bacilli;o__Izemoplasmatales;f__Izemoplasmatales
d__Bacteria;p__Firmicutes;c__Clostridia;o__uncultured;f__uncultured
d__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Enterobacterales;f__Yersiniaceae
d__Bacteria;p__Firmicutes;c__Bacilli;o__Lactobacillales;f__Lactobacillaceae
d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Flavobacteriales;f__Flavobacteriaceae
d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Porphyromonadaceae
d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__uncultured
d__Bacteria;p__Verrucomicrobiota;c__Lentisphaeria;o__Victivallales;f__Victivallaceae
d__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Burkholderiales;f__Oxalobacteraceae
d__Bacteria;p__Verrucomicrobiota;c__Verrucomicrobiae;o__Opitutales;f__Puniceicoccaceae
d__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridia;f__Hungateiclostridiaceae
d__Bacteria;p__Firmicutes;c__Bacilli;o__Acholeplasmatales;f__Acholeplasmataceae
d__Bacteria;p__Firmicutes;c__Clostridia;o__Oscillospirales;f__[Clostridium]_methylpentosum_group
d__Bacteria;p__Firmicutes;c__Clostridia;o__Oscillospirales;f__uncultured
d__Bacteria;p__Actinobacteriota;c__Coriobacteriia;o__Coriobacteriales;f__uncultured
d__Bacteria;p__Firmicutes;c__Incertae_Sedis;o__DTU014;f__DTU014
d__Bacteria;p__Synergistota;c__Synergistia;o__Synergistales;f__Synergistaceae
d__Bacteria;p__Firmicutes;c__Clostridia;o__Peptostreptococcales-Tissierellales;f__Peptostreptococcales-Tissierellales
d__Bacteria;p__Firmicutes;c__Clostridia;o__Oscillospirales;__
d__Bacteria;p__Firmicutes;c__Clostridia;o__Lachnospirales;f__Defluviitaleaceae
d__Bacteria;p__Firmicutes;c__Bacilli;o__Lactobacillales;f__Enterococcaceae
d__Bacteria;p__Verrucomicrobiota;c__Lentisphaeria;o__Victivallales;f__vadinBE97
d__Bacteria;p__Actinobacteriota;c__Actinobacteria;o__Actinomycetales;f__Actinomycetaceae
d__Bacteria;p__Firmicutes;c__Bacilli;o__Bacillales;f__Bacillaceae
d__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Enterobacterales;__
d__Bacteria;p__Firmicutes;c__Bacilli;o__Lactobacillales;__
d__Bacteria;p__Actinobacteriota;c__Coriobacteriia;o__Coriobacteriales;f__Atopobiaceae
d__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Enterobacterales;f__Hafniaceae
d__Bacteria;p__Bacteroidota;c__Bacteroidia;__;__
d__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridia;f__Gracilibacteraceae
d__Bacteria;p__Firmicutes;c__uncultured;o__uncultured;f__uncultured
d__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Burkholderiales;f__Comamonadaceae
d__Bacteria;p__Firmicutes;c__Bacilli;o__Erysipelotrichales;__
d__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Burkholderiales;f__Alcaligenaceae
d__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Burkholderiales;f__Neisseriaceae
d__Bacteria;p__Firmicutes;c__Clostridia;o__Caldicoprobacterales;f__Caldicoprobacteraceae
d__Bacteria;p__Firmicutes;c__Clostridia;o__Eubacteriales;f__Eubacteriaceae
d__Bacteria;p__Patescibacteria;c__Saccharimonadia;o__Saccharimonadales;f__Saccharimonadaceae
d__Bacteria;p__Firmicutes;c__Bacilli;o__Staphylococcales;f__Gemellaceae