Thesis

STABILITY AND DYNAMICS OF THE HEALTHY SKIN MICROBIOME

A longitudinal observational study over two years

submitted by

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Declaration of Academic Integrity

I hereby confirm that the present diploma thesis is the result of my own independent scholarly work. I also confirm that in all cases, where material from the work of others (in books, articles, essays, dissertations, and on the internet) is acknowledged, quotations and paraphrases are clearly indicated. No material other than that cited in the reference list has been used. I have read and understood the Medical University's regulations and procedures concerning plagiarism.

Graz, 02.03.2023 *Adina Vanessa Lehnen m.p.*

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Content

Abbreviations

Anosim	Analysis of similarity
ANOVA	Analysis of Variance
AOA	Ammonia oxidising archaea
ASV	Amplicon sequence variant
BMI	Body-Mass-Index
Bp	Base pair
°C	Temperature in degree Celsius
DADA	Divisive Amplicon Denoising Algorithm
DNA	Desoxyribonucleic acid
e.g.,	exempli gratia (for example)
LDA	Linear Discriminant Analysis
LEfSe	Linear Discriminant Analysis Effect Size
MAFFT	Multiple Alignment using Fast Fourier Transform
nd	not detectable
No.	Number
OTU	Operational taxonomic unit
PCoA	Principal coordinates analysis
PCR	Polymerase Chain Reaction
pH	Potential of hydrogen
QIIME	Quantitative insight into Microbial Ecology
RDA	Redundancy analysis
RESCRIPt	Reproducible sequence taxonomy reference database management

rRNA	Ribosomal ribonucleic acid
SPSS	Statistical Package for the Social Sciences
TAE	Tris-acetate-EDTA
TEWL	Transepidermal water loss
TSS	Total sum normalisation
UV	Ultraviolet

Glossary

16S rRNA	RNA component of the 30S subunit of a prokaryotic ribosome
Adonis	A multivariate ANOVA based on similarity test
FASTQ format	Text-based format for storing a biological sequence and its quality scores
FastTree	Tool that creates approximately-maximum-likelihood phylogenetic trees from alignments of nucleotide or protein sequences
Kolmogorov-Smirnov-Test	Nonparametric statistical test of the equality of probability distributions
Kruskal-Wallis-Test	Nonparametric statistical test used to compare more than two independent samples
Mann-Whitney-U-Test	Nonparametric statistical test used to compare two independent samples
Naïve-Bayes	Algorithm that uses Bayes´ Theorem to classify objects
Pearson index	Correlation coefficient that measures the linear correlation of two data sets
p-value	Probability of test results at least as extreme as the result observed, given that the null hypothesis is correct
Regression analysis	Statistical process for the evaluation of the relationship between one dependent and one or more independent variables
Shapiro-Wilk-Test	Statistical test of normality distribution
Shannon-Index	Measurement index of the diversity of species in a community
Sklearn	Scikit learn, a machine learning library for Python
t-value	The calculated difference represented in units of standard error
UniFrac	Distance metric used for comparison of biological communities

List of Figures

List of Tables

Zusammenfassung

Das Hautmikrobiom, bestehend aus Bakterien, Archaeen und Eukaryonten (z.B. Pilze), wird seit einigen Jahren intensiv erforscht. Es konnten vor allem in Bezug auf Bakterien und Pilze in vielen Studien mikrobielle Taxa ausgemacht werden, welche normalerweise gesunde Haut an verschiedenen Körperstellen besiedeln. Man fand heraus, dass sowohl interne als auch externe Einflussfaktoren zu Verschiebungen in der Zusammensetzung des Hautmikrobioms führen können, was einen Einfluss auf die Pathophysiologie von bestimmten Hauterkrankungen haben könnte. Die genaue Rolle ist jedoch in vielen Fällen noch nicht erforscht, z.B. bei Psoriasis, einer chronisch entzündlichen Hauterkrankung.

In unserer Studie wurde das Hautmikrobiom sowie wichtige hautphysiologische Parameter von 12 erwachsenen Probanden (Altersgruppen B1: 20-40 Jahre, B2: 60-80 Jahre) mit gesunder Haut an je vier Körperstellen über sechs Zeitpunkte innerhalb von zwei Jahren untersucht und im Hinblick auf verschiedene demographische und Lebensstilfaktoren verglichen. In meiner Diplomarbeit konzentriere ich mich auf die Daten des bakteriellen und fungalen Mikrobioms, die Auswertung des archaeellen Mikrobioms findet im Rahmen einer anderen Arbeit statt.

Die häufigsten Gattungen waren *Cutibacterium*, *Burkholderia*, *Staphylococcus*, *Corynebacterium*, *Streptococcus* und *Malassezia*. Es konnte ein signifikanter Einfluss von BMI, Hauthygiene, Jahreszeit und Körperstelle auf die Zusammensetzung des Hautmikrobioms nachgewiesen werden. Auch die interpersonelle Variabilität im Hautmikrobiom der Probanden zeigte sich signifikant. Die beiden Altersgruppen und Geschlechter wiesen typische Mikrobiota auf, die Diversität wurde jedoch nicht signifikant von diesen Metadaten beeinflusst. Bezüglich der Hautphysiologie zeigte sich ein signifikant höherer pH-Wert und ein niedrigerer transepidermaler Wasserverlust bei den älteren und weiblichen Testpersonen. Darüber hinaus waren die Proben aus den Sommermonaten durch eine höhere Hautfeuchtigkeit gekennzeichnet. Die häufigsten Taxa unserer Studie sowie deren Vorkommen bezüglich verschiedener Metadaten deckten sich größtenteils mit denen der Literatur. Insgesamt zeigte sich, dass vor allem externe, dynamische Faktoren einen Einfluss auf das Hautmikrobiom hatten und es ist weitere Forschung an größeren Kohorten und in Verbindung mit der Darm-Haut-Achse nötig, um bestenfalls neue Erkenntnisse als Basis für weitere Studien zur Pathophysiologie chronisch entzündlicher Hauterkrankungen zu gewinnen.

Abstract

The skin microbiome, consisting of bacteria, archaea and eukaryotes has been increasingly researched for several years. Particularly for bacteria and fungi, studies have identified taxa that typically colonise the skin at various body sites. Both internal and external factors can lead to shifts in the composition of the skin microbiome, which could play a relevant role in the pathophysiology of certain skin diseases.

In our study, the skin microbiome as well as important skin physiological parameters of 12 individuals with healthy skin (age groups B1: 20-40 years, B2: 60-80 years) were examined on four body sites each over six time points within two years and compared regarding various demographic and lifestyle factors. In this thesis I focus on the bacterial and fungal data, as the evaluation of the archaeal microbiome takes place in the context of another thesis. The collected data and results will also be used to compare the skin microbiome of healthy people with that of people suffering from psoriasis, and ideally to gain new insights as a basis for further studies on the pathophysiology of this skin disease.

The most common genera were *Cutibacterium*, *Burkholderia*, *Staphylococcus*, *Corynebacterium*, *Streptococcus* and *Malassezia*. A significant influence of BMI, skin hygiene, season and body site on the composition of the skin microbiome was identified. There was also a significant interpersonal variability in the skin microbiome. The two targeted age groups and sexes had typical microbiota, but diversity was not significantly affected by these metadata categories. There was a significantly higher skin pH and lower transepidermal water loss in the older, female subjects. In addition, samples from the summer months were characterised by higher skin moisture than those from winter.

The most common taxa in our study, as well as their abundance with respect to various metadata, were mostly consistent with those in the literature. Overall, mainly external, dynamic factors showed an influence on the skin microbiome and further research on larger cohorts and in connection with the gut-skin axis is needed to ideally gain new insights as a basis for further studies on the pathophysiology of chronic inflammatory skin diseases.

1 Introduction

1.1 Structure and Functions of the Human Skin

The skin, as with 1.85 m^2 surface area the largest organ of the human body, functions as a protective interface against external threats. These range from pathogens to toxic chemicals, UV radiation and mechanical injuries (1).

Another important function of the skin is the regulation of homeostatic processes such as water content and body temperature. Cutaneous glands make this perspiration possible through the secretion of sweat (2).

From an anatomical point of view, the skin consists of two main layers: the superficial epidermis and the deep dermis, where the hypodermis is located underneath (Figure 1). Together with the skin appendages (hair, nails, sebaceous and sweat glands as well as their ducts), they build the integumentary system (1). Each layer is constructed differently and has its own characteristics and functions. Hence, they should be explained in more detail here.

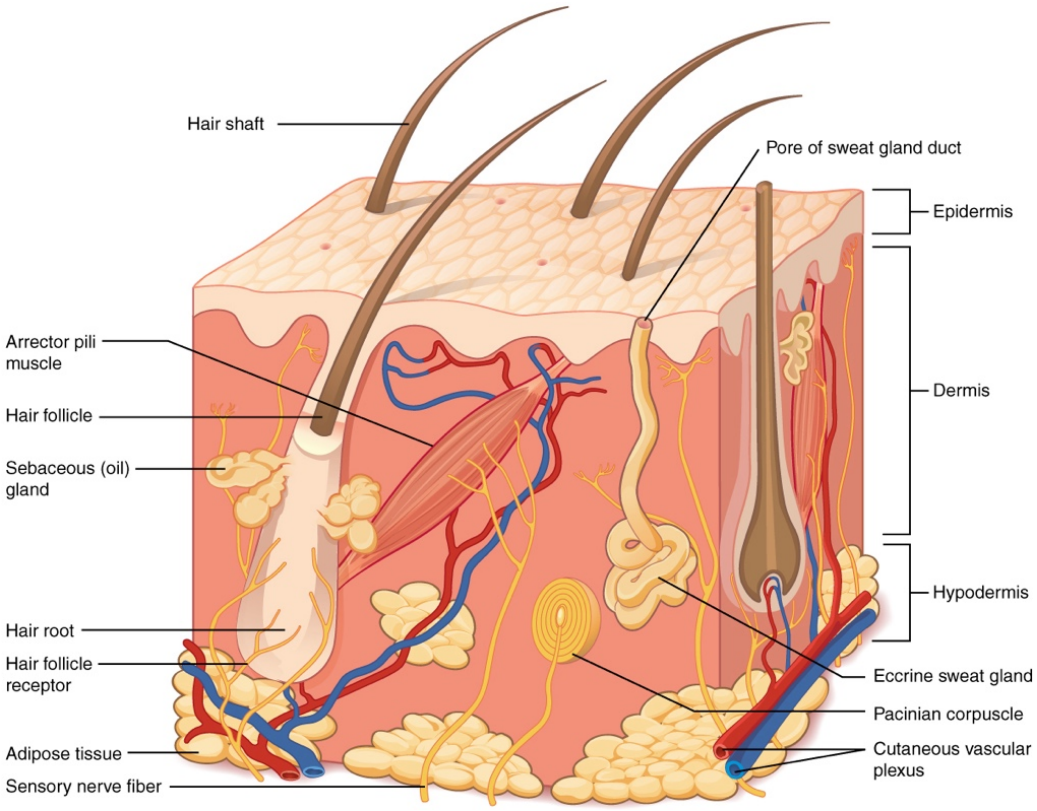

Figure 1: The human skin anatomy with its different layers and skin appendages (3)

Epidermis

The epidermis, the external layer, is largely responsible for the skin colour, texture, and moisture of the human skin (4). It consists of four main layers that represent different stages of cell development (5).

Beginning from the deepest basal layer, stem cells divide and become keratinocytes. After that, they undergo a 4-week migration and differentiation process to the uppermost layer, where they subsequently become cornified (4) and are terminally enucleated squamous epithelial cells that are interconnected to amplify the skin barrier (6). After completion of keratinization in the stratum corneum, the cells will be shed after apoptosis. Through that process, the epidermis is renewed repetitively in the course of time (1).

On cellular level, the epidermis contains 95% keratinocytes (1). They produce the protein keratin, which helps to maintain the flexibility of the skin, and at the same time, protects it from stress and external damage (5). Furthermore, the keratinocytes secrete antimicrobial peptides and build tight junctions to control microbial infections (7). Merkel-Cells and Melanocytes can be found in the basal layer of the epidermis. Merkel-Cells contain granules that secrete content like neuroendocrine cells. Melanocytes are responsible for the production of melanin and thus for the skin colour and protection from UV-radiation induced injuries. Langerhans cells, another epidermal cell type, are antigen processing and presenting (4).

Dermis

The dermis, located underneath the epidermis, forms a structural framework (5). It contains mainly fibroblasts, collagen, and elastin fibres, but also vessels and skin appendages such as hair follicles, glands, and nerve endings. Collagen and elastin are responsible for the strength and elasticity of the skin (2). The production of those proteins is conducted by fibroblasts, the primary cell type in the dermis.

The dermis is composed of the papillary stratum, which is near the epidermis and is characterized by fibrocytes, collagen and vessels, and the thicker reticular stratum with a denser collagen accumulation (4).

The hypodermis (or subcutaneous tissue) is connecting the cutis (epidermis and dermis) with deeper tissues like muscles or bones and functions as protective layer (5). It mainly consists of subcutaneous fat, blood vessels and nerves (4).

Skin appendages

Skin appendages, a group of glands and hair follicles that support the maintaining of the skin's functions pressure and temperature sensation, removal of threats, perspiration, and thermoregulation are also main part of the skin´s structure (1).

Sebaceous glands always occur together with hair follicles. Together with the arrector pili muscle and nerve endings they form a so-called pilosebaceous unit. This unit is important for temperature regulation, sensation, and the maintenance of the skin (4). By secretion of sebum, a lipid complex, onto the hair shaft, the sebaceous gland keeps the hair soft and flexible (1). Furthermore, the sebum protects the skin from drying out and functions as a barrier against pathogens (4). Body sites with a high density of sebaceous glands are the face, chest and back (8). The number and distribution of sebaceous glands remains stable the whole life, but their activity depends on age and hormone levels (5).

Sweat glands are exocrine glands that can be categorized into two major types: eccrine and apocrine (2). These glands are embedded in the deep dermis and subcutis with their ducts crossing the epidermis to the skin surface (4).

The most frequent sweat glands, present all over the body, are the eccrine glands. Their sweat is clear and consists mainly of water and salts (2). Different from apocrine and sebaceous glands, eccrine sweat glands drain their secretion directly onto the skin surface. The highest density of eccrine sweat glands, which are active from birth on, can be found at the palms, the soles of the feet, the forehead, and armpits (1). Their main function is thermoregulatory perspiration, which is regulated by different external and internal parameters e.g., external and body temperature, humidity, physical fitness, or emotions. Those parameters also affect the individual composition of eccrine sweat. Furthermore, the secreted sweat provides an acidic skin milieu, which had a protective effect on bacterial colonization and growth on the skin (2).

Apocrine glands are only present in hairy regions of the axilla, nipples, genital and anal, where they secrete their sweat into the hair canal (8). The main stimulation of those glands is hormonal, which is the reason why their activity begins only during puberty. Unlike eccrine sweat, apocrine sweat is milky and contains viscous substances such as lipids,

lactate, and proteins. On exposure to microorganisms, the secret becomes odorous. The exact function of apocrine sweat is not yet known in detail (2).
Depending on the topographical characteristics in different body sites, the skin environment is classified either as dry (e.g., palms, forearm, buttock), sebaceous/oily (e.g., forehead, back, upper body), or moist (e.g., axilla, elbow) (5).

1.2 Human microbiome on the skin

1.1.1 Microbial taxa on the skin

Human skin is colonised by a large community of microorganisms: bacteria, archaea, eukaryotes (fungi, mites) and viruses (9).
The skin microbiome can be grouped into two groups: resident microorganisms (the core microbiome) and transient microorganisms. Resident microorganisms are typically found in the skin and called commensals, which means that they are usually non-pathogenic and probably even beneficial for the host. Transient microorganisms, e.g., on the other hand, do not permanently colonize the host skin but stay there for some time (hours to days). Normally, they are also not harmful for the host. (10).
The following is a brief overview of the microorganisms living on the skin and their known characteristics and functions. Subsequently, the work will concentrate exclusively on bacteria and fungi.

Bacteria

The molecular characterisation of the bacterial skin microbiome of adults on genomic level reveals the presence of four main phyla: *Actinobacteria*, *Firmicutes*, *Bacteroidetes* and *Proteobacteria*. The most abundant genera on the skin are *Corynebacterium*, *Cutibacterium* (formerly: *Propionibacterium*) and *Staphylococcus*. Research has shown that, although the diversity is usually low at phylum level, the skin microbiome is characterised by a high diversity on species level. Also, the actual individual composition of microbiome was observed to vary over time, which shows that the human skin microbiome is dynamic (10).
Furthermore, the composition of the skin microbiome is strongly age dependent. the colonisation of the skin with microorganisms starts at birth and differs depending on the

birth mode. Vaginal birth will allow the colonisation of the baby´s skin with vaginal microorganisms, whereas a caesarean section will rather support the establishment of common skin residents due to the lack of contact to the birth canal and its microorganisms. In addition, the similarity of the baby's skin microbiome with that of the mother is much greater in children born vaginally than in those born by caesarean section (11). After this first establishment of skin microbiome, colonization of microorganisms on the body continues with an influence of physiological and biochemical characteristics of the skin (5). During puberty, there is a change in skin physiology due to the release of sexual hormones, which results in higher sebum production and a dominance of lipophilic *Cutibacterium* and *Malassezia* (12). By adulthood, a final state of balance in the skin microbiome diversity is established unique for each individual (10).

In general, interpersonal variation is higher than intrapersonal variation regarding the bacterial and fungal communities that harbour the skin (13). In adulthood, relative stability of an individual's skin microbiome over time has been observed (14).

Sex is found to be another criterion for differences in microbial diversity on human skin which probably has to do with sex-specific skin anatomy and physiology (e.g., thin thickness, androgen levels, gland activity) (5).

Furthermore, the composition and diversity of the microbes depends on the skin site with their different skin appendages, as there are specific bacteria associated with the microenvironments of the skin (moist, dry, sebaceous) (8). The topographical analysis of the bacterial skin microbiome shows that sebaceous skin sites (e.g., face, upper body and back) are dominated by *Cutibacterium* and *Staphylococcus* (15,16). In moist areas like the inner elbow or axilla mainly *Corynebacterium*, β-Proteobacteria and *Staphylococcus* can be found. On dry sites like the volar forearm and buttock the variation of genera is higher, with a high colonization by *Cutibacterium* and *Corynebacterium* (5,17). Figure 2 shows exemplarily the typical topographical distribution of the most common skin commensals and the corresponding site characteristic (sebaceous/oily, moist, dry).

Fungi

Apart from bacteria, fungi are also common residents on the human skin. The fungal skin microbiome differs mainly depending on the body site (18,19). *Malassezia*, a polymorphic yeast, is with 80% the most abundant cutaneous fungus on the human body (10). Other common fungal skin commensals are *Candida*, *Aspergillus*, *Penicillium* and *Cryptococcus*,

but all with much lower abundances than *Malassezia* (20). *Malassezia* is present all over the body (Figure 2), but particularly on the scalp (10), followed by core and arms (17). The highest fungal diversity on human skin can be found on the feet (Figure 2) with high shares of *Aspergillus*, *Cryptococcus*, *Rhodotorula* and *Epicoccus* in addition to *Malassezia* (5,18). Similar to the initial bacterial colonisation, the skin of new-borns immediately after birth was observed to be colonised with *Malassezia* (21), *Candida*, *Cladosporium*, *Fusarium* and *Cryptococcus and the composition* (22). Furthermore, the initial fungal composition is dependent on the delivery mode, with different species of *Candida* being predominant for the first 30 days of life (22). Additionally, the diversity of the skin mycobiome decreases with age (22).

Archaea

Archaea form a minority of up to 4% of the microbes on human skin (23). It has been shown that mainly representatives of *Thaumarchaeota* (now classified as *Thermoproteota* (24) and mainly referred to as ammonia oxidising archaea - AOA) are found on the skin. It is inferred that this group of archaea can oxidise ammonium from sweat and thus lower the pH of the skin (9). AOA are also frequently found in hospital environments and clean rooms (23). In previous studies, a more constant occurrence was observed on the skin of women than men (25). In addition, an increased prevalence of AOA has been observed in people over 60 or under 12 years of age (9).

Viruses

Various families of eukaryotic viruses (e.g., *Polyomaviridae*, *Papillomaviridae*, *Adenoviridae*, *Herpesviridae* and *Circoviridae*) could be found on healthy human skin (26). Results also show a high diversity of the viral skin microbiome in general (27). There is furthermore a high temporal variation of different types of eukaryotic viruses. Moreover, phages (e.g., *Staphylococcus phages, Streptococcus phages*), viruses that infect bacteria or archaea, could be observed on human skin (28). Studies show that the composition of the skin virome is depending more on the individual that on other factors like biogeography or skin physiology (14).

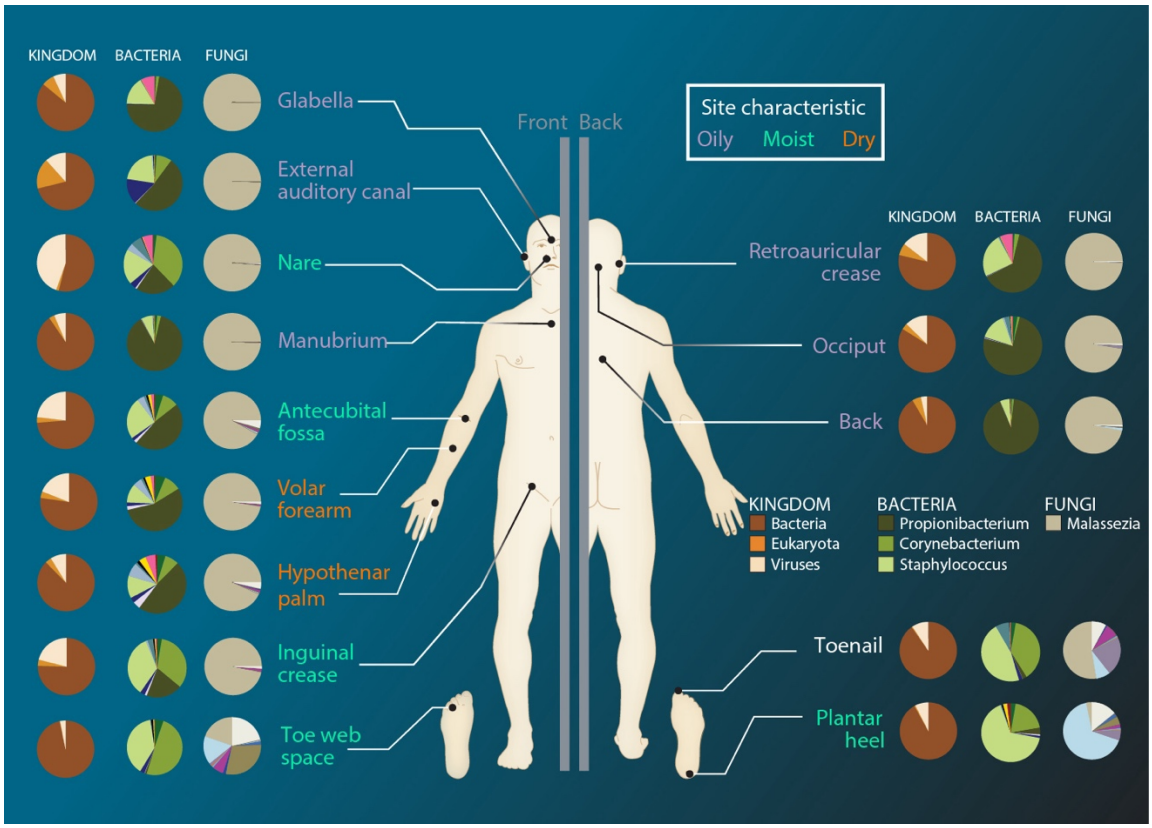

Figure 2: Classification of body sites according to skin physiology and proportional representation of the most common taxa on healthy human skin illustrated by pie charts (29)

1.1.2 Impact of the Microbiome on the skin barrier

There is a complex relationship between the host and the skin microbiome, with microbes being substantially involved in physiological processes of the skin: enhancement of differentiation and epithelialisation, direct protection against pathogens, encouraging tissue repair and stimulation of immune responses (16,30).

The skin microbiome builds a barrier with some bacteria using inhibitory mechanisms against pathogenic microorganisms. Those antagonistic mechanisms often interact effectively together with the host immune response. Furthermore, the physical barrier of keratinocytes is positively influenced and strengthened by the microbiome. The skin barrier is also maintained by the acidic skin pH, which is formed by bacteria and their produced fatty acids (16). Those findings suggest that if changes in diversity and abundance of commensals take place, it can result in disturbances of the skin barrier and subsequently reinforce or encourage skin diseases (10).

The symbiotic relationship and continuous connection between the microbes and the host is possible through complex signalling from the innate and adaptive immune system (10). Many innate immune responses can be triggered by skin-associated bacterial and fungal communities. The adaptive immune system includes resident T cells, whereby it has been shown that certain bacteria can recruit the T cells and stimulate their activity (16).

1.3 Link between skin diseases and the microbiome

There are several factors that could have an impact on certain skin diseases such as atopic dermatitis or psoriasis. These include lifestyle changes, personal hygiene routines or antibiotic use (15). An altered skin microbiome is often found in diseased skin, as microbial communities change when their niche is disrupted. It is not yet fully clear if the change of the microbial community is a cause or a consequence of the skin disease, but there is evidence that these changes may contribute to the pathogenesis of skin diseases (16). It is still debatable which microbes are beneficial, neutral, or harmful to the host. *Staphylococcus epidermidis*, for example, contributes to the modulation of the immune response in skin regeneration and wound healing and is consequently classified as beneficial for the host in this respect (30). On the other hand, the bacterium is capable to form biofilms that favour infection associated with foreign material (e.g., medical devices or prostheses) and is therefore often identified as the driving microorganism in these infections (31,32). Moreover, an association between the fungal skin commensal *Malassezia* and skin diseases such as seborrheic dermatitis or pityriasis versicolor could be identified (22).

Furthermore, it could be shown that there is a link between host-microbiome interactions and other organs in the human body (e.g., lungs, intestine) (16). Several studies indicate a bidirectional connection of the so-called gut-skin axis (17). The influence of the skin on the intestinal microbiome was shown, for example, by a study on narrow band ultraviolet light exposure of the skin, which resulted in an increase in α- and β-diversity of the faecal microbiome composition (33). The gut microbiome can also influence the skin through various mechanisms. On the one hand, an intact intestinal barrier prevents pathogens from entering the bloodstream, protecting the highly vascularised skin. On the other hand, the intestinal microbiome produces, for example, metabolites that have an anti-inflammatory effect (17). Accordingly, this finding also plays a role in the pathogenesis of skin diseases. It can be assumed that exposure of the skin to pathogens can potentially lead to systemic

responses and, conversely, the gut microbiome can influence the inflammatory responses of the skin (16).

1.4 Aim of this thesis

The aim of this thesis was to explore the main bacterial and fungal taxa of the skin of the study participants and to examine the influence of time and skin site on their abundances and the skin physiology markers. Another goal was to identify possible correlations between certain microbial taxa and recorded metadata of the participants.
In the following, the term skin microbiome relates only to bacteria and fungi, as my data include exclusively these microorganisms. I would also like to emphasise that the focus was finding significant associations of the most abundant taxa, and the bacterial and fungal taxa were therefore considered together in one dataset in all analyses.

The hypotheses of this work refer to significant differences between the metadata groups regarding skin physiological parameters and the skin microbiome. Hereafter, the individual hypotheses relating to the metadata categories are stated.

Age

$H1_0$: Skin pH increases with age, whereas sebum content, TEWL and skin moisture decrease with age.

$H2_0$: Age group B1 has a higher diversity of the skin microbiome than B2 with different typical microorganisms on the skin.

Sex

$H3_0$: Skin pH and sebum content is higher in men, whereas TEWL is higher on the skin of women.

$H4_0$: The composition of the skin microbiome between men and women differs significantly.

BMI

$H5_0$: Obesity is associated with a difference in microbial composition compared to healthy weight.

Skin hygiene

$H6_0$: Daily showering reduces the diversity of the skin microbiome and affects its composition.

Individuals

$H7_0$: There is significant interpersonal variability in the skin microbiome of the subjects.

Time

$H8_0$: There is no significant difference in skin physiological parameters over time.

$H9_0$: The skin microbiome is stable in composition and diversity regarding time.

Body site

$H10_0$: The body sites differ significantly with respect to skin physiological parameters, diversity, and the composition of the skin microbiome.

The results help us to understand the influences of different internal and external factors on the skin microbiome and may provide a basis for research on the role of the skin microbiome in the pathogenesis of skin diseases.

2 Material and Methods

2.1 Ethical consideration

Subjects of this study were recruited in the frame of the project "Detection of Archaea on healthy and diseased skin" with the ethical vote EK: 27-289 ex 14/15 and all ethical requirements were followed and satisfied.

2.2 Study design

In a longitudinal study design, four different healthy body sites (forehead, décolleté, arms and back) of seven women and five men had been sampled at six different time points over almost two years (Table 1). According to their age, the participants were divided into two groups (B1: 20-40 years, B2: 60-85 years). Every subject filled a questionnaire with different categories e.g., height and weight, skin type and detailed questions about their respective lifestyle (e.g., eating habits, smoking, alcohol consumption, skin care, medication, animal contact and hobbies). Potential changes over the sampling timeframe were documented as well.

Table 1: Overview of the sampling time points and seasons

Time point	Date	Season
t1	05 – 08/2018	Summer
t2	06/2019	Summer
t3	08/2019	Summer
t4	10/2019	Autumn
t5	12/2019	Winter
t6	02/2020	Winter

2.3 Skin measurements and sampling

The skin measurement and sampling for time point t1 was performed by Daniela Brunner and for the time points t2-t6 by Stefanie Duller, both members of the AG Moissl-Eichinger. The sampling was carried out on the following body sites: forehead, décolleté, arms and back (Figure 3).

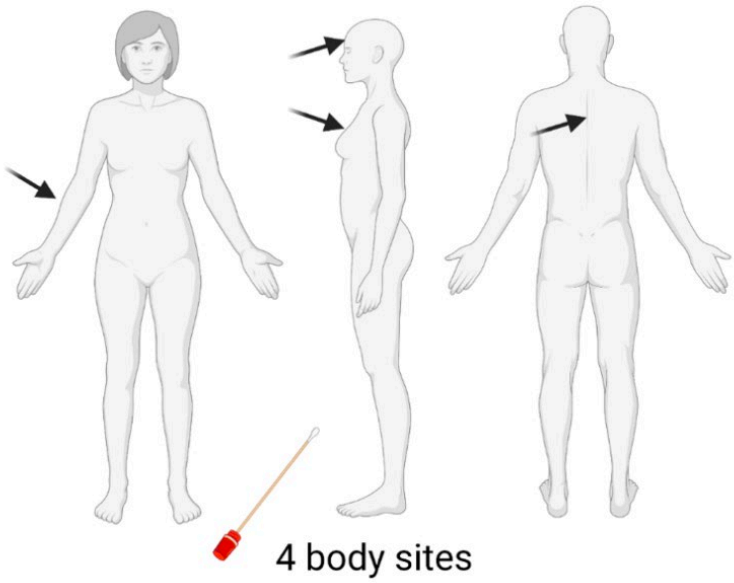

Figure 3: Sampling sites forehead, décolleté, arms and back

First, the skin temperature on each site was measured with an infrared thermometer.
After that, the sampling was performed with pre-moistened swabs (0.9 % DNA-free NaCl (w/v)) and sponges. For the first time point, forehead, décolleté and arms were sampled with swabs and the back with a sponge. For the other time points (t2-t6), the sampling method was changed and only the forehead was sampled with a pre-moistened swab. After sampling, the samples were frozen at -80 °C until DNA extraction.
In each session, several skin parameters were measured with the Cutometer® MPA 580 on all sampling sites: transepidermal water loss (Tewameter), skin moisture (Corneometer), pH (pH-meter), and sebum content (Sebumeter). The interpretation of those parameters was done based on the recommended specifications by the manufacturer Courage + Khazaka electronic GmbH (https://www.courage-khazaka.de/de) (Tables 3-6).

Table 2: Materials for sampling

Chemicals	**Supplier**	**Location**
NaCl	VWR international GmbH	USA
Consumables	**Supplier**	**Location**
BD BBL™ Culture Swabs™ EZ	Becton, Dickinson and Company	USA
Surface-sampling Sponge	VWR Chemicals	Germany
Instrumentation	**Supplier**	**Location**
Cutometer® MPA 580	Courage + Khazaka electronic GmbH	Germany
Infrared Thermometer	Future Founder	China

Table 3: Interpretation of transepidermal water loss (Tewameter)

Transepidermal water loss [g/hm^2]	**Interpretation**
0-10	Very healthy skin
10-15	Healthy skin
15-25	Normal skin
25-30	Stressed Skin
> 30	Critical skin

Table 4: Interpretation of skin moisture (Corneometer)

Skin moisture [Corneometer Unit CU]	**Interpretation**
< 30	Very dry skin
30-40	Dry skin
> 40	Adequately moist skin

Table 5: Interpretation of skin pH (pH-meter)

pH	Interpretation
3.5-4.49	Acidic skin pH
4.5-5.5	Normal skin pH
5.51-7	Alkaline skin pH
> 7	Too alkaline skin pH

Table 6: Interpretation of sebum content (Sebumeter)

Forehead	Décolleté, Back	Arms	Interpretation
< 100	< 55	0-6	Dry skin
100-220	55-130	> 6	Normal skin
> 220	> 130	---	Oily skin

2.4 DNA Extraction

After defrosting the samples on ice, DNA extractions was done using the PureLink™ Microbiome DNA Purification Kit (Thermo Fisher Scientific Inc.). DNA extractions from time point t1 were performed by Daniela Brunner, all other DNA extractions (time points t2-t6) were conducted by Stefanie Duller.

For the swab samples, the instructions of the manufacturer's protocol were followed (https://www.thermofisher.com/order/catalog/product/A29790#/A29790), whereas for the sponges, the DNA-extraction was performed with the following modification:

For the preparation of the lysate, 1 mL of the liquid sample extract was concentrated by centrifugation (14,000 rpm, 4 °C, 10 min.). After that, the supernatant was removed, 70 µL were transferred to the bead beating tube together with 800 µL of S1-Lysis Buffer. Further processing was performed according to the manufacturer´s protocol. Furthermore, one extraction blank (kit control) for each run was added to control for the DNA contamination of the used reagents of the kit. Finally, the DNA was stored at -20 °C until further downstream applications.

Table 7: Material for DNA extraction

Kit	**Supplier**	**Location**
PureLink™ Microbiome DNA Purification Kit	Thermo Fisher Scientific Inc.	USA
Consumables	**Supplier**	**Location**
DeckWorks pipet tips (0.1-10 µL, 1-100 µL, 100-1000 µL)	Corning Inc.	USA
Eppendorf Tubes® Safe-Lock, PCR clean (1.5 mL)	Eppendorf AG	Germany
Instrumentation	**Supplier**	**Location**
Pipettes (0.1-2 µL, 2-10 µL, 10-100 µL, 100-1000 µL)	Eppendorf AG	Germany
BioVortex V1	peQLab Biotechnology GmbH	Germany
Thermo Shaker TS-100C	BioSan	Latvia
Vortex-Genie 2	Scientific Industries Inc.	USA
Centrifuge 5427 R	Eppendorf AG	Germany
ThermoScientific™ Safe2020 Workbench	Thermo Fisher Scientific, Inc.	Lithuania

2.5 PCR (Polymerase Chain Reaction)

For the amplification of the 16S rRNA genes, polymerase chain reactions (PCR) were conducted. Therefore, 2 µL of the extracted DNA was used as a template. For the master mix, the following primers with adapters for Illumina© sequencing were added to the reagents in appropriate concentrations (Table 8-9):

Illu 515F: 5´- TCGTCGGCAGCGTCAGATGTG – (primer sequence) - 3`

Illu 926R: 5´- GTCTCGTGGGCTCGGAGATGT – (primer sequence) - 3`

(34,35).

Table 8: Primers for PCR

Primer	Sequence	Length (bp)	Supplier	Country
515F	TAT AAG AGA CAG GTG YCA GCM GCC GCG GTA A	19	Eurofins MWG Synthesis GmbH	Germany
926R	GTA TAA GAG ACA GCC GYC AAT TYM TTT RAG TTT	20	Eurofins MWG Synthesis GmbH	Germany

After that, the template was put together with the master mix into 8-strip PCR reaction vials. The amplification was processed with the thermal cycler Biometra TAdvanced with a defined temperature profile (Table 10). If the result in the gel electrophoresis was under detection limit, PCR was repeated with 0.5 µL, 1µL or 5 µL template.

Table 9: PCR reagent mix for 16S rRNA gene amplification

Reagent	Concentration	Volume per reaction (µL)	Final concentration
Buffer with MgCl2	10X	2	1X
Forward Primer Illu-515F	10 µM	0.4	200 nM
Reverse Primer Illu-926R	10 µM	0.4	200 nM
dNTP mix	2.5 mM each	1.6	200 µM each
ExTaq® Polymerase	5 U/µL	0.1	0.5 U
Template	10-30 ng		
PCR-grade water		Complement to reach a total volume of 25 µL	

Table 10: Amplification process of the 16S rRNA gene

Step	Temperature in °C	Time in sec	Cycles
Initial denaturation	94	180	1
Denaturation	94	45	38x
Annealing	55	45	
Elongation	72	90	
Final elongation	72	600	1
Hold	4	∞	

Table 11: Material for PCR

Chemicals	**Supplier**	**Location**
dNTP-Mix	Takara Bio Inc.	Japan
10x ExTaq® Buffer	Takara Bio Inc.	Japan
ExTaq® DNA-Polymerase	Takara Bio Inc.	Japan
PCR-grade water	Analytik Jena AG	Germany
Consumables	**Supplier**	**Location**
DeckWorks pipet tips (0.1-10 µL, 1-100 µL, 100-1000 µL)	Corning Inc.	USA
Eppendorf Tubes® Safe-Lock, PCR clean (1.5 mL)	Eppendorf AG	Germany
Rotilabo® - 8-strip PCR reaction vials (0.2 mL)	Carl Roth GmbH + Co. KG	Germany
Instrumentation	**Supplier**	**Location**
Pipettes (0.1-2 µL, 2-10 µL, 10-100 µL, 100-1000 µL)	VWR International GmbH	Germany
Combi Spin PCV-2400 Grant-bio	Grant Instruments	United Kingdom

Mini centrifuge MCF-2360	LMS Co.	Japan
Biometra TAdvanced Thermocycler	Analytik Jena AG	Germany
UVC/T-M-AR DNA/RNA UV-Cleaner Box	BioSan	Latvia

2.6 Gel Electrophoresis

To examine the PCR products, agarose gel electrophoresis was performed. Therefore, a 3% agarose gel was prepared. First, 3% agarose (w/v) mixed with 1x TAE buffer (diluted 50X TAE electrophoresis buffer with double distilled H_2O) was heated in the microwave until the agarose dissolved. During this procedure, the evaporated water was replaced with double distilled H_2O several times to maintain the concentration. 20 mL of the gel together with 2 µL of Roti® GelStain, a DNA intercalation dye, was filled into a 20 mL gel chamber. Inserts for the slots were added. After hardening of the gel and removing of the inserts, 1x TAE was brought into the chamber as running buffer and the gel was loaded with 3 µL sample mixed with 1 µL DNA loading buffer. Furthermore, 2 µL of FastRuler Low range DNA ladder was used as size and quantification marker. Subsequently, the gel was set up in the Electrophoresis Power supply Consort EV261 for 30 minutes at 70 V and 400 mA. Afterwards, the gel was recorded with FluorChem FC3.

Table 12: Material for Gel Electrophoresis

Chemicals	**Supplier**	**Location**
CSL-AG500 LE Agarose Multi-Purpose Agarose	Cleaver Scientific Ltd	United Kingdom
50x TAE (Tris-acetate-EDTA) Electrophoresis Buffer	Thermo Fisher Scientific Inc.	Lithuania
5x DNA loading buffer blue	VWR international GmbH	Germany
Roti®-GelStain	Carl Roth GmbH + Co. KG, Germany	Germany
FastRuler Low Range DNA Ladder, ready-to-use	ThermoFisher Scientific Inc.	Germany

Consumables	Supplier	Location
Parafilm M PM-996	Bemis Inc.	USA
DeckWorks pipet tips (0.1-10 µL)	Corning Inc.	USA
Instrumentation	**Supplier**	**Location**
Gel chamber 20 mL	Regensburg Werkstätten Biologie	Germany
Pipettes (2-10 µL)	Eppendorf AG	Germany
Balance 1204 MP	Sartorius Lab Instruments GmbH & Co. KG	Germany
Micromaxx Microwave inverter	Medion AG	Germany
Electrophoresis Consort Power Supply EV261	Scie-Plas Ltd.	United Kingdom
FluorChem FC3 System	ProteinSimple Inc.	USA

2.7 Sequencing, data analysis and visualisation

The library construction and next-generation sequencing (Illumina MiSeq®) was performed by the Core Facility Molecular Biology team at the Center of Medical Research (Medical University of Graz, Austria). In a first step, DNA concentrations of the generated amplicons were normalized with a SequalPrep^{TM} normalisation plate (Invitrogen) and then each sample was indexed with a unique barcode sequence using 8 cycles of indexing PCR. These indexed samples were pooled and purified by gel cuts, before the library was sequenced on an Illumina MiSeq® instrument and the MiSeq® Reagent Kit v3 with 602 cycles (2 x 301 cycles).

The resulting 16S rRNA gene sequences in FASTQ format were processed by Alexander Mahnert using QIIME2 (36) versions 2020.8 – 2021.8. After importing demultiplexed FASTQ files into the QIIME2 environment using a manifest file, features of amplicon sequence variants (ASVs) were defined by DADA2 (37) and non-mergeable reads as well as chimeric sequences were removed. Due to the heterogenic lengths of the primer constructs resulting from prokaryotic and eukaryotic marker gene targets, no read truncations were set at this step. The resulting representative sequences were classified by a pre-trained Naïve-

Bayes classifier trained on the curated and trimmed SILVA138 database (38) using RESCRIPt and the feature-classifier classify-sklearn plugin (39). For phylogenetic metrics like UniFrac (40), a rooted phylogenetic tree was generated with FastTree2 (41) based on a masked MAFFT alignment. Detection and removal of potential contaminants from Kit reagents, sample handling or PCR negative controls was achieved with decontam (42) and the prevalence method set to a threshold of 0.5. After filtering the feature table core, phylogenetic diversity metrics of α- and β-diversity were calculated with the core metrics plugin in QIIME2. Respective datasets like the feature table, taxonomic annotations, distance matrices and the metadata file were exported to allow further analysis and visualisations of the dataset.

The data analysis was performed using Calypso (43) version 8.84, an online software for visualisation and interpretation of microbial communities. At first, the data was filtered. The top 20'000 taxa were included, and Cyanobacteria and Chloroplasts were removed. Furthermore, the samples were normalized by total sum normalization (TSS) and square root transformation to force the dataset into a normally distributed composition.

After that, multiple statistical tests were carried out using several analysis tools in Calypso. For evaluating and displaying the α-diversity, Shannon indices were calculated and compared with the Diversity tool. β-diversity was examined with PCoA (principal coordinates analysis), using UniFrac distance matrix, RDA (redundancy analysis), Adonis and Anosim, using the Multivariate tool. With the Basic Quantitative Visualisation tool, the microbial community composition was examined and with the Rank tests of the Group analysis tool, relative taxa abundances across the sampling groups were compared. Furthermore, Linear Regressions (Pearson index) and Repeated Measure Analyses were carried out to identify correlations between the occurrence of certain taxa and metadata categories.

For the analysis of the metadata, IBM SPSS statistics 27 software (44) was used. First, descriptive statistics were calculated to describe the physical characteristics of the subjects. To examine the numerical scaled data for normal distribution, Kolmogorov-Smirnov and Shapiro-Wilk tests were performed. Mann-Whitney-U (2 variables) and Kruskal-Wallis (> 2 variables) tests for non-parametrical data were carried out to investigate correlations between metadata categories. In order to describe the degree of correlation more in detail, the effect strength (45) was calculated:

$$r = \frac{|z|}{\sqrt{n}}$$

with r: effect strength, z: z-Score and n: number of samples. An effect strength $r > 0.5$ can be categorized as strong $0.3 > r > 0.1$ as medium and $r \leq 0.1$ as weak effect (45).
Furthermore, linear regressions were calculated to further examine certain correlations between skin physiological parameters and other metadata.

3 Results

3.1 Overview on the 16S rRNA gene amplicon dataset

Before filtering in QIIME2, 336 samples (51 controls, 285 samples) with a total of 8’589 features were included. The minimum number of reads per sample was 2 and the maximum number of reads per sample was 34’698 with a mean of 9’277 and a median of 8’673 reads.

After quality filtering, removal of potential contaminants and normalisation, the finally analysed dataset contained 284 samples. According to standard quality threshold cut offs, 279 of them were included in Calypso. Furthermore, 334 taxa on family level, 690 taxa on genus level and 7’627 taxa on ASV (amplicon sequence variant) level were considered throughout the downstream analysis. Figure 4 shows an overview on the uploaded data after filtering in Calypso. One sample from the back of time point t6 (B1B20ZHt65, min=1’011) contained the minimal number of reads per sample, and another sample from the décolleté of time point t1 (B2B1MFt11, max=33’805) the maximum.

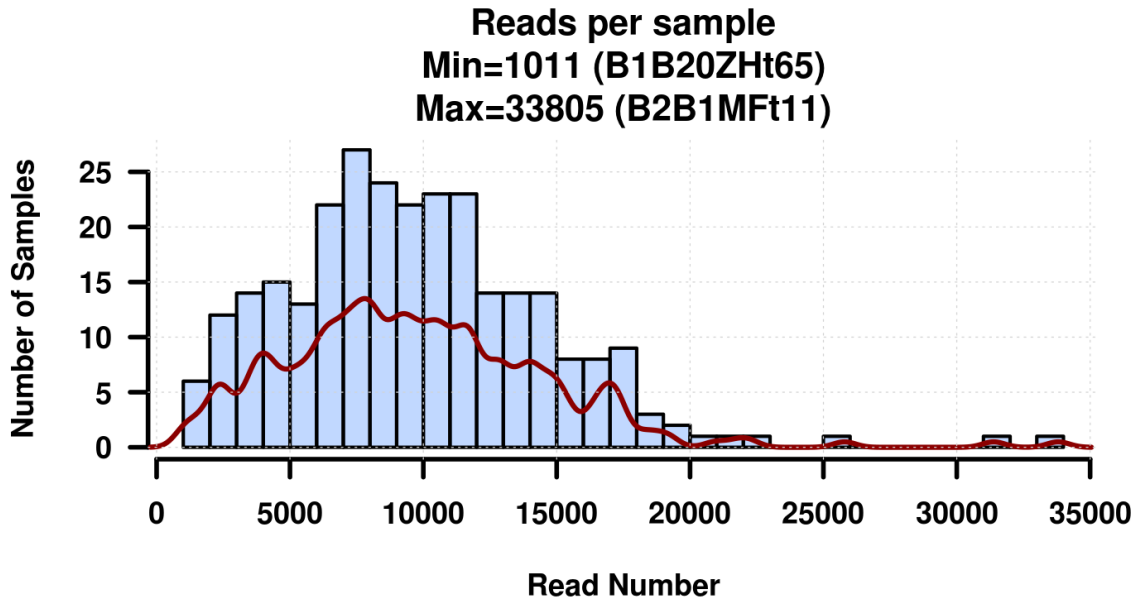

Figure 4: The filtered microbiome data showed a minimum of 1'011 reads per sample and a maximum of 33'805 reads per sample.

3.2 Analysis of acquired metadata

From the questionnaire and the skin measures, 79 different metadata categories were created. To display the physical characteristics of the probands, descriptive statistics were conducted using SPSS (Table 13). On average, the subjects were 50 years old with a minimum of 27 and a maximum of 77 years. Their BMI had a mean of 25, and on average, they were 169 cm tall and weighed 72 kg.

Table 13: Descriptive statistics of the test subjects

Metadata category	N	Min	Max	Mean	Standard deviation
Age	284	27	77	49.72	17.92
BMI	284	21	30	24.89	2.29
Size in cm	284	157	186	168.56	9.99
Weight in kg	284	55	94	71.76	12.93

Furthermore, relevant numerically scaled metadata were tested for normal distribution using Shapiro-Wilk and Kolmogorov-Smirnov (Lilliefors-corrected) Tests in SPSS (Table 14). All metadata had a significance of $p<0.001$ in both tests, what indicates non-normally distributed data.

Table 14: Kolmogorov-Smirnov-Test and Shapiro-Wilk-Test on the metric metadata categories

Metadata category	Kolmogorov-Smirnov	p	Shapiro-Wilk	p
Age	0.27	<0.001	0.82	<0.001
BMI	0.21	<0.001	0.92	<0.001
Height in cm	0.18	<0.001	0.89	<0.001
Weight in kg	0.21	<0.001	0.86	<0.001
Tewameter	0.14	<0.001	0.81	<0.001
Skin pH	0.13	<0.001	0.80	<0.001

Sebumeter	0.30	<0.001	0.64	<0.001
Corneometer	0.09	<0.001	0.96	<0.001
Skin Temperature	0.17	<0.001	0.94	<0.001

3.3 Overview on bacterial and fungal communities on human skin

The microbiome data were transformed in Calypso (see section 2.7 for further details), so that statistical tests for normally distributed data were used in the following. Moreover, I would like to emphasise that the following chapters deal with the analysis of compositional data and the formulation of abundance refers to the qualitative character of the data, not the actual quantity.

In order to get a brief overview on the microbial communities across the different body sites and time points, group analysis tests with indications of the relative abundances on phylum and genus level were carried out.
The five most abundant phyla of the dataset (in descending order) were *Actinobacteriota*, *Proteobacteria*, *Firmicutes*, *Bacteroidetes* and *Basidiomycota* (Figure 5).
Compared with the other body sites, the subject´s foreheads had significantly higher abundance of *Actinobacteriota*, *Basidiomycota* and *Fusobacteriota* (T-test, $p<0.001$), but the lowest abundance of *Proteobacteria* (T-test, $p<0.001$). Furthermore*, Bacteroidetes* were significantly higher abundant both on arms and on the forehead (T-test, $p<0.01$). Signatures of *Basidiomycota*, on the other hand, occurred to significantly higher proportions on the forehead than the other body sites (T-test, $p<0.001$).

In terms of the seasonal distribution, it is remarkable, that only *Proteobacteria* were more abundant at sampling events from summer and autumn (t1-t4) than compared to those samples which were obtained in winter (T-test, $p<0.001$). However, for all the other top 5 phyla it was the opposite (T-test, $p<0.05$).

On genus level, the five most abundant taxa (in descending order) were *Cutibacterium*, *Burkholderia*, *Staphylococcus*, *Corynebacterium,* and *Streptococcus.*

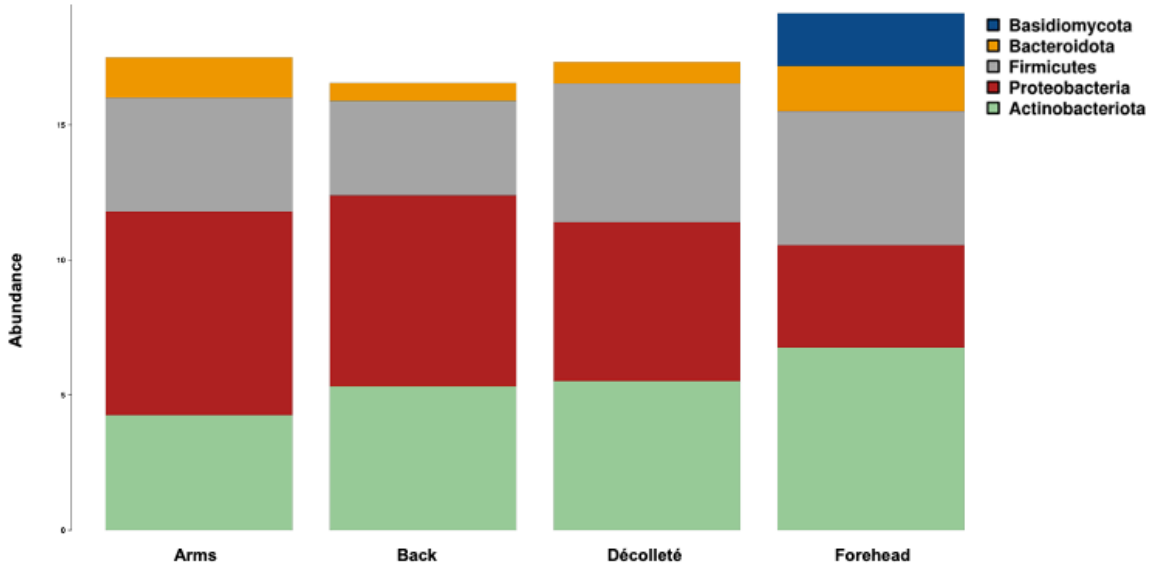

Figure 5: The Bar chart shows the distribution of the top 5 phyla on the body sites. Basidiomycota occurred almost exclusively on the forehead.

3.4 Age

No relevant differences in α- and β-diversity regarding age groups

To examine differences and similarities regarding the age groups, α- and β-diversity analyses were carried out. With regard to α-diversity, there was no significant difference between the age groups B1 and B2 (Shannon index, ANOVA, p=0.78).
The multivariate analyses on ASV level showed that grouping of samples according to age groups had very weak coefficients in the similarity analyses (RDA Var 14.78, p=0.001; Anosim R=0.061, p=0.001; Adonis R2=0.034, p=0.0003).

Higher skin pH and lower transepidermal water loss could be observed on the skin of older subjects

According to the Mann-Whitney-U-Test (Table 15), age group B2 (60-85 years) had significantly higher skin pH values than B1 (20-40 years) (pH-meter, p<0.001, r=0.25). However, neither the Mann-Whitney U-test of the female participants regarding skin pH showed a significant difference between B1 and B2, nor the test of the male participants

(p=0.139). In addition, the transepidermal water loss (Tewameter, p<0.001, r=0.24) of group B2 was significantly lower than in group B1. There were no significant differences between the two age groups in terms of sebum content and skin moisture.

Table 15: Mann-Whitney-U-Test on the age groups regarding skin physiological parameters

Skin measurement	p	Effect strength r	Average Rank B1	Average Rank B2
Sebumeter (Sebum content)	0.81	-	141.3	143.67
pH-meter (Skin pH)	<0.001	0.25	121.99	162.44
Corneometer (Skin moisture)	0.85	-	141.57	143.40
Tewameter (Transepidermal water loss)	<0.001	0.24	141.93	107.35

Significant differences in the abundance of certain genera according to age

Of the top 20 genera (Rank test, p<0.05), ASVs of *Corynebacterium*, *Streptococcus* and *Neisseria* were identified to be more common on the skin of the older participants of group B2. *Cutibacterium*, *Enhydrobacter*, *Lawsonella* and *Micrococcus* occurred to significantly higher proportions on the skin of the younger age group B1.

Additionally, the LEfSe (linear discriminant analysis effect size method) analysis of the top 20 taxa supported the result of the rank test revealing *Corynebacterium* and *Streptococcus* being characteristic for B2 with a LDA score over 4, while *Cutibacterium*, *Lawsonella* and *Enhydrobacter* were characteristic for the younger participants of B1. In this context, the LDA score indicates the effect size of the respective taxa on the age groups.

The regression analysis (Pearson index) of the top 20 showed significant correlations between the abundance of specific taxa and age. Only relevant correlation coefficients R>0.3 or R<-0.3 were considered in the following (Table 16).

Lawsonella, *Cutibacterium* and *Enhydrobacter* had a significantly negative correlation with age, whereas *Corynebacterium* showed a positive correlation coefficient.

Table 16: Regression analysis (Pearson index) of the relevant top 20 genera in relation to the age groups

Taxa	Positive Samples	R	p	Mean Abundance
Lawsonella	120	-0.459	5.8e-16	0.543
Cutibacterium	273	-0.3931	9.5e-12	3.975
Enhydrobacter	164	-0.3286	1.9e-08	0.841
Corynebacterium	257	0.316	6.9e-08	2.554

3.5 Sex

No relevant differences in α- and β-diversity regarding a subject´s sex

Comparable to the age, α-diversity did not differ significantly regarding the sex of a subject (Shannon index, ANOVA, p=0.12). Furthermore, the β-diversity analyses showed a significant but weak variation between the samples of women and men (RDA Var 14.71, p=0.001; Adonis R2=0.0404, p=0.0003; Anosim R=0.058, p=0.001).

Higher skin pH and lower transepidermal water loss on the skin of women

To examine the data for gender differences in skin physiological measurements, a Mann-Whitney-U-Test was performed (Table 17). It turned out that the female participants had a significantly higher skin pH (pH-meter, $p<0.001$, $r=0.361$), but a significantly lower transepidermal water loss (Tewameter, $p<0.001$, $r=0.318$) than the male subjects. Apart from that, there were no significant differences between the sexes in terms of sebum content and skin moisture.

Table 17: Mann-Whitney-U-Test on the sex regarding skin physiological parameters

Skin measurement	p	Effect strength r	Average Rank male	Average Rank female
Sebumeter (Sebum content)	0.208	-	149.66	137.26

pH-meter (Skin pH)	<0.001	0.361	107.84	167.86
Corneometer (Skin moisture)	0.379	-	137.49	146.16
Tewameter (Transepidermal water loss)	<0.001	0.318	150.05	104.14

Significant differences in abundance of microbial genera regarding sex

The LEfSe analysis (top 20) identified several typical taxa on the skin of the female participants. Especially signatures of *Streptococcus* and *Acinetobacter* signatures were found to be increased with a LDA score over 4. On the other hand, *Cutibacterium*, *Enhydrobacter*, *Finegoldia* and *Lawsonella* were more abundant on male skin. For all those taxa, the rank test (Top 20) displayed a highly significant difference (Figure 6, Rank test, p=0.001).

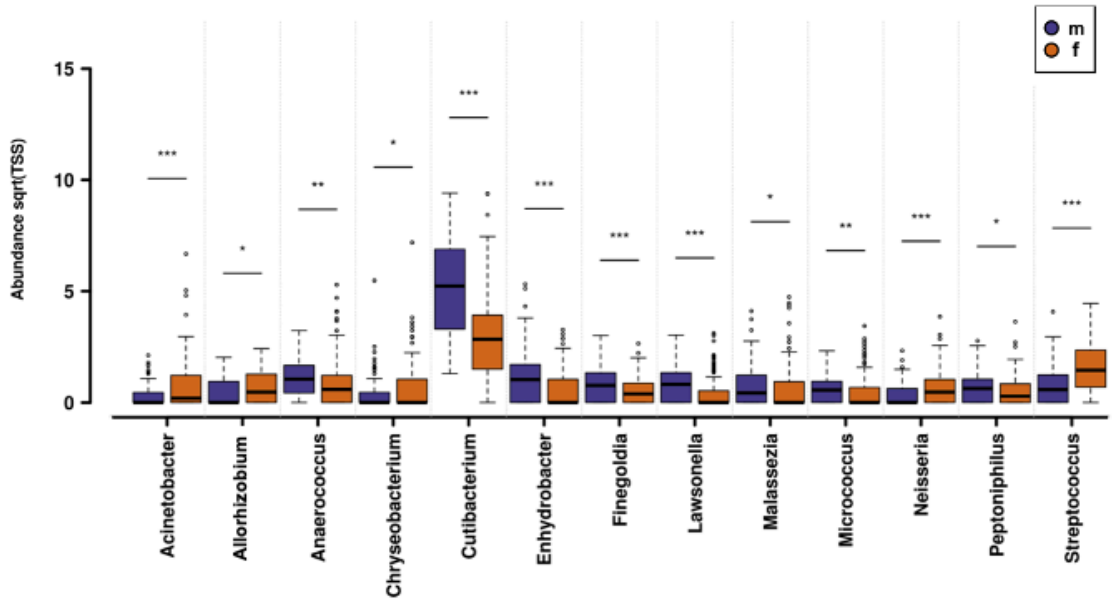

Figure 6: Differences in the occurrence of the top 20 genera regarding sex (Rank test, p<0.05)

3.6 Weight, height, and BMI

For all participants, their BMI was calculated as follows, depending on their height and the weight given:

$$BMI\ [\frac{kg}{cm^2}] = \frac{Weight\ [kg]}{Height\ [cm]^2}$$

(46).

Furthermore, the calculated BMIs were interpreted as shown in Table 18. In the dataset, participants had BMIs classified as healthy weight (140 samples) or overweight (144 samples).

Table 18: Classification of BMI into categories (47)

BMI	Category	Number of Samples
< 18.5	Underweight	0
18.5-24.9	Healthy weight	140
>25.0	Overweight	144

According to Mann-Whitney-U-Test, age group B1 had significantly lower BMI values ($p<0.001$, $r=0.21$), higher values for height ($p<0.001$, $r=0.54$) and lower values for weight ($p<0.001$, $r=0.23$) than age group B2 (Table 19).

Table 19: Mann-Whitney-U-Test on the age groups regarding BMI, height and weight

Metadata	p	Effect strength r	Average Rank B1	Average Rank B2
BMI	<0.001	0.21	125.7	158.83
Height in cm	<0.001	0.54	187.41	98.83
Weight in kg	<0.001	0.23	161.47	124.06

Higher α-diversity in overweight than participants with healthy weight

According to the BMI, the overweight participants had a higher α-diversity than those with healthy weight (Figure 7, Shannon index, ANOVA, p=1.8e-06). Richness was also differing

significantly (p=8.5e-09), whereas evenness did not result in a significant distinction (p=0.68).

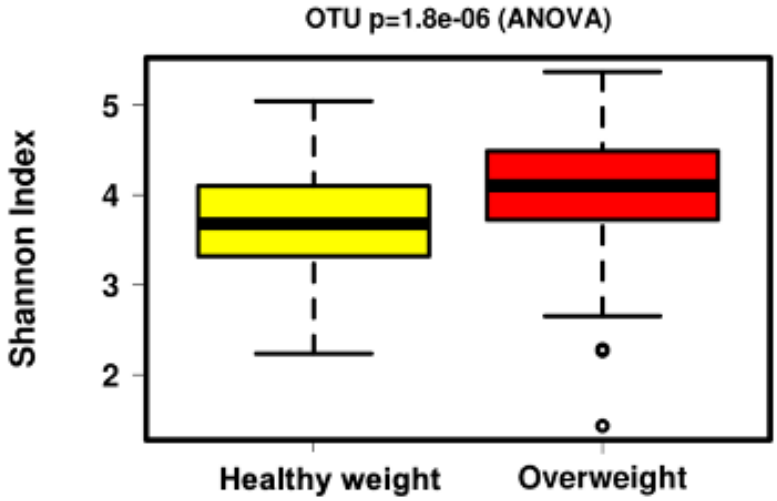

Figure 7: Significant difference of the Shannon index (ANOVA) between the subjects regarding the BMI category

On the other side, the β-diversity tests showed a significant, but low variation (RDA Var 10.69, p=0.001; Adonis R2=0.0184, p=0.001, Anosim R=0.029, p=0.001).

Typical genera on the skin of overweight and healthy weight participants

The LEfSe analysis of the top 20 genera showed some characteristic taxa for overweight participants with LDA over 4: *Corynebacterium*, *Peptinophilus* and *Chryseobacterium*. *Burkholderia* and *Cutibacterium* occurred to higher proportions on the skin of subjects with a healthy BMI.

The rank test of the top 20 genera ($p<0.01$) displays that the overweight participants had a significantly higher abundance of e.g., *Corynebacterium*, *Streptococcus*, and *Finegoldia* than subjects with healthy weight (Figure 8). In contrast, no genus (top 20) showed significantly higher proportions on the skin of people with healthy weight.

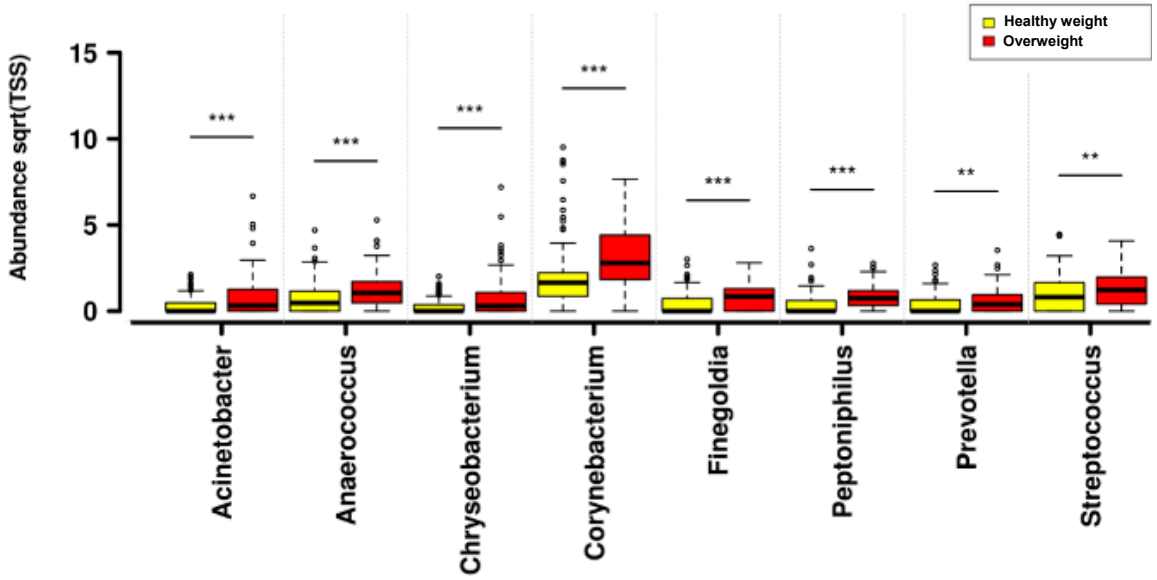

Figure 8: Differences in the occurrence of the top 20 genera regarding BMI category (Rank test, p<0.05)

3.7 Skin physiology

3.7.1 Sebum content

Significant changes in α- and β-diversity regarding sebum content

For the Sebumeter measures, the samples with dry skin had a significantly lower diversity than the samples with regular sebum content (used synonymously with the manufacturer's designation normal sebum content) (Figure 9, Shannon index, ANOVA, p=1.3e-06; Richness p=1.1e-05; Evenness p=0.022).

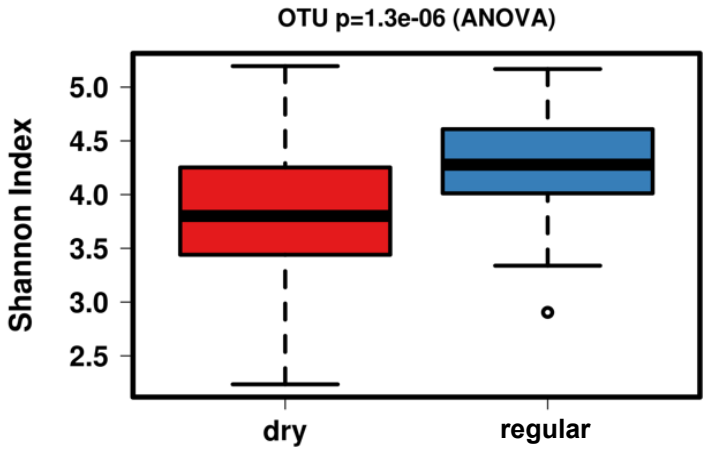

Figure 9: Significant difference of the Shannon index (ANOVA) between the samples regarding sebum content

PCoA and RDA analyses (Figure 10) showed a difference in β-diversity between skin with regular sebum content and dry skin with a low, but significant variation coefficient in all tests carried out (RDA, Var 15.24, p=0.001; Adonis R2=0.132, p=0.0003, Anosim R=0.448, p=0.001).

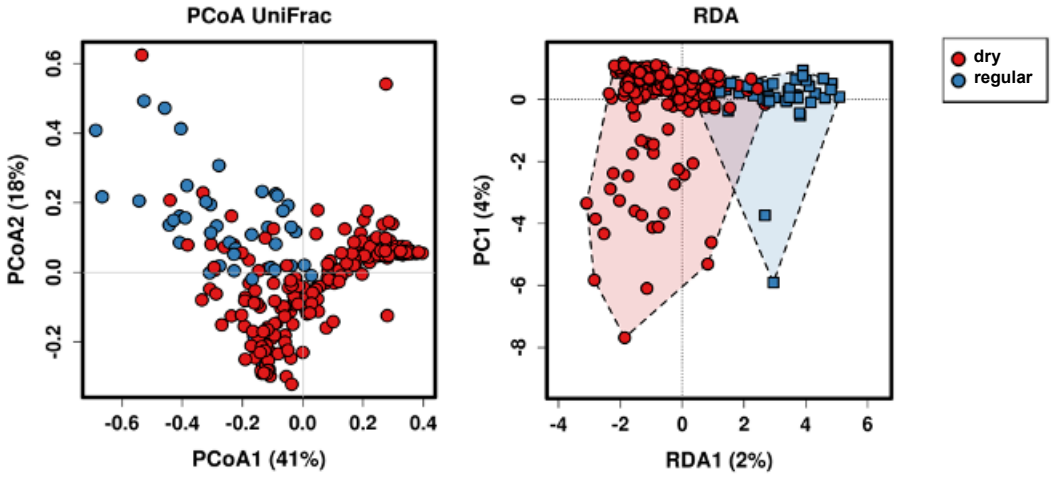

Figure 10: Significant difference in the β-diversity (PCoA, RDA, p=0.001) of the samples regarding the sebum content of the skin

Typical genera on dry skin and skin with regular sebum content

Many taxa were typical for skin with regular sebum content in the LEfSe analysis (top 20 genera). *Malassezia* and *Neisseria* had the highest LDA score over 4. On dry skin, *Burkholderia* and *Allorhizobium* were the most predominant taxa.

Burkholderia and *Allorhizobium* were also the only genera of the top 20 rank test that were found more frequently on dry skin, all the other top 20 genera were more abundant on skin with regular sebum content (Figure 11, Rank test, $p<0.05$).

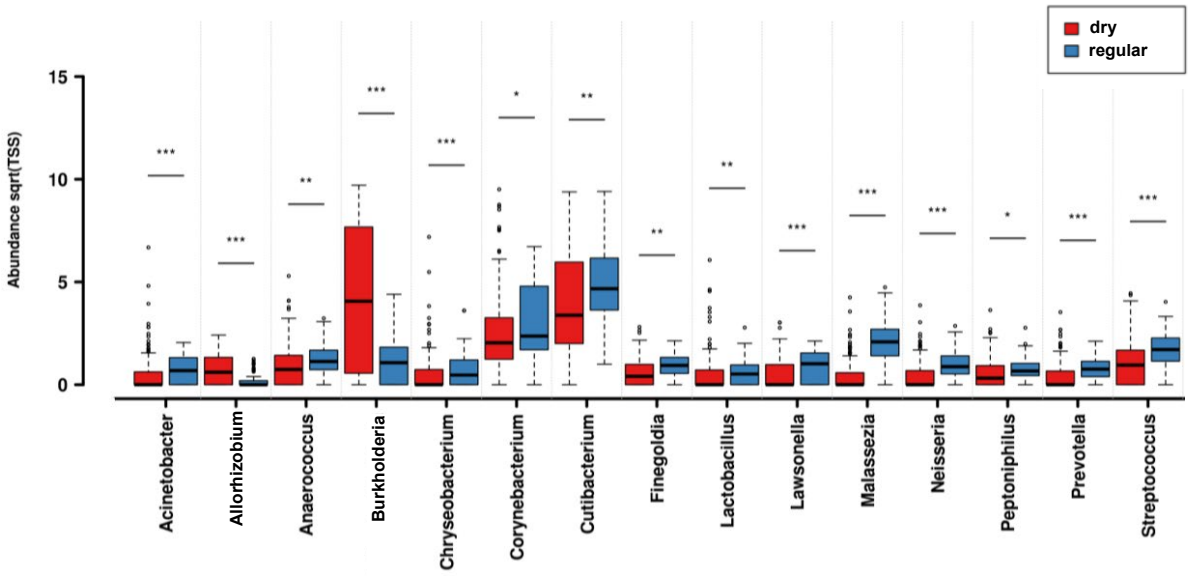

Figure 11: Differences in the occurrence of the top 20 genera regarding sebum content (Rank Test, p<0.05)

The analysis of possible connections between the occurrence of genera and the level of sebum content showed two taxa with significant, relevant correlation:
Malassezia and *Neisseria* had both positive correlation coefficients ($R>0.3$) with sebum content (Table 20, Figure 12).

Table 20: Regression analysis (Pearson index) of Malassezia and Neisseria in relation to sebum content

Taxa	Positive Samples	R	p	Mean Abundance
Malassezia	138	0.434	3.1e-14	0.661
Neisseria	129	0.323	3.4e-08	0.492

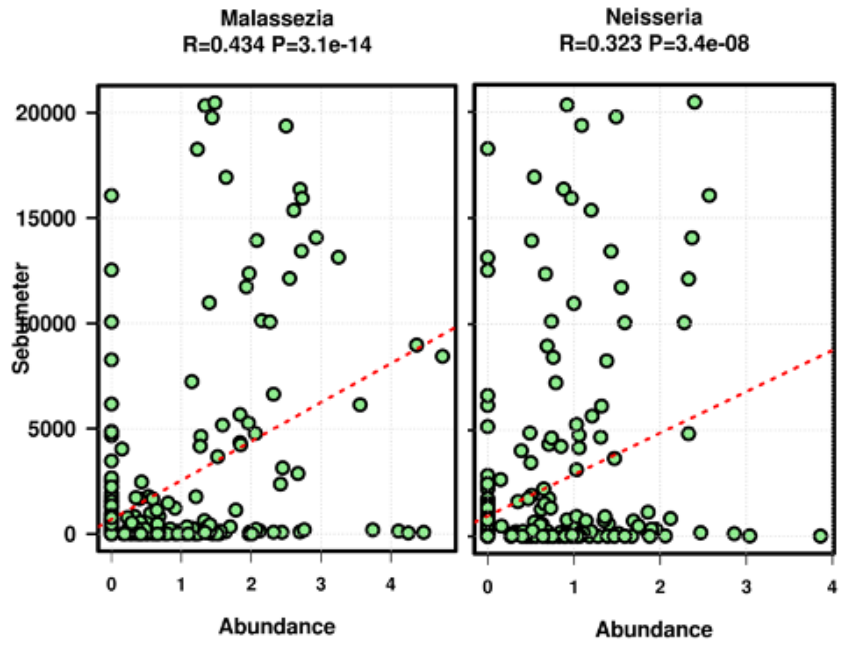

Figure 12: Positive and significant correlation (Pearson index) between the occurrence of Malassezia / Neisseria and increasing sebum content

As the signatures of both taxa occurred mainly on the forehead (T-test, $p<0.05$ Figure 13), the regression analysis was carried out again just for the samples of this body region (70 samples). As *Malassezia* did not show a significant correlation in this setting anymore ($R=0.022$, $p=0.86$, mean abundance 1.894, positive samples 56), the association might be more likely with the body site than with sebum content. *Neisseria*, on the other hand, continued to show a positive significant correlation with respect to the sebum content ($R=0.318$, $p=0.0099$, mean abundance 0.858, positive samples 50).

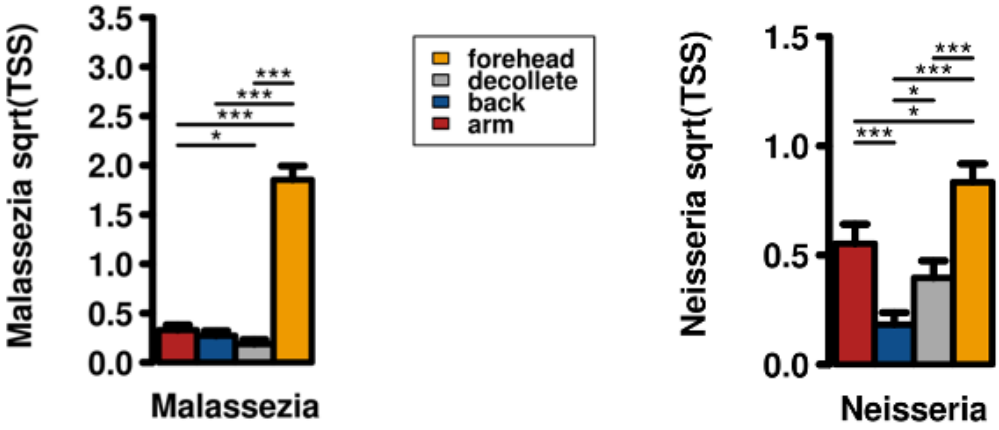

Figure 13: T-Test ($p<0.05$) of the occurrence of Malassezia and Neisseria on the body sites

3.7.2 Skin pH

No significant difference of α-diversity, low differences in β-diversity regarding skin pH

When analysing the samples regarding skin pH, the acidic samples had a non-significant higher microbial diversity compared to the skin samples with a regular (used synonymously with the manufacturer's designation normal skin pH) or alkaline pH (Shannon index, ANOVA, p=0.16).
The β-diversity analyses showed significant, but not considerable variations between the samples grouped by skin pH (RDA Var 14.95, p=0.005; Adonis R2=0.0331, p=0.0003; Anosim R=0.051, p=0.041).

Typical taxa for acidic skin

The LEfSe top 20 analysis showed that *Lawsonella* and *Aerococcus* were typical for acidic skin with a LDA score over 4. However, no other skin pH category revealed any characteristic taxa from the top 20 genera. The rank test of the top 20 genera showed that there was not a single taxon exclusively associated with a specific skin pH category (Rank test, $p<0.05$).

3.7.3 Skin moisture

No significant difference of α-diversity for different moisture contents and only low differences in β-diversity regarding skin moisture

Skin moisture showed no significant difference in α-diversity among the different subgroups (Shannon index, ANOVA, p=0.66) and the variation of the samples grouped by skin moisture was significant, but only weak (RDA Var 12.42, p=0.001; Adonis R2=0.0368, p=0.0003; Anosim R=0.07, p=0.001).

Typical taxa for dry and moist skin

According to LEfSe analysis of the top 20 genera, a typical taxon on very dry skin was *Corynebacterium*, on moist skin *Burkholderia* and *Allorhizobium* with all LDA scores over 4.
The rank test of the top 20 genera regarding the skin moisture also confirms the preference of *Burkholderia* and *Allorhizobium* for moist skin, and *Corynebacterium* and *Enhydrobacter* being more abundant in dry and very dry skin samples (Rank test, $p<0.05$).

3.7.4 Transepidermal water loss

No significant difference of α-diversity, low differences in β-diversity regarding transepidermal water loss

For the TEWL (transepidermal water loss), there was no significant difference in α-diversity (Shannon index, ANOVA, $p=0.33$).
As almost all samples were categorised as very healthy in terms of transepidermal water loss and also showed high similarities, it was not surprising that the variations of the multivariate analyses were also low (RDA, Var 12.64, $p=0.415$; Adonis $R2=0.0414$, $p=0.00267$; Anosim $R=0.177$, $p=0.001$).

Typical taxa for the categories of transepidermal water loss

Allorhizobium was representing the samples with low TEWL values (categorised as very healthy) in the top 20 LEfSe analysis with a LDA score over 4, whereas *Malassezia* and *Lawsonella* often occurred on stressed skin (with high TEWL values). The rank test of the top 20 genera showed the same tendencies, but only with a significance of $p<0.05$ and with the restriction that 83.6% of the samples with valid TEWL values were classified as very healthy. 41 samples had to be excluded because of a technical issue with the Tewameter (measurement error of unknown cause) during sampling.

3.8 Personal hygiene

Higher α-diversity of skin microbiome composition on participants who did not shower daily

The participants who did not shower daily had a significantly higher α-diversity (Figure 14, Shannon index, ANOVA, p=3.1e-09), whereas skin cream usage did not reveal a significant difference (Shannon index, ANOVA, p=0.66). Evenness (p=0.0073) as well as richness (p=1.3e-10) was significantly lower on skin that was washed daily.

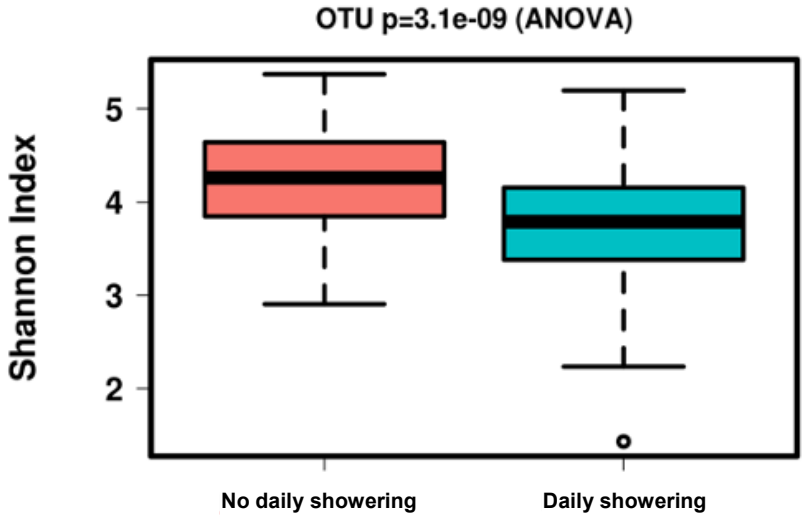

Figure 14: Significant difference of the Shannon index (ANOVA) between the samples regarding daily showering

Regarding β-diversity of daily hygiene, there was a significant, but low variation of the samples neither for daily showering (RDA Var 18.03, p=0.001; Anosim R=0.066, p=0.021; Adonis R2=0.0254, p=0.0003) nor regular skin cream usage (RDA Var 13.99, p=0.001; Anosim R=0.049, p=0.019; Adonis R2=0.0378, p=0.0003).

Hygiene dependent taxa abundances

Staphylococcus was the only taxon of the top 20 genera, which was more abundant on the skin of participants who showered daily. All other taxa in Figure 15 (e.g., *Anaerococcus*,

Corynebacterium, *Finegoldia*) showed significantly ($p<0.01$) higher abundance on skin of participants who did not shower daily.

Regarding the regular usage of skin cream, the rank test ($p<0.05$) of the top 20 genera (Figure 16) showed that *Streptococcus* was significantly more abundant on participants who used skin cream daily, whereas for *Cutibacterium*, *Lawsonella* and *Peptoniphilus* it was the opposite ($p<0.01$).

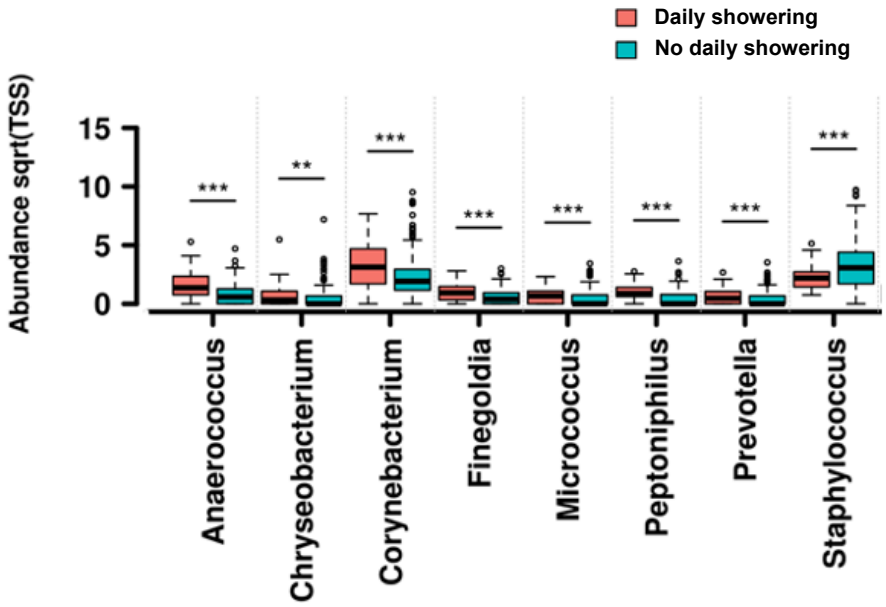

Figure 15: Differences in the occurrence of the top 20 genera regarding daily hygiene (Rank test, $p<0.05$)

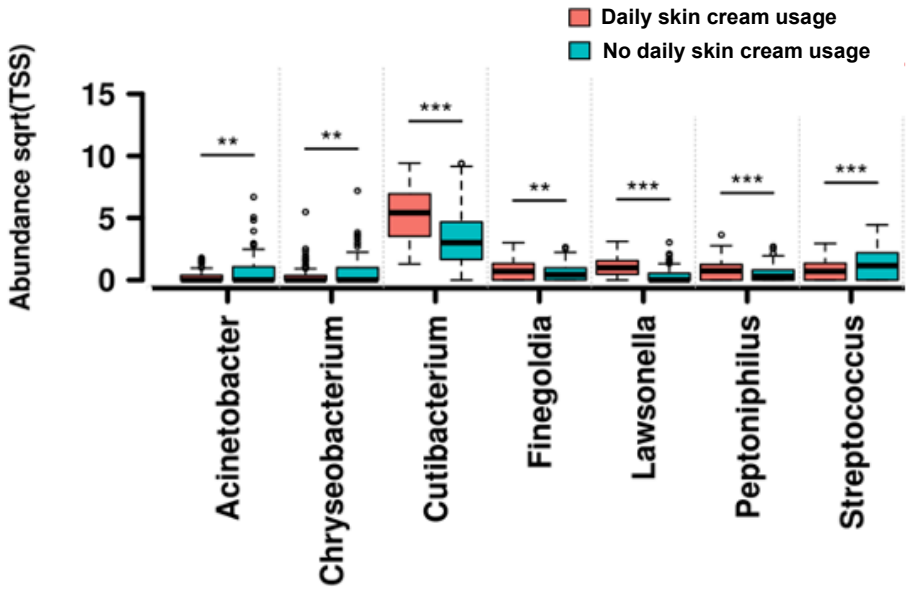

Figure 16: Differences in the occurrence of the top 20 genera regarding daily skin cream usage (Rank test, $p<0.05$)

3.9 Individuals

Significant but low differences in α- and β-diversity for the individual´s microbiome

The difference in α-diversity between the individuals was significant (Shannon index, ANOVA, p=1.8e-09, Richness p=5.8e-12, Evenness p=0.0022) (Figure 17).

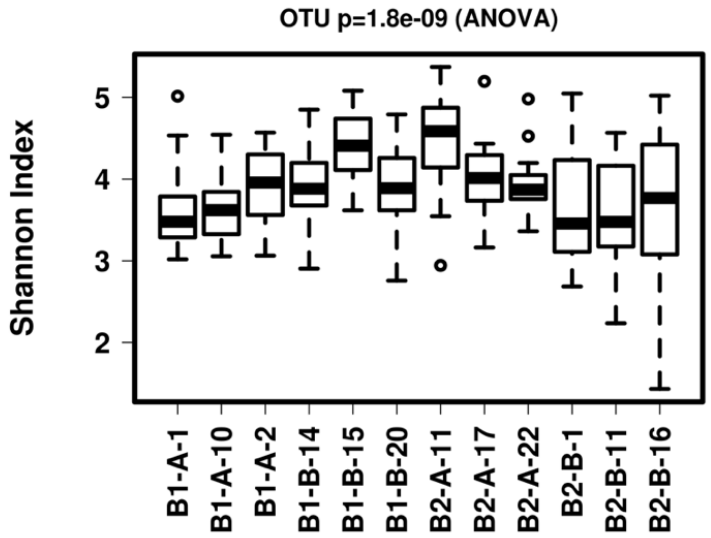

Figure 17: Significant difference of the Shannon index (ANOVA) between the samples regarding individuals

For the individuals, the PCoA and RDA plots showed especially four participants that seemed to have a dissimilar skin microbiome compared to all the others (B1-B-20, B2-B-1, B2-A-11, B1-B-15), what becomes visible through measuring points further away from those of the other subjects (Figure 18).

Grouping per individual was significant for all three tests carried out (RDA Var 118.2, p=0.001; Anosim R=0.147, p=0.001; Adonis R2=0.173, p=0.0003).

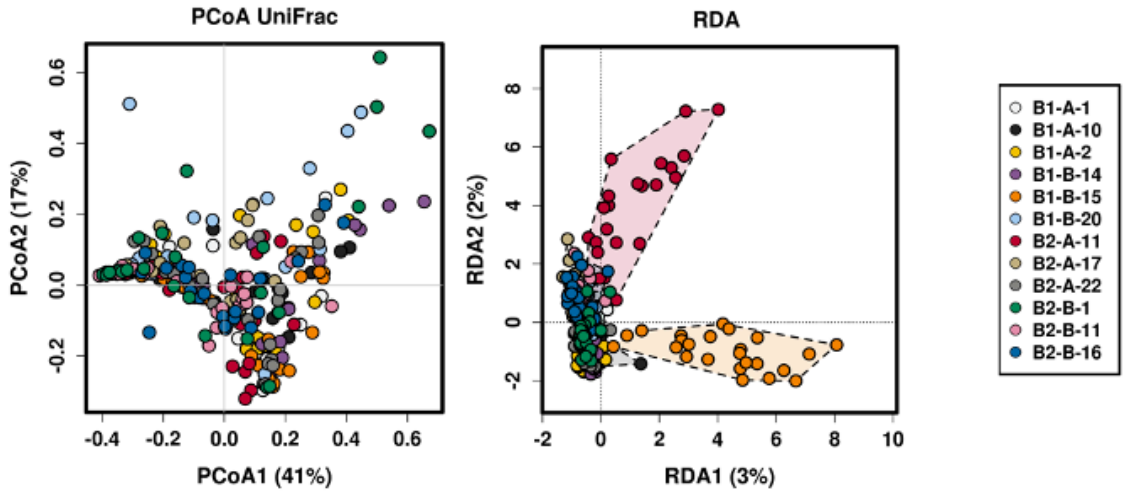

Figure 18: Significant difference in the β-diversity (PCoA, RDA, p=0.001) of the samples regarding individuals

Differences in skin microbiome composition of the individuals

LEfSe analysis of each individual showed just a few different taxa from the top 20 genera that were typical for certain subjects with a LDA score of over 4: *Chryseobacterium* and *Acinetobacter* occurred often on B2-B-16, *Peptoniphilus* on B1-A-10, *Lawsonella* on B1-A-10 and *Cutibacterium* was typical on the skin of B1-A-1.

For 16 of the top 20 genera, there were highly significant (Rank test, p<0.001) differences in their proportion on the skin from different individuals (Figure 19). It is striking, that two participants almost had no occurrence of *Streptococcus*, one of the top 5 taxa. On the other hand, *Burkholderia* was the only top 5 taxon not being present in the rank test, thus showed no significant difference in occurrence among the participating subjects (ANOVA, p=0.35).

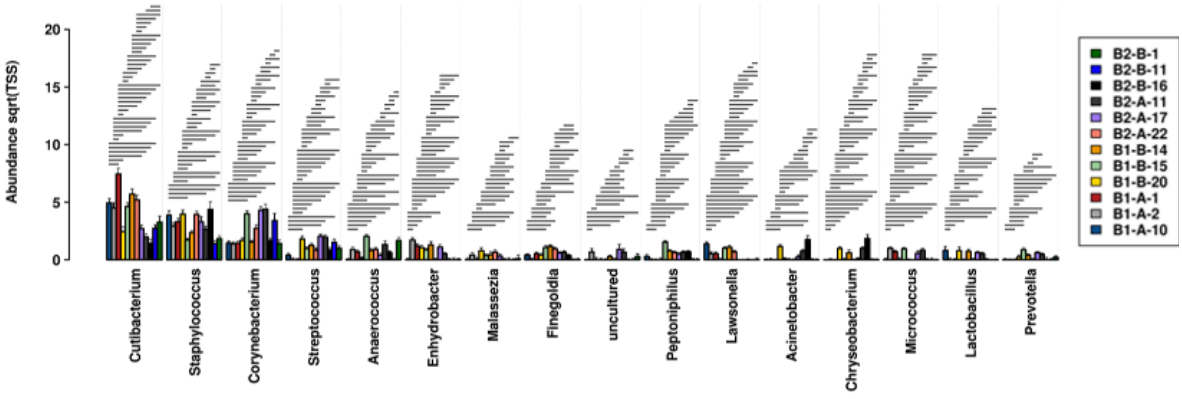

Figure 19: Differences in the occurrence of the top 20 genera regarding individuals (Rank test, p<0.01)

3.10 Time

Significant differences between t1 and other time points regarding the sebum content and skin pH

Even if there was a general significant difference between the time points regarding the sebum content (Figure 20, Kruskal-Wallis-Test Sebumeter, $p<0.001$) and the skin pH (Figure 21, Kruskal-Wallis-Test pH-meter, $p<0.001$), a closer look revealed only a significant change between t1 and other time points (Tables 21, 22).

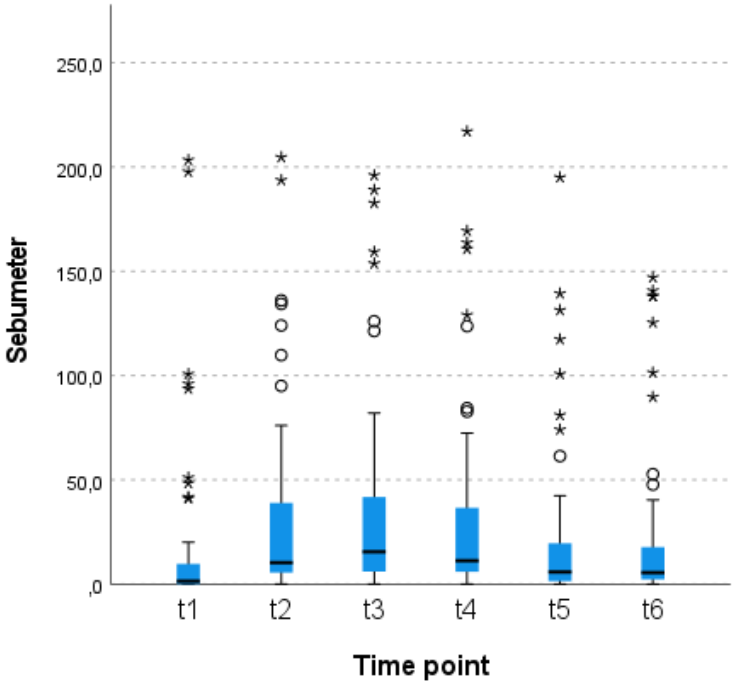

Figure 20: Significant difference between the time points regarding sebum content (Kruskal-Wallis-Test, p<0.01)

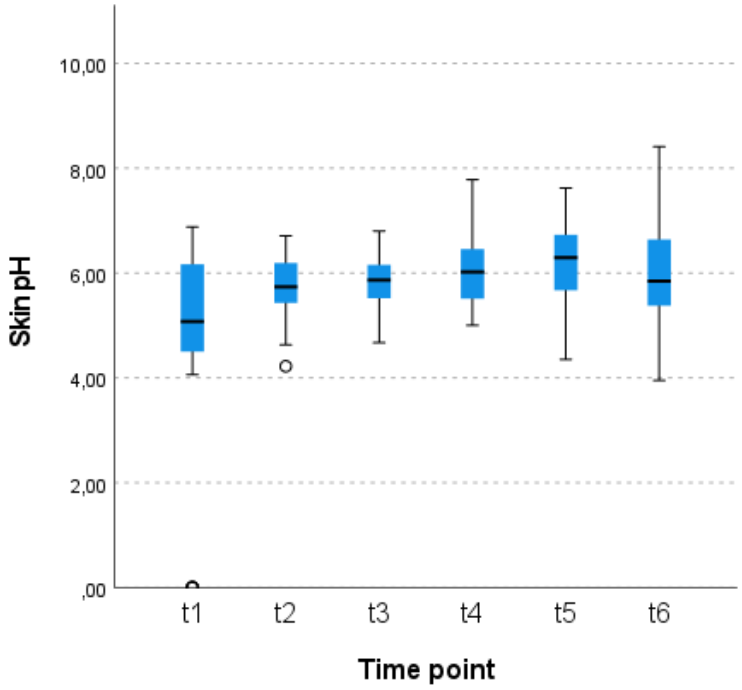

Figure 21: Significant difference between the time points regarding skin pH (Kruskal-Wallis-Test, p<0.001)

Table 21: Kruskal-Wallis-Test on the time points regarding sebum content

Time points	Bonf.corr. p	Effect strength r
t1 – t2	0.001	0.42
t1 – t3	0.001	0.47
t1 – t4	0.001	0.42

Table 22: Kruskal-Wallis-Test on the time points regarding skin pH

Time points	Bonf.corr. p	Effect strength r
t1 – t3	0.03	0.32
t1 – t4	0.01	0.35
t1 – t5	0	0.43
t1 – t6	0	0.54

Significant seasonal influence on the skin moisture

Over time, an increased occurrence of moist skin samples in the summer (t1-t3) and dry and very dry skin samples in the winter months (t5-t6, Figure 22) with a significant difference could be observed (Kruskal-Wallis-Test, Corneometer, $p<0.001$). Table 23 shows the significant differences between the individual time points.

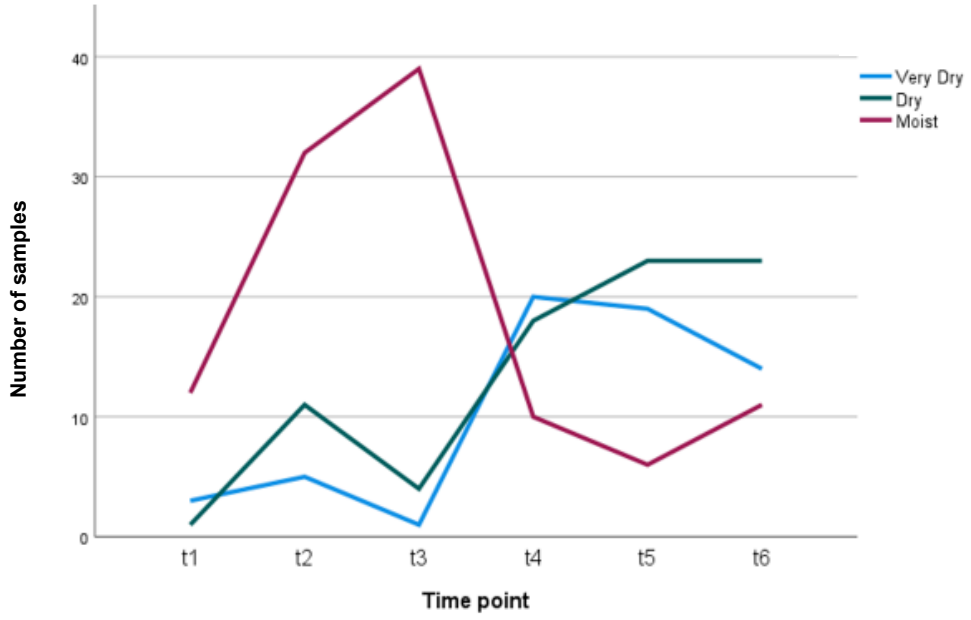

Figure 22: There were more samples with a high skin moisture in summer and more dry and very dry samples in the winter months.

Table 23: Kruskal-Wallis-Test on the time points regarding skin moisture

Time points	Bonf.corr. p	Effect strength r
t1 – t2	0	0.72
t1 – t3	0	0.98
t1 – t6	0.01	0.35
t2 – t4	0	0.46
t2 – t5	0	0.48

t2 – t6	0.004	0.37
t3 – t4	0	0.73
t3 – t5	0	0.75
t3 – t6	0	0.64

Seasonal differences in the bacterial and fungal diversity

There was no significant difference in α-diversity between the different sampling time points (Figure 23, Shannon index, ANOVA, p=0.24). While richness was very similar (p=0.88), evenness differed significantly (p=0.022). Considering grouped time points according to different seasons, changes in α-diversity were still not significant, but more visible than for individual time points (Figure 24, Shannon index, ANOVA, p=0.077; Richness, p=0.51; Evenness, p=0.0014).

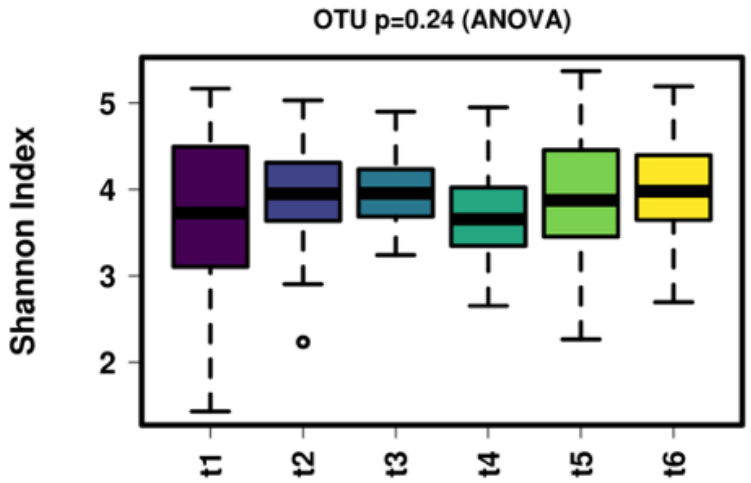

Figure 23: No significant difference of the Shannon index (ANOVA) between the samples regarding the time points

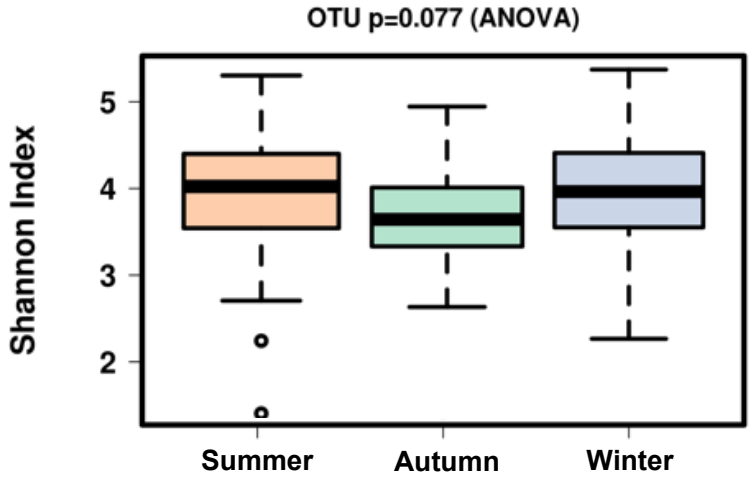

Figure 24: No significant difference of the Shannon index (ANOVA) between the samples regarding the seasons

When analysing the PCoA plot of the time course (Figure 25), it was remarkable that the samples of the winter months (t5 and t6) seemed to be more similar according to their β-diversity than to the samples obtained from summer and autumn samples (t1-t4). The RDA plot (Figure 25) also showed a different cluster for t1 than for the other samples, but with very low percentages on the axes of the plot (RDA, Var 31, p=0.001). Adonis and Anosim indicated a significant clustering per time point as well, but with low explained variances (Adonis, R2=0.149, p=0.0003; Anosim, R=0.159, p=0.001).

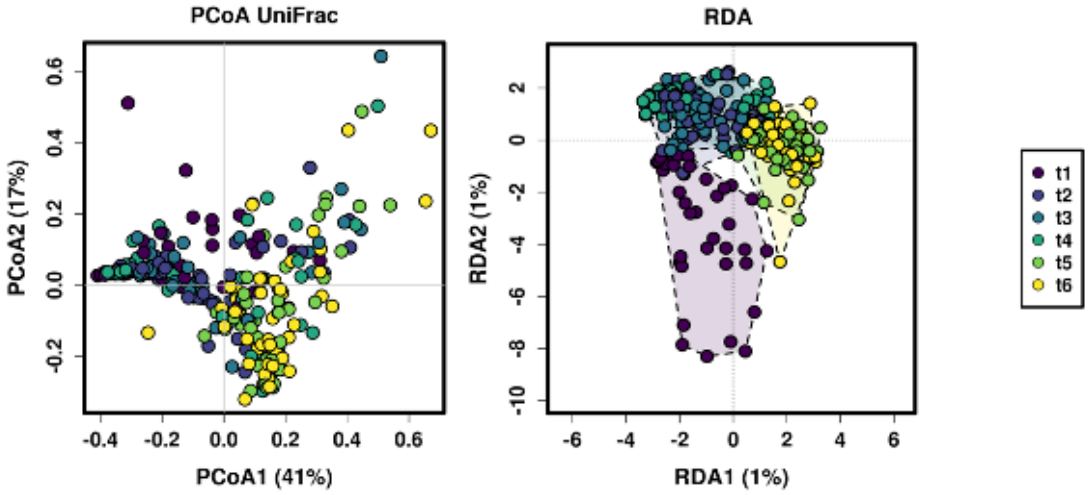

Figure 25: Significant difference in β-diversity (PCoA, RDA, p=0.001) between samples from different time points

High seasonal influence on some of the most common skin bacteria and fungi

Figure 26 shows a Bubble plot of the top 10 genera over time. While most of the genera showed an even distribution over time, *Burkholderia* occurred almost exclusively in t1-t4, which represent the summer and autumn months.

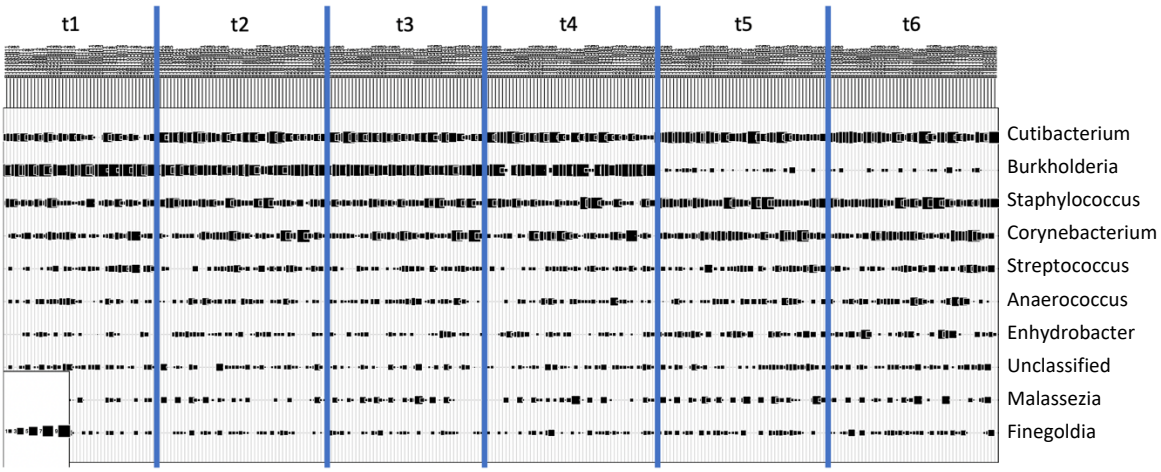

Figure 26: Bubble plot of the top 10 genera over the six time points

The ANOVA analysis of *Burkholderia* (Figure 27) reinforces this impression, since it shows very clearly a significantly higher abundance in summer than in winter (ANOVA, p=6.3e-46).

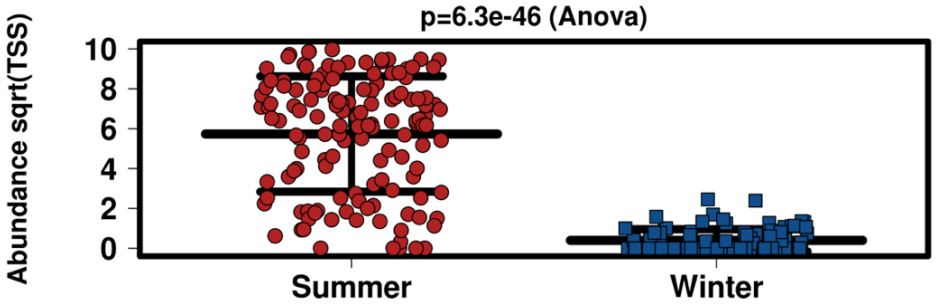

Figure 27: The ANOVA analysis shows that Burkholderia occurred significantly more often in the summer months (t1-t3) than in winter (t5-t6).

Figure 28 shows the rank test of the Top 20 genera over time (Rank test, $p<0.05$). *Cutibacterium*, *Staphylococcus*, *Corynebacterium* and *Enhydrobacter* had significant higher abundances in t5 and t6, during winter, whereas for *Burkholderia* and *Allorhizobium* higher proportions were visible in the warm seasons of the year (t1-t4).

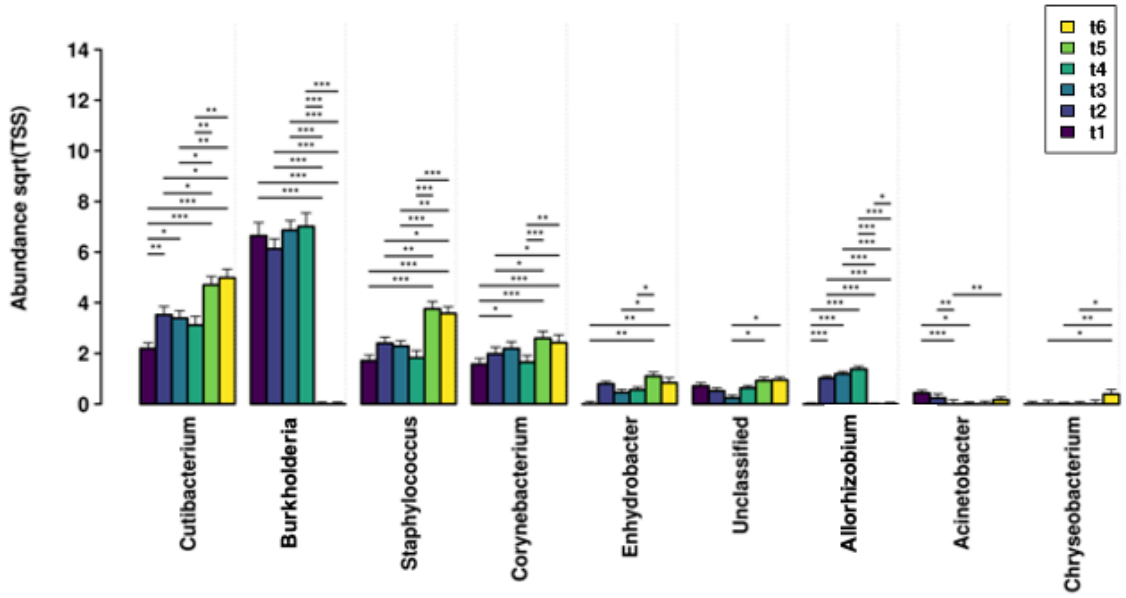

Figure 28: Differences in the occurrence of the top 20 genera regarding the time points (Rank test, p<0.05)

Since the differences seemed to be seasonal, the rank test was repeated regarding the seasons running into significance for autumn, summer, and winter (Rank Test, p<0.01). All the seasonal changes in the taxa mentioned above were clearly visible for this test as well.

A mixed effect linear regression model confirmed the temporal behaviour of the taxa which were already observed in the rank test:

- *Burkholderia* occurred almost only in t1-t4 (Bonf. corr., p=0).
- *Allorhizobium* behaved similarly, with a significant occurrence mainly in t2-t4 (Bonf. corr., p=0).
- *Cutibacterium* (Bonf. corr., p=2.5e-11), *Staphylococcus* (Bonf. corr., p= 5.3e-6), *Corynebacterium* (Bonf. corr., p=2.1e-4) and *Enhydrobacter* (Bonf. corr., p=0.0028) followed a contrary behavior with the highest occurrence in winter (t5-t6).

Furthermore, regression on the day of sampling (Figure 29) showed a correlation coefficient (Pearson index) of R=-0.45 for *Burkholderia* (p=0, mean abundance 3.84, positive samples 216) and a positive correlation coefficient of R=0.37 for *Cutibacterium* (p=0, mean abundance 3.98, positive samples 273).

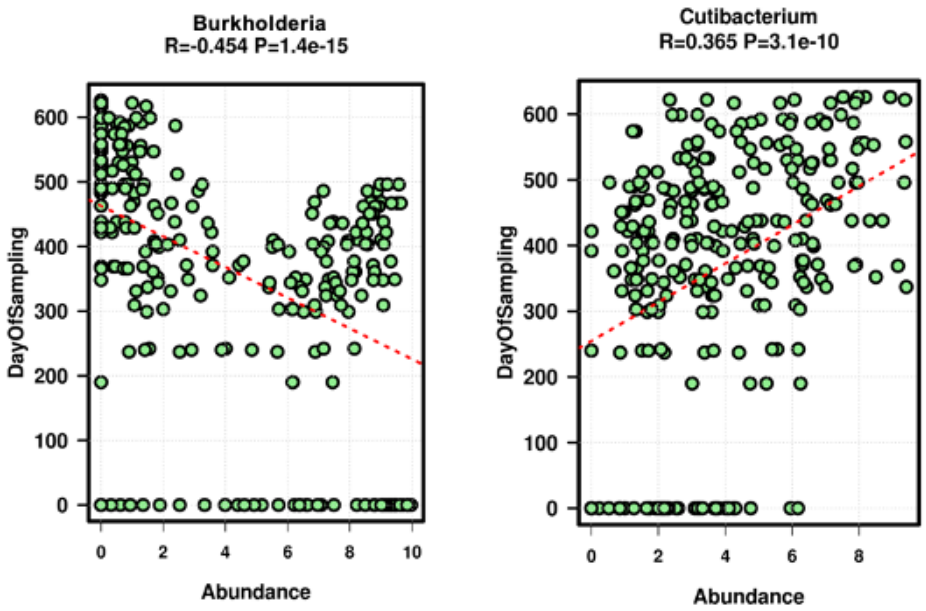

Figure 29: Burkholderia had a significant negative correlation (Pearson index) between its occurrence and the day of sampling, whereas Cutibacterium showed a significant positive correlation (Pearson index).

A LEfSe analysis for the different seasons demonstrated several characteristic genera even for the top 10 taxa. This observation confirms the strong influence of the season on the bacterial and fungal composition shown before. *Burkholderia* showed to be a typical genus for summer with a LDA score of over 5, whereas *Cutibacterium*, *Corynebacterium*, *Staphylococcus*, *Enhydrobacter* and *Finegoldia* were typical for winter with LDA scores over 4.

3.11 Sampling region

No significant differences in skin pH regarding different sampling sites

The Kruskal-Wallis-Test showed no significant difference between the body sites regarding the skin pH (pH-meter, p=0.051).

Significantly higher sebum content on the forehead than on the other body sites

For the following analyses, 39 samples had been excluded, since the Sebumeter could not provide any measure due to technical issues (nd).

Figure 30 shows that almost all skin samples were categorised as dry. Only 18% of all measured samples were classified as regular, occurring almost exclusively on the forehead.

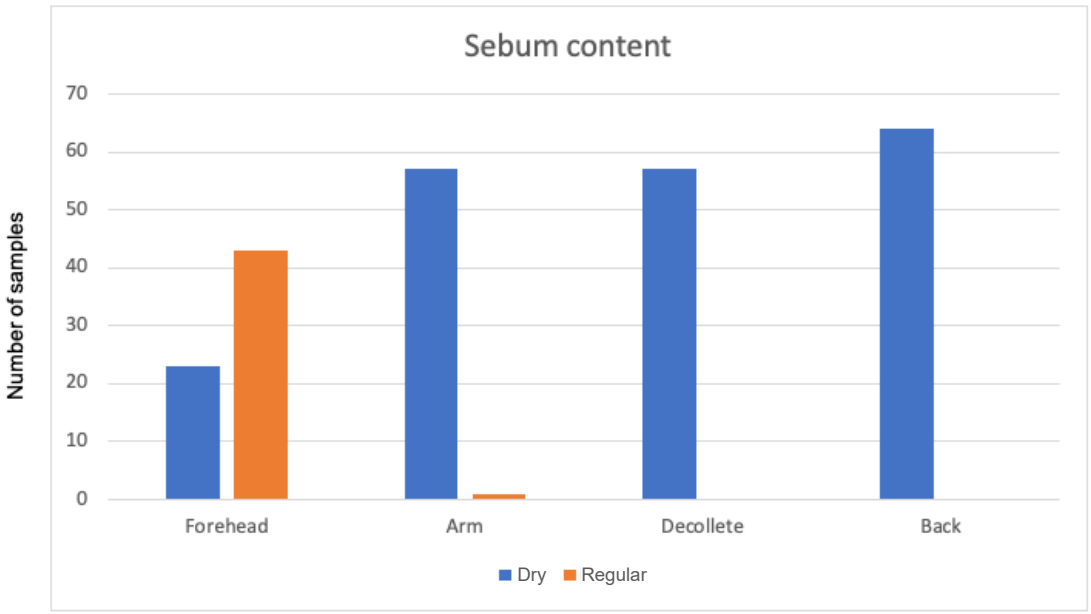

Figure 30: Samples of the forehead had higher sebum content than the other body sites. Most of the samples were categorised as dry.

According to the Kruskal-Wallis-Test (Figure 31), there was a significant difference between the body sites regarding the sebum content, with highest values on the forehead (Sebumeter, $p<0.001$). Significant differences occurred between forehead and arm (Bonf.corr. $p=0$, $r=0.78$), forehead and back (Bonf.corr. $p=0$, $r=0.72$), as well as forehead and décolleté (Bonf.corr. $p=0$, $r=0.69$).

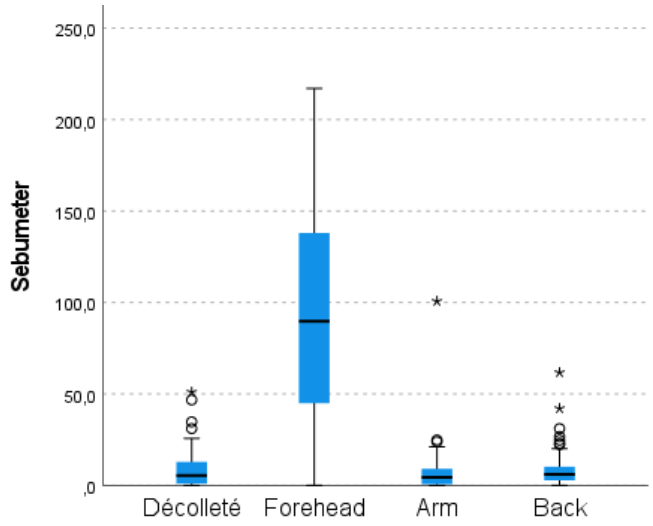

Figure 31: Testing for differences between the body sites regarding sebum content using Kruskal-Wallis-Test

Significant difference between the body sites regarding the skin moisture

For the following analyses, 8 samples had been excluded, since the Corneometer could not provide any measure due to technical issues (nd).

The distribution of the samples by skin moisture across the body sites (Figure 32) showed that the forehead (57%) and arms (73%) had mostly dry and very dry samples, whereas back (53%) and décolleté (53%) had a higher proportion of moist samples.

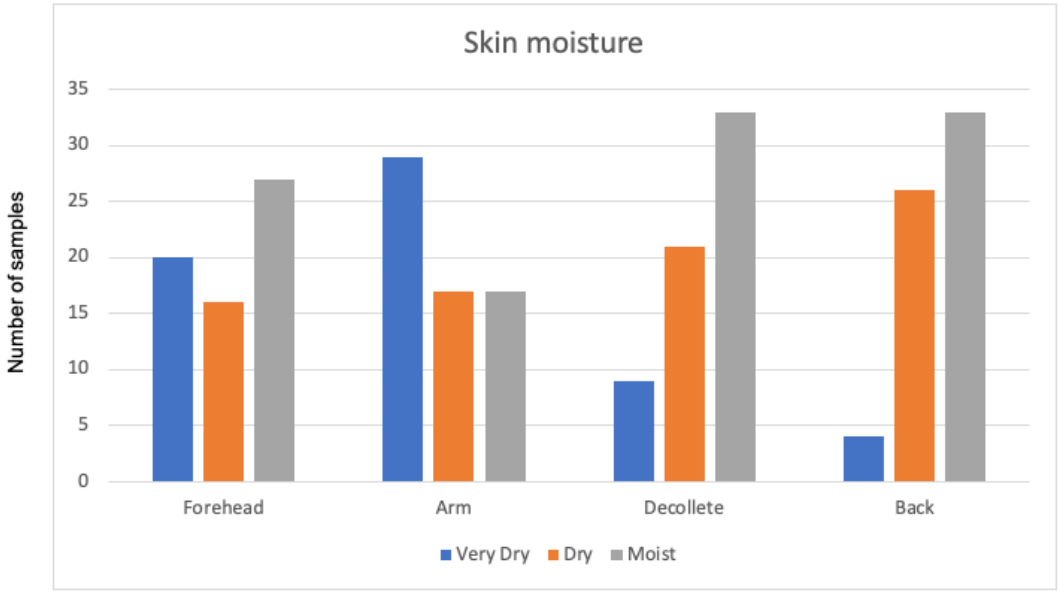

Figure 32: Distribution of samples regarding skin moisture across the body sites with samples categorised very dry, dry, or moist.

According to the Kruskal-Wallis-test, there was a significant difference between the body sites regarding the skin moisture (Kruskal-Wallis-Test, Corneometer, $p<0.001$). Taking a closer look, only arm and décolleté (Bonf.corr. $p=0.005$, $r=0.28$) and arm and back (Bonf.corr. $p=0.003$, $r=0.29$) differed significantly (Figure 33).

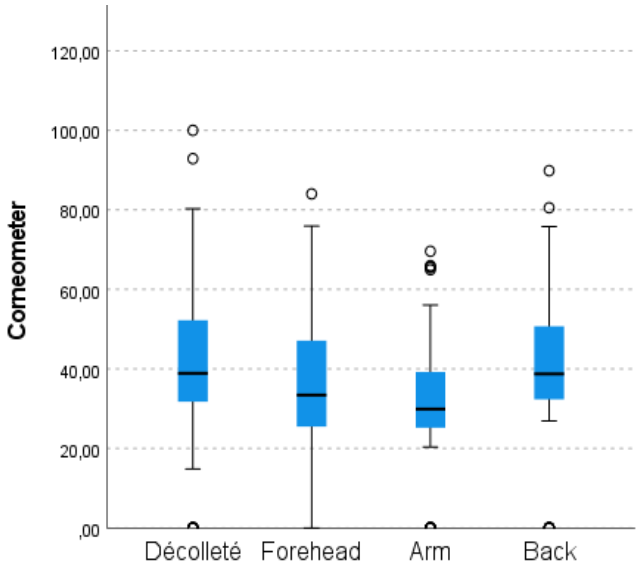

Figure 33: Testing for differences between the body sites regarding skin moisture using Kruskal-Wallis-Test

Forehead differed from the other body sites in terms of transepidermal water loss

For the analyses on transepidermal water loss, 41 samples were excluded, because no measurement for these samples was possible due to technical issues (nd).

Almost all the remaining samples (95%) were categorised as very healthy or healthy according to the Tewameter measures. Although, a different distribution of transepidermal water loss categories is visible in Figure 34.

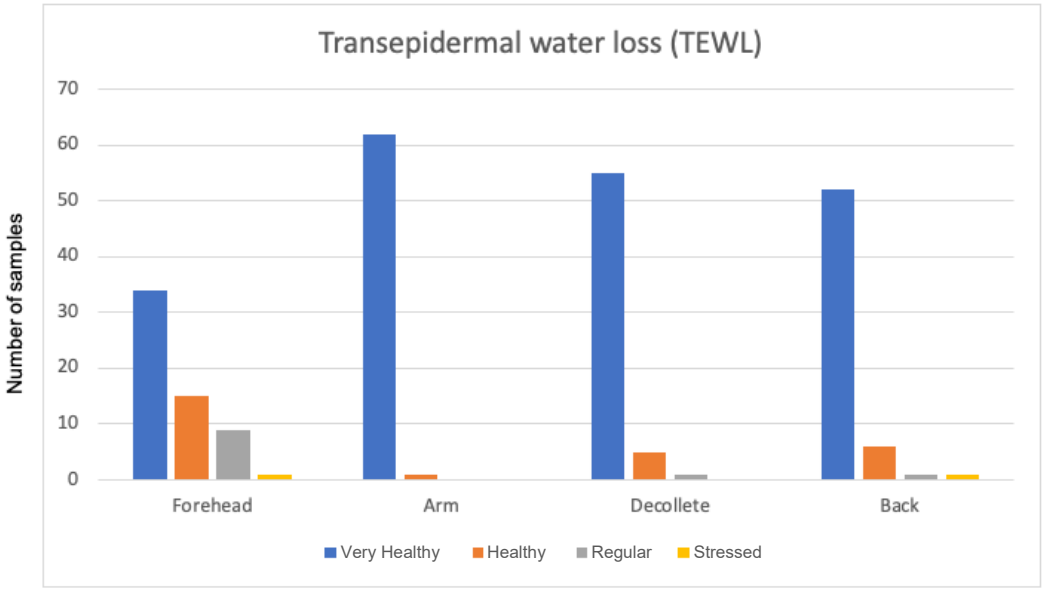

Figure 34: Forehead differing from the other body sites regarding TEWL with more samples categorised as healthy, regular, or stressed.

The difference between the body sites regarding the transepidermal water loss was significant (Kruskal-Wallis-Test, $p<0.001$). There were significant differences between forehead and arm (Bonf.corr. $p=0$, $r=0.38$), forehead and back (Bonf.corr. $p=0.001$, $r=0.35$), and forehead and décolleté (Bonf.corr. $p=0$, $r=0.4$). Figure 35 shows, that the samples from the forehead had the highest values of transepidermal water loss, with lower values being categorized as healthier skin (Table 3).

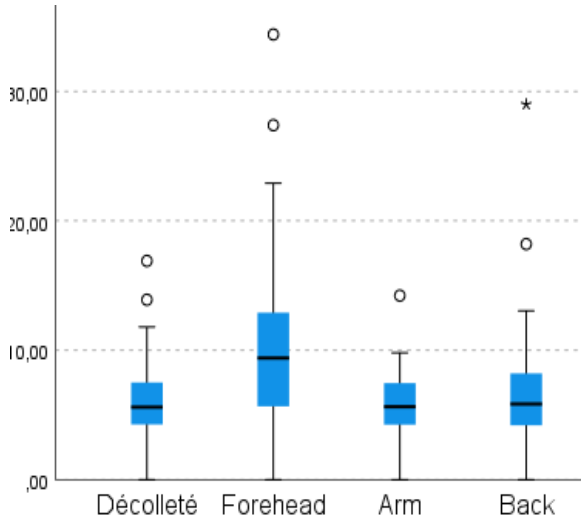

Figure 35: Testing for differences between the body sites regarding TEWL using Kruskal-Wallis-Test

Highest microbial diversity on the forehead among all sampled body sites

When looking at the α-diversity on different body sites (Figure 36), a significant difference could be observed, with forehead and arms showing the highest, and samples from the back the lowest α-diversity (Shannon index, ANOVA, p=3.2e-10; Richness, p=1.1e-09; Evenness, p=0.0042).

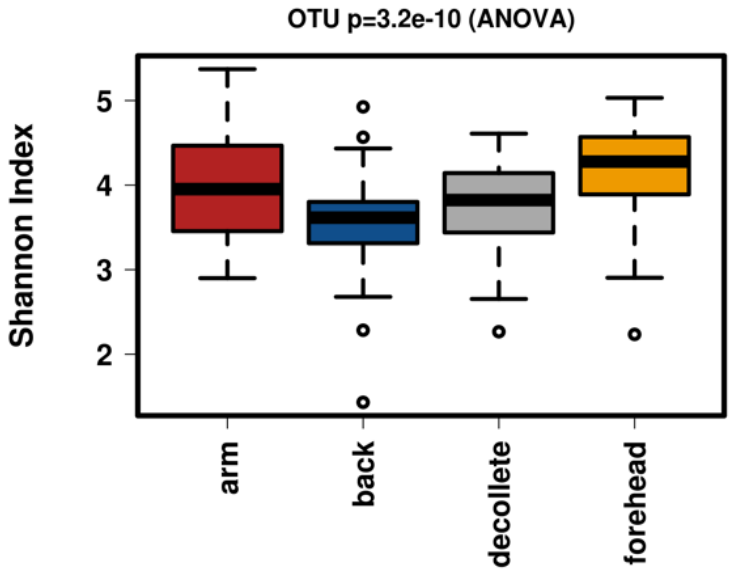

Figure 36: Significant difference of the Shannon index (ANOVA) between samples regarding the body sites

To analyse the differences and similarities of the bacterial and fungal communities within the different body sites, principal coordinates analysis (PCoA) on ASV level was carried out. The PCoA analysis (Figure 37) showed that samples from the forehead had a different bacterial and fungal community composition, while the other three sites were more similar. Nevertheless, despite significant influence of the location on the bacterial and fungal composition in the RDA (Figure 37), the axes revealed only a low percentage, which indicates that the variation was not really considerable (RDA, Var 27.96, p=0.001). Based on Anosim and Adonis, grouping per sampling site was significant and a reasonable fraction of diversity could be explained by this grouping (Anosim, R=0.199, p=0.001; Adonis, R2=19.4, p=0.0003).

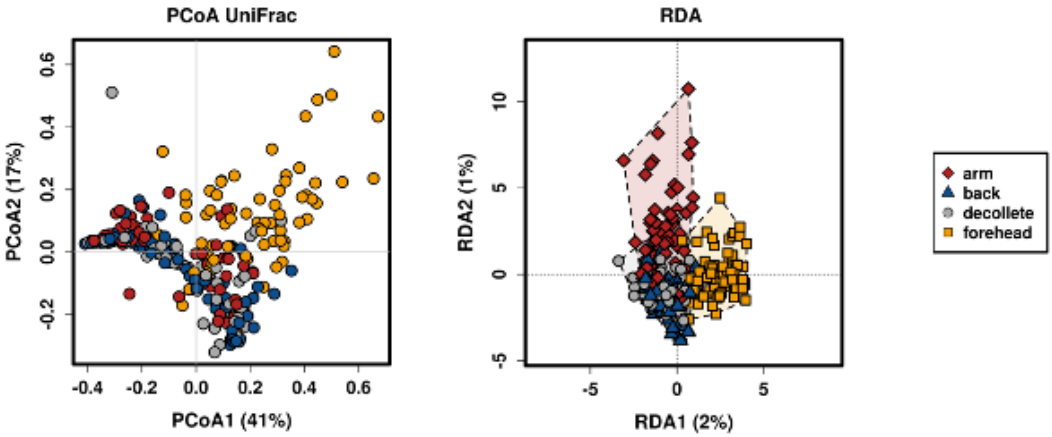

Figure 37: Significant difference in β-diversity (PCoA, RDA, p=0.001) between the samples regarding the body sites

When looking at individual sampling time points, it can be stated that the samples from forehead differed consistently from the other body sites at all six time points in the PCoA (Figure 38). Grouping per sampling site was clearly significant in RDA, Adonis and Anosim analysis (Table 24). The variation coefficients were higher in t1-t3, and decreased in t5-t6, which showed that the samples of the body sites differed more from each other during the summer than during winter.

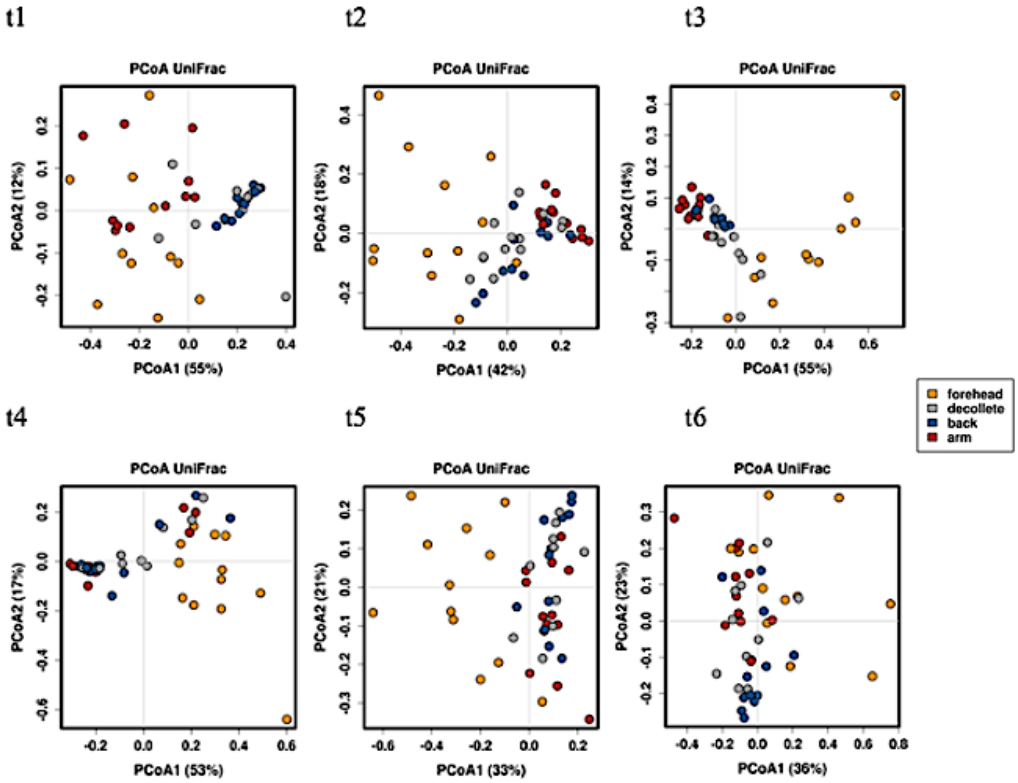

Figure 38: β-diversity analysis of the different body sites over time. The variation stayed constant over time.

Table 24: RDA, Adonis and Anosim analyses of the body sites over time

Timepoint	No. of Samples	RDA	Adonis	Anosim
t1	43	Var 61.42, p=0.001	R2=0.406, p=0.0003	R=0.363, p=0.001
t2	48	Var 63.44, p=0.001	R2=0.332, p=0.0003	R=0.316, p=0.001
t3	44	Var 70.62 p=0.001	R2=0.455, p=0.0003	R=0.368, p=0.001
t4	48	Var 57.02 p=0.001	R2=0.307, p=0.0003	R=0.234, p=0.001
t5	48	Var 52.46 p=0.023	R2=0.279, p=0.0003	R=0.252, p=0.001
t6	48	Var 54.92 p=0.017	R2=0.211, p=0.0003	R=0.177, p=0.001

Taxonomic characteristics of the body sites

The rank test on the top 20 genera with $p<0.05$ throughout all four body sites showed that *Cutibacterium* occurred to highest proportions on the forehead, whereas on arms, back and décolleté, *Burkholderia* was the most abundant taxon on genus level (Figure 39).
For most taxa such as *Cutibacterium*, *Corynebacterium*, *Malassezia* and *Peptoniphilus*, the forehead was the body region with the highest abundance.
It is conspicuous that *Burkholderia* and *Allorhizobium* were the only genera in this analysis that consistently occurred in relatively small proportions on the forehead (T-test, $p<0.001$). Furthermore, *Malassezia* is standing out with an extremely high significant abundance on the forehead compared to the other body sites (T-test, $p<0.001$).

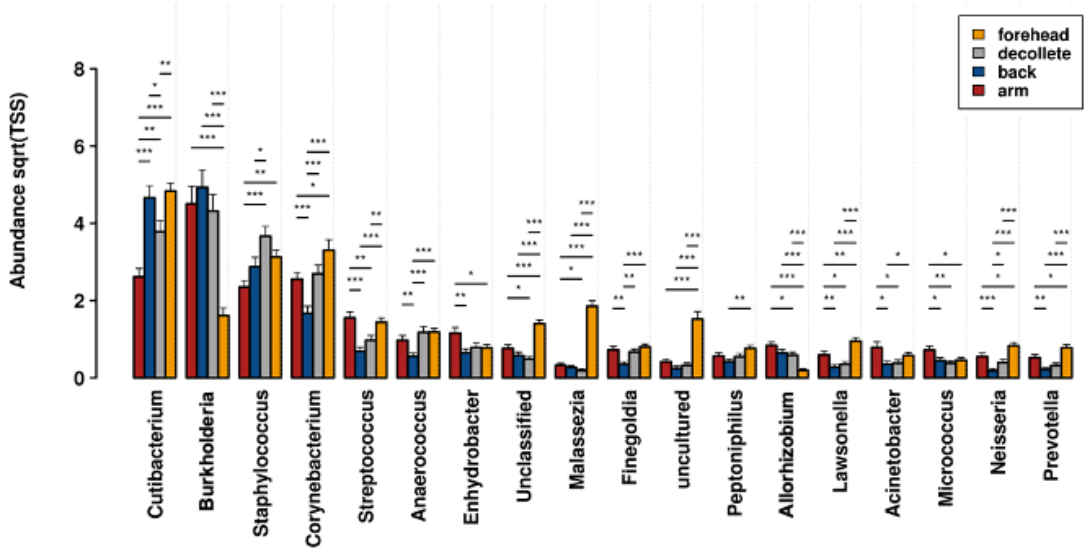

Figure 39: Differences in the occurrence of the top 20 genera regarding body sites (Rank test, p<0.05)

According to the LEfSe of the top 20 genera (Figure 40), samples from the forehead had plenty of characteristic genera with a LDA score over 4 (*Malassezia, Corynebacterium, Neisseria, Lawsonella, Prevotella*), whereas the other body sites only showed two characteristic taxa per region. The strongest association with the highest LDA score was evident between *Burkholderia* and samples from the back, followed by *Cutibacterium*. Typical genera of the décolleté were *Staphylococcus* and *Aerococcus* and on the arm *Allorhizobium* and *Finegoldia* were identified as characteristic taxa.

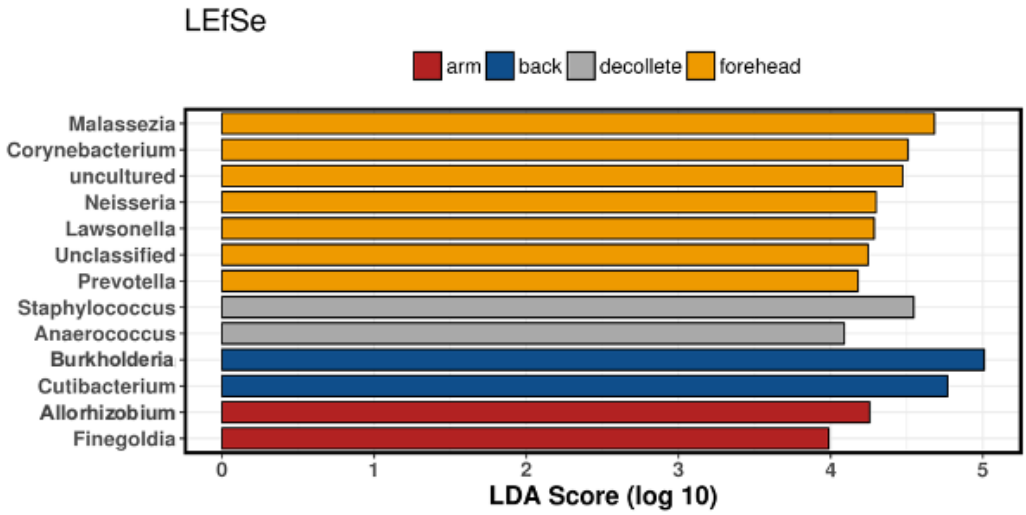

Figure 40: LEfSe analysis of the top 20 genera regarding the different body sites

4 Discussion

In this study, a total of 336 samples from 12 subjects were retrieved from four different skin sites (forehead, décolleté, arms and back). For each sample, considering skin measurements (skin pH, skin moisture, transepidermal water loss and sebum content) were performed and the bacterial and fungal skin microbiome was analysed.
The goal of this thesis was to understand longitudinal dynamics of the skin microbiome on different healthy skin sites, in the context of skin physiology and other metadata related to health, behaviour or lifestyle of the sampled subjects.
The hypotheses claim differences and similarities in skin physiology and the skin microbiome in relation to metadata categories and are discussed further in this chapter.
Moreover, the results will be compared with previous study findings about the healthy skin microbiome and addresses possible reasons for discrepancies. The discussion also considers the results in relation to common skin diseases and typical associated changes in the skin microbiome and skin physiology.

4.1 General findings on the bacterial and fungal microbiome

The four most abundant bacterial phyla in our study were *Actinobacteriota*, *Proteobacteria*, *Firmicutes* and *Bacteroidetes* and the most abundant genera were *Cutibacterium*, *Burkholderia*, *Staphylococcus*, *Corynebacterium,* and *Streptococcus.*
Regarding the fungal microbiome, *Basidiomycota* was the most abundant phylum and *Malassezia* the only fungal genus being present in the analyses.
These findings are consistent with previous studies, except for the occurrence of *Burkholderia*, which has not been reported as a common genus on human skin (15,48-52). However, in previous studies, relevant proportions of *Burkholderia* could be observed e.g., subepidermal on face and palm (53), epidermal on hands (49,54) and on ankles of zebu owners in Madagascar (55).
Burkholderia, a genus of Gram-negative, aerobic bacteria classified as member of β-Proteobacteria includes over 40 species. It is found in several ecological niches such as soil, water, animals, and human and is known for symbiosis with various fungi and plants (56). Some *Burkholderia* species show high biotechnological potential as they produce enzymes

and bioactive substances that can promote plant growth, health, and reduce pollutants. In humans, species of the *Burkholderia cepacia* complex are opportunistic pathogens in patients with immunodeficiency and cystic fibrosis (57).
Unfortunately, no exact species of the genus *Burkholderia* could be identified in the sequencing of our study, which would have been interesting for a more precise classification of the conspicuously high occurrence of this genus.
Contamination as a possible reason for the high appearance of *Burkholderia* needs consideration as well. The negative controls in a skin microbiome study using also another PureLink DNA extraction kit showed a large amount of *Burkholderia* (58). The ASVs of *Burkholderia* processed in our analyses were not identified as potential contaminants by their comparison to processed DNA extraction controls or PCR controls. Hence, they were not removed from the dataset as they cannot be perceived as an obvious contamination in our dataset. Nevertheless, the fact that other signatures of *Burkholderia* were found among potential contaminating ASVs could still indicate a potential source for contamination. In addition, potential batch effects during sequencing cannot be ruled out as well.

4.2 Influences on the skin microbiome

The composition of the skin microbiome is known to be dependent on many factors such as skin physiology (e.g., moisture, skin pH and sebum content), age and external factors (e.g., weight, diet and antibiotic use) (17). The following section takes up the hypotheses of this thesis and puts my findings into the context of previous studies.

4.2.1 Age

$H1_0$: Skin pH increases with age, whereas sebum content, TEWL and skin moisture decrease with age.

$H2_0$: Age group B1 has a higher diversity of the skin microbiome than B2 with different typical microorganisms on the skin.

In our data, participants of the older age group (60-80 years) had a significantly higher skin pH and lower transepidermal water loss than younger subjects (20-40 years), but there were no significant differences in terms of sebum content and skin moisture. As only skin pH follows $H1_0$, the hypothesis needs to be rejected.

In general, there are contradictory results in the literature with regard to age and skin physiological parameters: some studies found increasing parameters with age, others decreasing ones, and others no significant correlations (59-62).

The observed relationship between skin pH and age is consistent with the findings of previous studies, in which there was a significant relationship between increasing skin pH and age (60). This trend was also seen in the study of Luebberding et al., but for women with a peak in the pre-menopausal group and a skin pH decrease in the age groups of 60-80 years old participants. Decreasing pH values in the older age group could be explained by the low postmenopausal oestrogen levels. However, this specific development of skin pH in women could not be confirmed in our analyses.

Furthermore, in this study, the women showed a significant reduction in sebum production with age, whereas the values of the men remained stable. The skin moisture and TEWL showed no significant correlation with age (61,62). In contrast, the study by Kim et al. found a decrease in TEWL and sebum content with age and suggested that this was related to changes in skin barrier function due to skin ageing (59).

Moreover, a previous study found a higher diversity of the skin microbiome in participants between 25 and 35 years than in adolescents and older people between 50 and 60 years (52). Our data found no significant age-related differences in terms of α-diversity, and only a weak difference in β-diversity. This result is contrary to hypothesis $H2_0$, which leads to a rejection of this hypothesis.

In our study, significantly more signatures of *Corynebacterium*, *Streptococcus* and *Neisseria* were identified on the skin of the older study group B2 (60-80 years) than on that of the younger group B1 (20-40 years). On the other hand, *Cutibacterium*, *Enhydrobacter*, *Lawsonella* were found to significantly higher proportions on the skin of subjects from group B1 than on those of participants from group B2.

4.2.2 Sex

$H3_0$: Skin pH and sebum content is higher in men, whereas TEWL is higher on the skin of women.

$H4_0$: The composition of the skin microbiome between men and women differs significantly.

The skin of women differs to men regarding hormone metabolism (higher testosterone in men, higher oestrogens in women), thickness of skin (higher in men), hair growth (more in men), sweat rate during exercise (higher in men), sebum production (higher in men), surface pH (higher in men), tactile and sensorial perception (less pain and temperature sensitivity in men) and wound healing (slower in men) (63). The study by Luebberding et al. also found an increased pH and a decreased TEWL on the skin of the male subjects (61,62).
In contrast to that, a significantly increased skin pH and decreased TEWL compared to men were found in the female subjects in our data. Regarding skin moisture and sebum content, we could not identify significant sex-specific differences. This contradicts the assumption in hypothesis *$H3_0$*, which leads to its rejection.
Furthermore, we found no significant difference in α-diversity between the sexes and only a very weak variation in β-diversity. Nevertheless, there were clearly significant sex-specific differences in the occurrence of genera: *Streptococcus* and *Acinetobacter* were found to significantly higher proportions on female skin, whereas signatures of *Cutibacterium*, *Enhydrobacter*, *Finegoldia* and *Lawsonella* appeared significantly more often on male skin. Although diversity differs not significantly between the sexes, there are significant sex-specific variations in the abundance of some of the most abundant taxa on the skin, leading to endorsement of hypothesis *$H4_0$*.
In a study of Ying et al. with 71 participants, *Cutibacterium*, *Corynebacterium*, *Anaerococcus* were significantly more abundant on the skin of men, whereas *Acinetobacter*, *Paracoccus* and *Sphingomonas* were found to significantly higher proportions on the skin of women. Hence, these observations only overlap with our own datasets in terms of the genus *Cutibacterium*. However, in this study, β-diversity in terms of sex was also greater than in the collected data (52). Moreover, a study of the skin surface microbiome on the palms of 24 women and 27 men showed a significantly higher diversity in women (49).

4.2.3 BMI

$H5_0$: Obesity is associated with a difference in microbial composition compared to healthy weight.

Our data showed significantly higher α-diversity and richness in the skin microbiome of overweight subjects.
This contrasts with the findings of Brandwein et al. who associated reduced microbial diversity with obesity. However, this was stated due to a significant difference in diversity between underweight and overweight people and they could not find a significant difference between people with healthy weight and overweight people. Typical genera on the skin of overweight participants were *Anaerococcus*, *Finegoldia* and *Peptoniphilus* and *Corynebacterium*, and all showed a positive correlation with BMI (64). In our data, *Corynebacterium*, *Streptococcus* and *Finegoldia* were genera that could be found significantly to higher proportions on the skin of overweight individuals. According to these results, hypothesis *$H5_0$* can be confirmed.
Obesity is not only associated with typical comorbidities such as type 2 diabetes, dyslipidaemia or atherosclerosis, but also with skin diseases, especially psoriasis. It has been found that the risk of developing psoriasis correlates with the BMI (65). Moreover, studies figured out that there is a significant difference between the composition of the gut microbiome of overweight people compared to lean people. Furthermore, an association between intestinal dysbiosis and obesity has been identified (66,67). An altered intestinal microbiome can lead to increased intestinal permeability and consequently to systemic inflammation. Furthermore, there is evidence of an interaction between the gut microbiota and its secreted metabolites with immunological, neurological and metabolic pathways (67). These correlations could all contribute to a change in the microbial composition of the skin of overweight people.

4.2.4 Skin hygiene

$H6_0$: Daily showering reduces the diversity of the skin microbiome and affects its composition.

The α-diversity of the subjects who did not shower daily was significantly increased compared to those who showered daily. This was reflected in both significantly increased evenness and richness. Besides, there was a significantly higher ASV occurrence of 7 of the top 20 genera (*Corynebacterium*, *Anaerococcus*, *Finegoldia, Chryseobacterium, Micrococcus, Prevotella* and *Peptoniphilus*) on skin that was not washed daily. Only *Staphylococcus* was found to significantly higher proportions on skin that was washed daily. Based on this result, hypothesis $H6_0$ is endorsed.

In a study that examined hand washing behaviour in relation to skin microbiome composition, *Staphylococcus*, *Streptococcus* and *Lactobacillus* were found to be more abundant on regularly washed skin. In contrast, *Cutibacterium*, *Neisseria*, *Burkholderia* and *Pasteurella* showed an opposed pattern and could be retrieved to higher magnitudes from hands that were washed less frequently (49). Compared to our data, there was only one concordance regarding the genus *Staphylococcus*, but a comparison is also only possible to a limited extent, as samples were taken from different skin sites and the washing behaviour was not standardised (e.g., uniform shampoo/soap, cleaning procedure). Without being able to make a precise statement due to the limitations mentioned above, it seems reasonable to assume that frequent skin washing could reduce diversity and thus promote dysbiosis on the skin. The increased occurrence of *Staphylococcus*, a genus that includes facultative pathogenic species, could support this hypothesis. An increase in the abundance of *Staphylococcus* (especially *Staphylococcus aureus*) on the skin and a reduced microbial diversity is also found in the chronic inflammatory skin disease atopic dermatitis (17). In this context, the hygiene hypothesis states that reduced contact with a biodiverse environment, especially in early childhood, is associated with an increased incidence of allergies and the skin disease atopic dermatitis (68).

4.2.5 Individuals

$H7_0$: There is significant interpersonal variability in the skin microbiome of the subjects.

The analysis of the individuals generally revealed a significant difference in diversity, which was, however, more pronounced in some subjects than in others. There were also significant differences in the abundance of four of the five most common genera. Only *Burkholderia* showed no significant individual variations, which is potentially an indicator for a

contamination. Since significant interpersonal differences were found in four out of five of the most common genera and the diversity also differs significantly, hypothesis $H7_0$ can be confirmed.

Previous studies on the human bacterial skin microbiome agree on a high interpersonal variability (32). In the study of Grice et al., the interpersonal variation depended on the skin sites. It was also shown that the intrapersonal variation was less than interpersonal differences in adults (15).

A study of the skin microbiome of cohabiting family members and dogs found out that people acquire their skin microbiome partly through interaction with their environment. Their microbiome showed similarities to the people and animals with whom they were in close contact (69). The stability of a healthy skin microbiome was further demonstrated by a study by Lax et al.: firstly, a clear association was found between a family´s skin microbiome and the environmental microbiome in their household, and secondly, it was figured out that when the family moved, the skin microbiome transferred rapidly to the new environment (70).

4.2.6 Time

$H8_0$: There is no significant difference in skin physiological parameters over time.

$H9_0$: The skin microbiome is stable in composition and diversity regarding time.

As there were no significant differences in α-diversity across the different sampling events, our data suggest that the microbiome was relatively stable over time. Our dataset also showed a weak but significant change in β-diversity over time. It turned out that samples from autumn and winter were more similar to each other than to those from summer. Another striking feature was a similarity deviation of the microbiome at time point t1, which took place in the summer but more than a year before the others. In addition, the samples were taken by a different person, which could have introduced additional technical bias to this sampling event.

In a study that analysed the facial skin microbiome of healthy volunteers over 2 years, relative stability in composition and diversity was also evident. However, the differences that could be identified in some subjects could be related to changes in skin physiology (71).

Regarding these parameters, in our study there were significantly more samples with high skin moisture in the summer months than in the winter months. The other skin physiological parameters (skin pH, TEWL and sebum content), on the other hand, showed no relevant significant changes over time. Since a significant difference between winter and summer in terms of skin moisture was identified, hypothesis $H8_0$ must be rejected.

When looking at the composition of the microbiome over time, it is noticeable that *Burkholderia* (and to a lesser extent also *Allorhizobium*) was significantly present to lower proportions in the winter samples. In contrast, *Cutibacterium*, *Staphylococcus*, *Corynebacterium* and *Enhydrobacter* behaved in the opposite way, with a significantly higher signature occurrence of signatures on the skin in winter than in autumn or summer. As some of the most common taxa showed a distinct seasonal distribution, hypothesis $H9_0$ is rejected.

Allorhizobium is a gram-negative α-*Proteobacterium* typically found in soil (72). In our data, this genus had a similar behaviour as *Burkholderia* regarding its occurrence, but in lower quantity.

There are various explanations for the occurrence of *Burkholderia* (and *Allorhizobium*) in a temporal context. A possible hypothesis for the exclusive appearance on the skin in the warm months could be increased contact with nature and transmission in summer and autumn, when bacteria of the genus *Burkholderia* (and *Allorhizobium*) get into contact with the skin via the environment, e.g., soil in the garden.

Since samples in which hardly any *Burkholderia* (and *Allorhizobium*) were found were taken during the last two sampling events, the hypothesis of contamination could still be supported by an associated methodological change (e.g., a new batch of test kits) at these dates.

In conclusion, the data show that signatures of *Burkholderia* occurred more frequently on skin with high moisture levels and on samples taken in the summer months. These findings are consistent with the observation that the skin samples from summer had higher moisture levels than the ones from autumn or winter. The fact that the ASVs of other typical genera (e.g., *Cutibacterium*, *Staphylococcus*) occurred significantly less in the summer months raises the question of displacement by *Burkholderia*.

4.2.7 Body sites

$H10_0$: The body sites differ significantly with respect to skin physiological parameters, diversity, and the composition of the skin microbiome.

When classifying our chosen sampling sites into the physiological environments dry, moist, and sebaceous, we came to the following groupings:

- forehead: sebaceous
- arms: dry (possibly also moist if sampling was also carried out on the inner elbow)
- décolleté: sebaceous
- back: sebaceous

As already mentioned above, the sampling site on the arm was not clearly defined regarding the skin physiological categorisation (hairy forearm = dry, inner elbow = moist) which made comparability with known literature difficult and unprecise.
In terms of diversity, the forehead was found to be the region with the highest richness and evenness, followed by arms, décolleté and back. This is partly controversial to some studies with the findings of sebaceous sites (including forehead) to be less diverse than dry and moist sites (8). Over time, all skin sites showed stability in terms of β-diversity, which is contrary to a study that stated site-dependency: areas with higher diversity (e.g., forearm) were less stable over time, whereas skin sites with lower diversity remained more stable over time (15).
A known predominant genus on skin classified as sebaceous is *Cutibacterium* (13,15,48), which was the most abundant genus on the forehead and second most abundant genus on back and décolleté in our data. The most common genus on arms, back and décolleté was *Burkholderia*. Since arms were classified as a dry skin area, this result fits at least partially with the literature, in which a mixed population of mainly β-Proteobacteria (to which *Burkholderia* belongs) and *Flavobacteriales* was found to be predominant on dry skin sites (15). Furthermore, the most abundant genera in a study of the microbiome on the forearm include *Cutibacterium, Staphylococcus*, *Streptococcus* and *Corynebacterium* (50), which after *Burkholderia* were also the most common on arms, back and décolleté in our data. Signatures of *Burkholderia* (and to a lesser extent also *Allorhizobium*) were least common

on the forehead, the body region with the significantly highest sebum production. In the rank test of genera in relation to sebum content, *Burkholderia* and *Allorhizobium* stood out as the only two of the top 20 taxa that preferred with high significance dry skin over skin with regular sebum production. This raises the assumption that *Burkholderia* (and *Allorhizobium*) might have been found solely to a low degree on the forehead compared to the other skin sites due to the high sebum content prevailing there.

According to literature, *Malassezia* is the dominant fungal genus on the skin of the human trunk and arms. Only the feet show a higher fungal diversity on genus level. It has been shown that the fungal composition on the skin is also dependent on topographical and skin physiological markers (18). In our data, *Malassezia* was also the only fungal genus that appeared in the analyses of the most common genera that were carried out. It was particularly striking that ASVs of *Malassezia* were found with high significance more often on the forehead than on other sampling sites, which was also confirmed in a study of Oh et al. (19). Furthermore, *Malassezia* showed a positive correlation with increasing sebum content of the skin, which fits with the observation that the forehead was also the sampling site with the highest sebum production. Overall, the body sites show clear significant differences in terms of skin physiology and composition of the skin microbiome, which is why hypothesis $H1_0$ can be endorsed.

4.3 Limitations

Even though amplicon studies, also known as PCR-based sequencing, have become a widely used method for the detection and characterisation of microorganisms in molecular biology research, there are some methodological limitations that should be considered. One limitation is that amplicon studies can only detect microorganisms that are present in the original sample (detection limit). Microorganisms that are not present in the original sample or are present in too low numbers cannot be detected because they are not amplified by PCR. Due to the uneven and individual distribution of the biomass on the skin, it is therefore possible that not all microorganisms present on the skin site are present in the sample obtained. Furthermore, skin samples are highly susceptible to environmental and reagent contamination, and usually only a low amount of DNA is obtained (73). A standardised procedure within a study and the performance of blank and control samples is therefore important in order to keep the influences as transparent as possible, minimise

contamination and avoid observations based on false positives (74). In our study, blank and control samples were collected and processed, but the standardisation of the sampling was only possible to a limited extent due to different people carrying out the sampling and a change in the sampling process after time point t1. A study of Manus et al. also found that the storage method could influence bacterial diversity, which is why a limitation can also be expected in this respect (75).

To study a ribosomal community profile, different primers are chosen depending on the target region of the 16S rRNA gene (or the 18S rRNA gene for fungi). Amplicon sequencing is typically limited to a specific set of regions of interest, rather than genome-wide analysis. There are different accurate taxonomic classifications for respective microorganisms depending on selected primers, but it is not possible to accurately distinguish all bacteria, archaea and fungi in the process. Thus, primer bias means that a primer cannot amplify all taxa equally well and consequently not all microorganisms present in the sample can be detected. Moreover, it can introduce screw the relative abundances of certain taxa in your sample. Some will be amplified better by the PCR settings, others only to a lesser extent (73).

Furthermore, PCR bias may occur due to primer specificity and efficiency, template concentration and PCR cycle conditions, which may lead to inaccurate results (74). To counteract this bias, PCR blanks were used, and gel electrophoresis was performed as quality control measure.

Additionally, the sequencing data obtained, and the analyses carried out are of qualitative (rather than quantitative) information what must be taken into account when interpreting the results. The limited resolution of the taxonomic classification (mainly genus only and not species or strain) also leads to limitations, as e.g., typical commensals cannot be distinguished from obligate pathogens of the same genus.

Moreover, a limitation arises from the choice of the tool for data evaluation and the types of performed analyses. The experience of the person carrying out the analysis also influences the interpretation of the data.

The interpretation of the study results is generally limited by the small number of participants which entails even smaller groups when divided into different subgroups based on the metadata. The subjects studied in this work originate from a larger cohort of skin-healthy subjects and were selected for further longitudinal studies because of an interesting skin archaeome. Therefore, strictly speaking, the recruitment of subjects cannot be considered

random. And since the archaea were excluded from my data set, their possible influence cannot be taken into account.

Regarding the analyses on the factor age, it must be considered that only two different age groups were compared, and the selected cohort only covers the ages 20-40 years and 60-80 years respectively. Older as well as younger people and people between 40 and 60 years were not included in the correlation analyses, which should be considered regarding the interpretation of the results. It should also be noted that the different skin sites have different skin physiological compositions and therefore a correlation between age and the general skin physiology of a subject, which summarises several skin sites, can only be interpreted to a limited extent. This could also be the reason why contradictory results can be found in the literature with regard to age and general skin physiological parameters.

Furthermore, time point t1 took place more than a year before the second time point (the other intervals were about two months), and the sampling was carried out by a different person. In addition, the use of swabs and sponges varied from the other time points in the first sampling. Any differences in time must therefore be considered with respect to this. Due to technical issues with the Tewameter during sampling, no TEWL values could be determined for 41 samples, which must be taken into account in the interpretation of results about transepidermal water loss. Although the analyses showed strong seasonal changes in the skin microbiome, the interpretation is limited in that sense that only three seasons are represented, and the samples were not evenly distributed across them.

Some results can only be interpreted to a limited extent due to missing information in the survey or imprecise definitions in the sampling procedure.

This includes skin hygiene, as the question about a daily shower does not consider the different products used (shampoo, shower gel, etc.) and the length of showering. Furthermore, it only distinguishes between daily showering and non-daily showering.

The interpretation of the results regarding the body site arm is limited by the fact that no precise definition of the exact sampling area was made. This would have been important because different parts of the arm have different skin-physiological characteristics (hairy forearm = dry versus inner elbow = moist). A comparison with existing literature data is therefore also only possible to a limited extent.

As already mentioned in the chapters above, there are some restrictions regarding the interpretation of the occurrence of *Burkholderia*. Unfortunately, no exact species of the genus could be identified in the sequencing of our study, which would have been interesting

for a more precise classification of the conspicuously high occurrence of *Burkholderia*. Despite the lack of evidence in the evaluation of our negative controls, some of the correlations found suggest a potential contamination. These include the sudden drop of abundance in the last two time points and the lack of individual variation among the subjects. Furthermore, there were signatures of *Burkholderia* that were filtered out of the controls, which reinforces the suspicion for contamination. In addition, potential batch effects during sequencing cannot be ruled out with certainty. Therefore, significant results of *Burkholderia* should be considered in the light of these limitations.

4.4 Conclusion and Outlook

Overall, the results of my research were largely consistent with the literature. The greatest deviation, both in terms of abundance and the strength of certain correlations (e.g., seasonal occurrence), concerned *Burkholderia*. In order to find out more about the causes of this unexpected high abundance, a de novo study of the same design with different DNA extraction kits and implementation of even more quality controls would be an option to address the question of a possible contamination. To test the hypothesis of possible transmission of *Burkholderia* from nature (e.g., soil) to the skin, a study with a cohort of subjects regularly working in the garden versus a cohort having little contact to the nature would be suitable. In addition, a comparison of body sites that have varying amount of contact with the environment (e.g., hands vs. lower back) would be interesting.
Furthermore, the impact of the Covid-19 pandemic (with mask-wearing, increased disinfection) would be an interesting aspect for further longitudinal analysis on the composition of the skin microbiome, preferably using the same test persons and sampling sites of our study.

The original aim of this work was to compare skin microbiome data from healthy subjects with those from people suffering from psoriasis. Psoriasis is a chronic inflammatory disorder of the skin, nails and joints leading to hyperplasia of the keratinocytes and overactivation of immune cells in the epidermis. It affects up to 11% of the population worldwide and is associated with some comorbidities as well as great psychological distress (7,76). As the acquisition of test subjects experienced a major delay due to the Covid-19 pandemic, the decision was made to work purely on the cohort of skin healthy subjects, consisting of a

younger (B1) and older age group (B2), with a more detailed focus on differences within this cohort regarding various metadata and over time. It would therefore be interesting to compare the data collected in the meantime from the psoriasis cohort with my results and to work out significant correlations, which could be used as a basis for further studies on the pathogenesis of psoriasis in relation to the skin microbiome. Innate and adaptive immune responses triggered by genetic and environmental factors are known to contribute to the pathogenesis of psoriasis. Scratching due to psoriasis-related itching can lead to sores on the skin, which can bring bacteria into the deep dermis or bloodstream and lead to an immune system inflammatory response (7). Recent studies have also shown a link between psoriasis and microbial dysbiosis of the skin and the gut. There are different, partly contradictory, results of over- and underrepresentation of bacteria on psoriatic skin. What consistently stood out in some studies was the reduced representation of *Cutibacterium* (77). Furthermore, increased colonisation of *Staphylococcus aureus* and lower abundance of *Streptococcus epidermidis* in psoriasis lesions have been observed (7).

Gut dysbiosis, which is associated with psoriasis and other comorbidities (e.g., obesity, hypertension, type 2 diabetes), can lead to a chronic inflammatory process. This results in an imbalance between T cells and suppressor T cells and especially amplification of Th17 cell activation leading to psoriasis-like skin inflammation (7,17,78,79). Moreover, increased levels of several cytokines (e.g., IL 17, IL 22) have been found due to changes in the skin microbiome in patients with psoriasis (77).

It is not yet clear whether changes in the microbiome are the cause or the consequence of skin diseases. However, a positive correlation has been shown between lesion severity and the occurrence of pathological microorganisms in psoriasis and atopic dermatitis, whereas restoration of physiological microbiome composition accompanies with clinical improvement (80). Thus, the therapeutic approaches of pro- and prebiotics are promising as they do not eradicate the entire microflora like antimicrobial therapies but focus on restoring a microbial balance (79).

Since many studies in recent years have shown that the gut-skin axis seems to play an important role in the development of skin diseases, I believe that the parallel consideration of gut and skin microbiome is essential for further research comparing skin-healthy subjects with those with psoriasis. In order to ensure that certain correlations are not distorted by external factors (such as diet, pets, contact with nature), it would be suitable to acquire a cohort of people with a similar living environment (e.g., monks, prison inmates, inhabitants of remote villages), even if this is difficult to implement.

In conclusion, further studies on larger cohorts and in connection with the gut-skin axis are needed to understand how the changes in the microbiome composition are linked to the respective pathological skin conditions and subsequently to derive targeted microbiome-based therapeutic approaches.

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6 Supplementary

Mikrobiom der Haut v.1 2018-03-012

Fragebogen

Stammdaten

Geburtsdatum		(TTMMJJJJ)
Geschlecht	Weiblich □	Männlich □

Ernährung

Mahlzeiten pro Tag		
geschätzte Energiezufuhr pro Tag		kcal
Fleischkonsum	Ja □	Nein □
Häufigkeit des Fleischkonsums pro Tag		
Besondere Ernährungsgewohnheiten	Ja □	Nein □
Wenn Ja welche? (z.B. Vegetarier, Veganer, Freeganer, Flexitarier, Rohköstler, Pescetarier, Pudding-Vegetarier, Lacto-Vegetarier, Ovo-Vegetarier, Frutarier)		

Körpereigenschaften

Körpergröße		cm
Körpergewicht		kg

Schwangerschaft (derzeit)	Ja □	Nein □
Stillperiode (derzeit)	Ja □	Nein □

Seite 1 von 4

Mikrobiom der Haut v.1 2018-03-012

Hauttypus (Haut-, Haar und Augenfarbe)

Keltischer Typ (Typ I: Hautfarbe sehr hell, Haar rötlich bis hellblond, Augenfarbe blau bis grün, Brustwarzen sehr hell, Sonnenbrand sehr häufig, Sommersprossen)	□
Nordischer Typ (Typ II: Hautfarbe hell, Haar blond bis hellbraun, Augenfarbe blau bis grün, Brustwarzen mäßig pigmentiert, Sonnenbrand häufig, oft Sommersprossen)	□
Mischtyp (Typ III: Hautfarbe mittel, Haar dunkelblond bis dunkelbraun, Augenfarbe blau, grün oder braun, Brustwarzen mäßig braun, Sonnenbrand manchmal, kaum Sommersprossen)	□
Mediterraner Typ (Typ IV: Hautfarbe braun oder olivfarben, Haar braun bis schwarz, Augenfarbe braun, Brustwarzen dunkel, Sonnenbrand selten, keine Sommersprossen)	□
Dunkle Hauttypen (Typ V: Hautfarbe dunkel bis hellbraun, Haar schwarz, Augenfarbe braun, Sonnenbrand kaum, keine Sommersprossen)	□
Schwarze Hauttypen (Typ VI: Hautfarbe dunkelbraun bis schwarz, Haar schwarz, Augenfarbe braun, nie Sonnenbrand, keine Sommersprossen)	□

Photohauttyp nach Fitzpatrick

Nach Sonnenbestrahlung beobachten Sie:

immer Rötung der Haut, nie Bräunung	□
immer Rötung, manchmal Bräunung	□
manchmal Rötung, immer Bräunung	□
niemals Rötung, immer Bräunung	□

Seite 2 von 4

Mikrobiom der Haut v.1 2018-03-012

Freizeitgestaltung

Sportliche Aktivität in Stunden pro Woche (ca.)		Stunden
Anzahl an Stunden im Freien pro Woche (ca.)		Stunden
Regelmäßige Gartenarbeit	Ja □	Nein □

Besondere Hobbies		
Haustiere	Ja □	Nein □
Wenn ja welche		
Regelmäßiger Kontakt zu Tieren	Ja □	Nein □
Wenn ja welche		

Rauchverhalten

Raucher	Ja □	Nein □
Wenn Ja – Anzahl der Zigaretten pro Tag		

Alkoholkonsum

Regelmäßiger Alkoholkonsum	Ja □		Nein □
Wenn Ja – Wieviel Liter pro Woche	Bier	Wein	Spirituosen

Hygieneverhalten

Tägliches Duschen	Ja □	Nein □
Regelmäßige Verwendung von Hautcremen	Ja □	Nein □
Wenn Ja – Welche Produkte		

Seite 3 von 4

Mikrobiom der Haut v.1 2018-03-012

Medikamente

Einnahme von Antibiotika in den letzten 2 Jahren	Ja □	Nein □
Einnahme/Verwendung von Antiseptika in den letzten 2 Jahren	Ja □	Nein □
Systemische antibiotische Therapie innerhalb der letzten 3 Monate	Ja □	Nein □
Lokale antimikrobiell-wirksame Therapie innerhalb der letzten 3 Monate	Ja □	Nein □
Einnahme von Statinen in den letzten 2 Jahren	Ja □	Nein □

Hauterkrankungen

Plaque und/oder exanthematische Psoriasis	Ja □	Nein □
Atopische Dermatitis	Ja □	Nein □
Dokumentation von PASI (psoriasis area severity index) vorhanden	Ja □	Nein □
Dokumentation von EASI (eczema area and severity index) vorhanden	Ja □	Nein □

Seite 4 von 4

Figure 41: Questionnaire of the test subjects