

THE DYNAMICS OF MICROBIAL TRANSFER
AND PERSISTENCE ON HUMAN SKIN

by

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A DISSERTATION

Presented to the Department of Biology
and the Graduate School of the University of Oregon
in partial fulfillment of the requirements
for the degree of
Doctor of Philosophy

June 2017

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Degree awarded June 2017

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DISSERTATION ABSTRACT

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Doctor of Philosophy

Department of Biology

June 2017

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The skin microbiome is a critical component of human health, however, little is understood about the daily dynamics of skin microbiome community assembly and the skin's potential to acquire microorganisms from the external environment. I performed a series of microbial transfers using three skin habitat types (dry, moist, sebaceous) on human subject volunteers. Microbial communities were transferred to recipient skin using a sterile swab 1) from other skin sites on the same individual, 2) from other skin sites on a different individual, 3) and from two environmental donor sources (plant leaf surfaces and farm soil). With these experiments I was able to test for the presence of initial transfer effects and for the persistence of those effects over the time period sampled (2-, 4-, 8-, and 24-hours post-transfer). The sebaceous skin community was associated with the strongest initial effect of transfer and persistence on the moist recipient skin site, and to a lesser extent the dry skin site. The soil donor community when transferred to dry skin resulted in the strongest initial transfer effect and was persistent over 8- and even 24-hours post-transfer. These experiments are the first in scope and scale to directly demonstrate that dispersal from other human or environmental microbial communities are plausible drivers of community dynamics in the skin microbiome.

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ACKNOWLEDGMENTS

I wish to express sincere appreciation and gratitude to my co-mentors, Professors Brendan Bohannon and Jessica Green. Without their faithful support this dissertation research and manuscript would not have been possible. In addition, I would like to thank Maggie Weitzman and Doug Turnbull at the University of Oregon Genomics Core Facility for their advice and assistance with high-throughput sequencing. Lastly, I'd like to acknowledge Dr. Roxana Hickey and Hannah Wilson for their substantial contributions to the experimental execution and library preparation, respectively, for the experiments in Chapter Three. This research was supported by a National Science Foundation Fellowship (GRFP) and by a grant from the Alfred P. Sloan Foundation awarded to the Biology and the Built Environment (BioBE) Center at the University of Oregon.

I dedicate this manuscript to my parents Jeanne and Barry Bateman and to my fiancée Brian Jones. I couldn't have achieved this without their unwavering support and commitment to my academic and personal success.

TABLE OF CONTENTS

Chapter	Page
I. INTRODUCTION	1
II. WITHIN-SUBJECT MICROBIAL TRANSFERS	7
Introduction.....	7
Materials & Methods	8
Experimental Design & Sample Collection.....	8
Illumina Library Preparation & 16s rRNA Gene Sequence Analysis	10
Results & Discussion	12
III. BETWEEN-SUBJECT MICROBIAL TRANSFERS	20
Introduction.....	20
Materials & Methods	21
Experimental Design & Sample Collection.....	21
Illumina Library Preparation & 16s rRNA Gene Sequence Analysis	23
Results & Discussion	24
IV. ENVIRONMENTAL MICROBIAL TRANSFERS	34
Introduction.....	34
Materials & Methods	35
Experimental Design & Sample Collection.....	35
Illumina Library Preparation & 16s rRNA Gene Sequence Analysis	38
Results & Discussion	39
V. CONCLUSION.....	49
APPENDICES	54
A. ILLUMINA SEQUENCING PRIMERS	54

Chapter	Page
B. GENERALIZED LINEAR MIXED MODELS.....	55
C. COMPOSITIONAL BAR PLOTS: WITHIN-SUBJECT MICROBIAL TRANSFERS	57
D. DIVERSITY.....	60
E. COMPOSITIONAL BAR PLOTS: WITHIN-SUBJECT AND BETWEEN-SUBJECT MICROBIAL TRANSFERS	61
F. COMPOSITIONAL BAR PLOTS: ENVIRONMENTAL MICROBIAL TRANSFERS.....	66
REFERENCES CITED.....	75

LIST OF FIGURES

Figure	Page
1.1. Sampling Regime for Within-Subject Microbial Transfers.....	10
1.2. Modeling Transfer Effect and Persistence for Within-Subject Transfers.....	13
1.3. Sebaceous-to-Moist Transfers Within-Subjects: Subjects P and T	15
1.4. <i>Propionibacteriaceae</i> Increases Significantly After Transfer of Sebaceous Donor Community but not Moist	16
1.5. Baseline Community Diversity and Composition for Skin Sites.....	18
2.1. Sampling Regime for Between-Subject Microbial Transfers.....	23
2.2. Modeling Transfer Effect and Persistence for Between-Subject Transfers.....	26
2.3. Sebaceous-to-Moist Transfers Between-Subjects: Subject A.....	27
2.4. <i>Propionibacteriaceae</i> Increases Significantly After Transfer of Sebaceous Donor Community Both Within and Between Subjects.....	29
2.5. Baseline Community Diversity and Composition for Skin Sites Recapitulated...	31
3.1. Sampling Regime for Environmental Microbial Transfers	38
3.2. Modeling Transfer Effect and Persistence for Environmental Microbial Transfers	40
3.3. Soil-to-Skin Transfer: Subjects S07 and S15.....	42
3.4. Relative Abundance of Taxa Enriched in Skin After Soil Transfer	43
3.5. Pairwise Comparisons for DESeq Analysis.....	44
3.6. Baseline Community Diversity and Composition for Leaves, Soil, and Skin.....	47

LIST OF TABLES

Table	Page
3.1. Taxa Identified Using DESeq as Enriched in Skin Compared to Soil Donor	45

CHAPTER I

INTRODUCTION

Human beings are naturally obligate hosts to greater than a trillion bacterial cells that are members of complex microbial populations found throughout the various nooks and crannies of the human body (Caporaso et al., 2011; Costello et al., 2009; Huttenhower et al., 2012; Noecker, McNally, Eng, & Borenstein, 2016; Sender, Fuchs, & Milo, 2016; Zhou et al., 2013). Although these vast assemblages are diverse – comprising bacteria, archaea, fungi, and viruses - to date bacterial members of the human microbiome are the best studied among these. The microbes associated with human beings can be specialized to live in the many kinds of habitats found on and in the human body; subsequently, they constitute only a subset of total phylogenetic diversity found in the surrounding environment (Martiny, Jones, Lennon, & Martiny, 2015; Mathieu et al., 2013; O'Dwyer, Kembel, & Green, 2012). Moreover, some of these microbial passengers serve to benefit the human host in a variety of ways (Cho & Blaser, 2012; Costello, Stagaman, Dethlefsen, Bohannan, & Relman, 2012; Dethlefsen, McFall-Ngai, & Relman, 2007; Lewis, 2013; Morgan et al., 2012; Weyrich, Dixit, Farrer, & Cooper, 2015).

The total surface area of human skin is relatively large (~1.5-2 m²) and as a consequence of host factors can be broadly categorized into three, distinct habitat types that feature largely the same taxonomic groups but at different relative abundances: dry (e.g. forearm, palm), moist (e.g. elbow, back of knee), and sebaceous (e.g. chest, back) (E A Grice et al., 2009; Elizabeth A Grice & Segre, 2011; McBride, Duncan, & Knox, 1977; Wilson, 2005). Culture-based estimates for the number of bacteria living at these sites range widely from 10⁴ cells/cm² in dry skin sites to 10⁷ cells/cm² in sebaceous sites, probably underestimating the total and live bacterial biomass on the skin (Wilson, 2005). A more encompassing and quantitative method, qPCR, estimates the total number of 16S rRNA gene copies (approximately proportional to total bacterial cell number depending on copy number variation in the community) in a given bacterial assemblage. In 2010, (Gao, Perez-Perez, Chen, & Blaser, 2010) sampled moist armpits, dry forearms, and sebaceous foreheads, and found the greatest quantity of 16S rRNA gene copies in the moist habitat followed by a significantly lower (< 2 log₁₀) quantity in sebaceous and dry

habitats sampled. Although not as abundant as in other body habitats, the bacterial cells on the skin contribute to critically important host ecosystem services, including the processing of lipids and other skin metabolites, pathogen resistance, and education of the immune system early in life. They are generally commensal or even symbiotic, but depending on context can contribute to the etiology of skin disease or promote host defense. Psoriasis, acne, and atopic dermatitis are all common skin disorders that have been associated with predictable alterations of the commensal bacterial community of the affected skin (Belkaid & Segre, 2014; G. A. Rook, 2013).

The abiotic conditions on skin are largely driven by the amount of occlusion experienced by the area and the number of sebaceous and sudiferous glands present. The sebaceous and sudiferous glands produce sebum and sweat, respectively, and these host substances provide much of the available resources to bacteria living on the skin (Wilson, 2005). The differential quantities of these resources distributed across the surface of a person's skin determine much of the variation in the bacterial communities found there. This scale of variation is at an intraindividual level, i.e. variation that occurs within a single host subject.

The second scale over which bacterial communities on the skin vary is that of variation between hosts, or interindividual variation. Variation within skin sites (intraindividual variation) is generally lower than variation between skin sites (interindividual variation); in other words, skin sites are more similar to each other across different people than disparate skin sites are to each other on the same person (Costello et al., 2009; Noah Fierer, Hamady, Lauber, & Knight, 2008). Despite this, individuals can be differentiated from one another either based on the overall structure of a skin community or based on low-abundance taxa that are shared across skin sites within the same individual (N. Fierer et al., 2010; Oh et al., 2014, 2016). Bacterial taxa appear to vary in their response to the skin microenvironment and an idiosyncratic, or unique, host individual. *Propionibacterium acnes*, for example, was found to be more individual- than site-specific, in contrast to *Staphylococcus epidermidis* strains that were found to be more site-specific (Oh et al., 2014).

The third scale over which bacterial communities vary is time. Focused efforts to design longitudinal studies of the skin microbiome are few, but slowly accumulating

(Blaser et al., 2012; Costello et al., 2009; E A Grice et al., 2009; Huttenhower et al., 2012; Zhou et al., 2013). In one particularly extensive longitudinal study with just a few body sites (left and right palms were the only skin sites; $n = 2$) sampled over 444 days and 396 time points, found high daily temporal variation that prevented elucidation of any “core” skin microbiome (taxa shared across all sampling events). They categorized many more OTUs (operational taxonomic units) as either persistent community members (appearing in a skin site and remaining for an extended period of time) or transient community members (appearing in a skin site and then quickly disappearing). They also noted that the taxa comprising these persistent and transient categories were significantly different. A more recent study with additional skin sites ($n = 17$) but only three time points suggests that skin microbial communities are largely stable over time (low taxonomic turnover), at least within a single individual (Oh et al., 2016). Overall, the variation in an individual’s skin site day-to-day tends to be less than the variation observed between people on any given day.

Thus, skin bacterial communities are highly variable in their composition relative to other body habitats, yet they maintain relatively low temporal variation despite the skin’s near-constant exposure to the external environment. But, is it that the environmental microbes are rarely encountered or acquired (dispersal-limited) or that they don’t persist long enough for us to consider them a member of the microbiota? Essential to elucidating skin microbiome dynamics and any associated health consequences is the understanding of whether dispersal from environmental sources to the skin is a plausible driver of these dynamics.

Recently the “hygiene hypothesis” or the “old friends” hypothesis has been proposed as a potential explanation for the dramatic and relatively recent increase in allergic diseases in the Western world; briefly, the lack of exposure to microbial agents in childhood is suspected to suppress normal immune system development such that autoimmune and allergic disorders are more likely to develop later in life (G. a. W. Rook, Lowry, & Raison, 2013; Strachan, 2000). A number of studies have indeed demonstrated connections between allergy, skin microbial community structure, immune development, and/or environmental sources of bacteria (especially green space, siblings, and pets) suggesting that human health can be significantly impacted by the presence or absence of

microbes in their immediate environment (Azad et al., 2013; Lehtimäki et al., 2017; G. A. Rook, 2013; Ruokolainen et al., 2015). Notably, the mechanism by which environmental microbial exposures act to impact health is currently being explored. It is still unclear whether and for how long environmental microbes are able to persist on the skin, or whether persistence or viability is even necessary to impact human health. If environmental microbes can indeed persist on the skin, they afford us a unique opportunity to understand how they modulate or amend the bacterial community to access ecosystem services the skin microbiome has the potential to provide.

General ecological theory tells us that selection and dispersal both act to determine the composition of any community; most likely the environmental conditions at a given skin site will constrain the diversity of bacterial taxa that can live there. Many descriptive, exploratory studies of the skin microbiome bear out the importance of environmental selection (Findley et al., 2013; E A Grice et al., 2009; Oh et al., 2014, 2016; Zhou et al., 2013). Given the external-facing role of the skin, dispersal will surely act to continually expose the skin to novel microbial community members, persistent or not. Is dispersal of microbial taxa from other sources ever sufficient to overwhelm the constraints of environmental selection on the skin microbiome? The transmission of pathogenic bacteria within a single individual and between individuals is well understood through the history of research on communicable bacterial diseases such as tuberculosis, pneumonia, and meningococcal meningitis (World Health Organization, n.d.); could commensal and/or symbiotic taxa be transmitted using similar mechanisms?

In an early study using an unconventional model system, we sampled roller derby skaters at a roller derby conference to determine how and to what degree teams' skin bacteria changed significantly after playing a bout in which they make direct skin contact with another team from a different geographic location (Meadow, Bateman, Herkert, Connor, & Green, 2013). We found that roller derby team members' upper arm skin bacteria homogenized with that of the opposing team after playing a bout with one another. One explanation for this outcome is that commensal microbial taxa were transferred during vigorous contact during play. A second explanation for this homogenization, however, is that the microbiota of opposing teams homogenized not due to direct skin-to-skin contact, but instead due to transfer from the shared environment (i.e.

the gym floor). Another recent and large study demonstrated that household members, especially couples, shared more of their commensal skin microbiota with each other than with individuals from different households (Song et al., 2013). Moreover, the effects of cohabitation seen in this study were stronger for skin than for oral or fecal microbiota. The researchers also found evidence for oral-to-skin microbiota transfer among couples, suggesting high frequency of contact among cohabitating partners and supporting both the hypothesis that intrapersonal microbial and interpersonal commensal microbial transfer are plausible modes of commensal microbial dispersal that can shape skin microbial communities. Again, from these studies it is impossible to determine whether the microbial transfer that we speculate is causing this homogenization among individuals is due to direct skin-to-skin contact between occupants or from commensal microbial transfer from items in the shared home environment. A third explanation could be that no significant commensal microbial transfer is occurring from other people or sources in the environment, but that certain bacteria are favored and selected in this shared environment and this is then reflected in shared community membership. These three explanations are not mutually exclusive; dispersal between occupants, dispersal from the shared environment, and selection due to shared environmental conditions likely all play a role here.

With the direct manipulation of human-associated microbial communities one could directly control microbial exposures, yet, there have been few attempts to do this due to non-trivial ethical and practical considerations. An experiment by (Costello et al., 2009) is one notable exception. To disentangle the contributions of environmental factors (selection) and microbial exposures (dispersal) to skin microbial community structure and composition, two skin habitats (sebaceous foreheads and dry inner forearms) were disinfected and subsequently reciprocally inoculated with sterile swabs and tracked over time (2, 4, and 8 hours post-inoculation) in 16 individuals. Foreheads and forearms were also inoculated with tongue (oral) microbial communities and tracked over time (2, 4, and 8 hours post-inoculation). From their results, the authors concluded that the sebaceous skin community was more constrained by its selective environment while the dry community was likely shaped by the controlled microbial exposure (Costello et al., 2009).

Building on this exciting and promising work, with my dissertation research I set out to determine a) whether the selective effects of intact (not disinfected) skin communities could be overwhelmed with microbial dispersal, and b) whether any effect of dispersal would persist over time. For my first chapter, I expanded the set of recipient skin sites studied in (Costello et al., 2009) to include the third, broad skin habitat (moist) and performed transfers of microbial donor communities from and to intact (i.e. not disinfected) skin sites within the same subject (n=10). For my second chapter, I investigated whether transfers of microbial donor communities from skin sites between subjects would exhibit the same effects as transfers of microbial donor communities from skin sites within those subjects (n = 4 pairs; 8 total). For my third chapter, I constrained the skin recipient community to the dry site type only, but I expanded the set of microbial donor communities to include those on the leaves of common indoor houseplants and farm soil (n = 16). I also asked whether the effect of transfer would persist beyond eight hours and whether the effect would persist after washing.

From this set of three experiments I aimed to determine if a) skin microbial communities are amenable to microbial transfer from sources likely to be encountered in human habitats, and b) if so, for how long does this transfer persist? I also hoped to determine which microbial taxa, if any, appear to a) differentially transfer to the skin, and b) persist on the skin after time and perturbation. Ultimately I aim to better understand how our physical interactions with a mostly microbial world serve to shape the microbial communities we find on ourselves, which provide us with essential ecosystem services, and that literally make up so much of who we are.

CHAPTER II

WITHIN-SUBJECT MICROBIAL TRANSFERS

Introduction

With this first chapter, I describe a set of microbial transfers of skin microbiota within a single host across three intact, skin site types. Although disinfection of the skin is a rational starting point for beginning to understand the acquisition of transient or environmental microbiota, unsterilized skin of human volunteers more closely resembles the normal state of the skin during daily contact with microbial donor sources in the environment. In addition, the microorganisms already living on the skin are predicted to have significant effects on the colonization success of invading microorganisms via both inter- and intra-specific interactions (Carriage et al., 2013; Christensen et al., 2016; Fitz-Gibbon, Tomida, & Chiu, 2013; Iwase et al., 2010; Libberton, Coates, Brockhurst, & Horsburgh, 2014; Rosenthal, Goldberg, Aiello, Larson, & Foxman, 2011; van Rensburg et al., 2015). Moreover adding an important, diverse skin site to the set of recipient skin sites adds to our understanding of the amenability of human skin to microbial dispersal.

Due to the possibility of direct and indirect interactions of the donor microbiota with the intact skin community, it is plausible that the effect of transfer will not be as large as that found for one of the transfers of skin microbiota to disinfected skin sites in the (Costello et al., 2009) study; namely, the forehead to forearm transfer (sebaceous-to-dry site transfer). Unfortunately, different distance metrics (UNIFRAC versus Bray-Curtis) were used to evaluate community similarity, which makes only qualitative comparison possible between these experimental transfers. Even if a significant effect of microbial transfer is observed at 2-hours post-transfer, it is also plausible that the combination of local environmental selection and direct/indirect interactions with the recipient skin community could result in a significant decay of community similarity to the donor community between 2-, 4-, and 8-hours post-transfer. These two hypotheses, 1) whether an initial effect of microbial transfer is observed in the intact, recipient skin community between 0 and 2-hours post-transfer, and 2) whether this effect of transfer

persists between 2-, 4-, and 8-hours post-transfer, will be tested in this first chapter with within-subject transfers across skin sites.

Materials & Methods

Experimental Design & Sample Collection

This study and its experimental protocols were approved by the IRB at the University of Oregon on December 23, 2013 and assigned IRB Protocol Number: 06082013. All researchers associated with this protocol are CITI certified to work with human subjects and associated sensitive information. Subjects were recruited from the Eugene, OR area with recruitment e-mails and campus announcements. After the subjects initiated contact they were asked to take an eligibility-screening questionnaire that assessed whether individuals met the requirements of the study. Subjects were required to be between 18-35, in good health and free of any skin conditions and/or infections, and have not taken antibiotics in the last 6 months. After passing the eligibility-screening questionnaire, subjects were given further, detailed instructions for their participation in the study. In addition to asking that the subjects refrain from bathing or applying topical items to the skin at least 12 hours prior to the start of the experimental period, subjects were asked to report if they were feeling unwell, or had begun taking new medications.

The study was performed on April 27th, 2014 in a small, semi-private conference room at the University of Oregon from 07:00 to 20:30. 10 subjects were ultimately included on the study day. Three skin sites (inner forearm, upper chest, back of the knee) were chosen for this experiment to represent three general skin habitat types (dry, sebaceous, and moist, respectively) while remaining cognizant of ease of access for the researchers and reasonable privacy for the human subject volunteers. First, grids were drawn on each subject using ethanol-disinfected custom plastic-vinyl stencils and thin-tipped permanent marker for the purpose of denoting equal “donor” and “recipient” areas of skin, and to establish equal, distinct areas for sampling the skin before the transfer and at 2, 4, and 8 hours after the transfer (Figure 1.1). Initial baseline (T0) microbial community swab samples were then obtained at each site for 10 subjects (each assigned a

letter: K, L, M, N, O, P, Q, R, and T). The 10 subjects' start times were staggered in pairs to make temporal sampling of multiple subjects more manageable for the researchers. After the baseline samples were collected, the microbial communities were picked up and immediately transferred with a swab for every pair of skin sites, including same-site control transfers, for a total of 9 transfers per subject.

For both sample transfers and sample collection, the same swabbing procedure was used. The swab is dipped into a sterile, saline solution (0.15 M NaCl; 0.1% Tween20) and rid of excess moisture by flicking the swab carefully. The swab is rotated while swabbing the skin firmly for approximately 10-15 seconds. For transfer the swab is then applied to the recipient area of skin and again rotated while swabbing firmly for approximately 10-15 seconds. For sample collection at baseline (0), 2-, 4-, and 8-hours post-transfer the swabs are immediately placed back into their sterile containers and frozen at -20 C until DNA extraction. After the transfer procedure is complete a sterile gauze dressing is lightly placed and taped to cover the area with minimal occlusion, and the subjects remain sedentary in a controlled, casual setting for 8 hours. Non-destructive swab samples are taken of each of the 9 transfer types on each subject at the requisite time point 2, 4, and 8 hours post-transfer. If needed the sterile gauze was replaced or additional tape is applied to secure the gauze in place. To avoid spatial correlations, the location of the sampling area for each time point was randomized within the sampling grid.

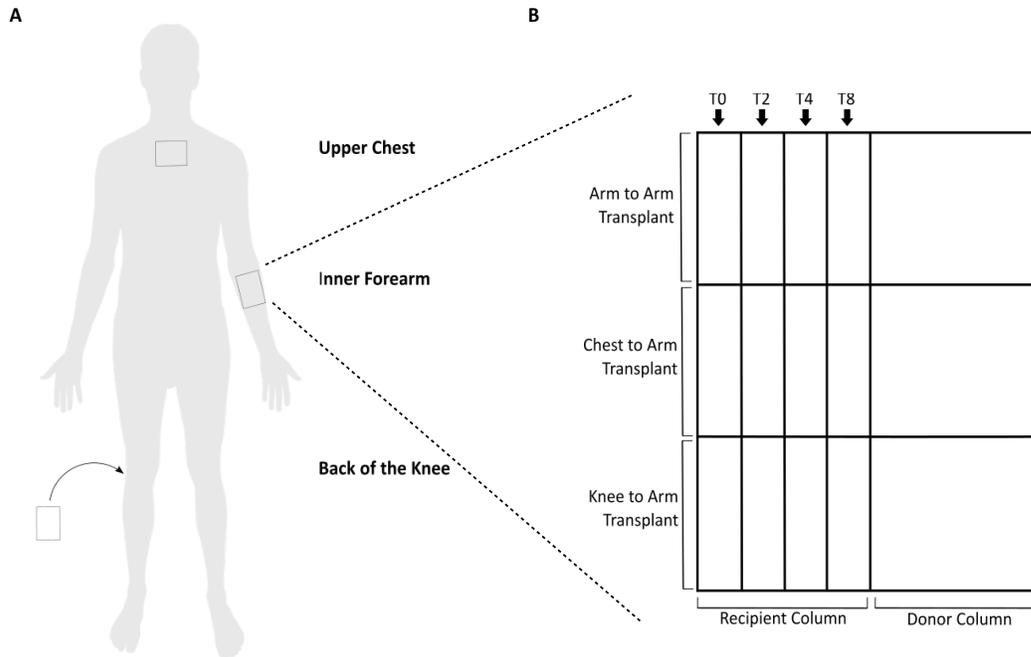


Figure 1.1: Sampling Regime for Within-Subject Microbial Transfers. Panel A shows the three skin sites on which microbial transfers were performed on each subject: the sebaceous upper chest, the dry inner forearm, and the moist back of the knee. At each skin site, a sampling grid was drawn on the subject as illustrated in an example “inner forearm” grid in Panel B. Each sampling grid at each site features two experimental microbial transfers that are inoculated with donor communities from the other two skin sites (pictured here as “chest to arm transplant” and “knee to arm transplant”), and one control transfer inoculated with a donor community from the same skin site (pictured here as “arm to arm transplant”). The Donor Column is used to provide the donor communities for inoculating two experimental transfers at the other two skin sites and the same-site control transfer. The Recipient Column is where a donor community is transferred with a sterile, nylon-flocked swab. The Recipient Column is further subdivided to enable non-destructive sampling at baseline (before transfer (0)) and at 2-, 4-, and 8-hours post-transfer. These subdivisions are randomized within the Recipient Column for each subject.

Illumina Library Preparation & 16s rRNA Gene Sequence Analysis

A total of 384 swab samples were processed and submitted for sequencing, including swab, kit controls, and negative controls to identify potential contaminants. After single-sample DNA extraction with the MoBio PowerSoil DNA Isolation Kit, DNA amplicons of the V3V4 region (319-806) of the 16S rRNA gene were prepared with two

PCR steps & custom phased primers according to the paradigm described by (Fadrosh et al., 2014), pooled and cleaned with the MinElute Gel Extraction Kit and 96 UF PCR Purification Kit, and subsequently underwent PE300 sequencing on the Illumina MiSeq platform at the Idaho/iBEST core.

8,476,527 un-demultiplexed raw sequence reads were received from the sequencing core. After trimming the custom spacer & primers with a custom script from each read (Appendix A) (forward “319” and reverse “806”) and joining the two index (barcoded) reads together the full barcode was joined to each read for quality assessment using Prinseq (Schmieder & Edwards, 2011). Based on the poor quality scores of the second read in particular, I moved forward with analysis of the first read only (region V3/Gene 319F). The forward read sequences were then quality filtered (q20; FASTX-toolkit) and demultiplexed with QIIME’s `split_libraries.py` resulting in 7,407,243 quality sequences (Caporaso et al., 2010). OTUs were picked using a custom workflow built with QIIME scripts: 1) group identical sequences with `pick_otus.py`, 2) pull representative sequences from step 1 with `pick_rep_set.py`, 3) pick OTUs against this representative set from step 2 at 97% sequence similarity threshold using `uclust` with `pick_otus.py` and Greengenes (version: `gg_13_8_otus`), 4) merge OTU maps from steps 1 and 3 with `merge_otu_maps.py`, 5) pick a final representative sequence set from the Greengenes reference sequence set using `pick_rep_set.py`, 6) assign taxonomy using Greengenes reference taxonomy using `assign_taxonomy.py`, 7) make the final OTU table using `make_otu_table.py`, and finally 8) add metadata to mapping file using the `biom` package and command `add-metadata`. The final OTU table had 4,632,390 sequences for 384 samples, with counts/sample: minimum=320, maximum=46106, mean=12063. The number of OTUs in the final OTU table = 8941. After contaminant taxa, chloroplast, archaeal, and mitochondrial sequences were removed the final OTU table had 8849 OTUs. Other QIIME scripts used include: `single_rarefaction.py` for rarefaction of the OTU table (rarefied to 1000 sequences/sample, dropping only two samples from the same subject); and `filter_otus_from_otu_table.py` to filter singleton OTUs from the OTU table and to remove contaminant taxa, chloroplast, archaeal, and mitochondrial sequences.

To identify contaminant taxa in the dataset that likely derive from a combination of sampling, reagent, and processing contamination, negative controls were sequenced

alongside the study samples. The average relative abundance of taxa in these controls were plotted against the average relative abundance of taxa in the study samples; the taxa that were significantly over-represented at relatively high abundances in the controls compared to the study samples were categorized as contaminants. This dataset had 7 such taxa identified in this way: OTU numbers 4342193 (*Caulobacteriales* genus *Phenylbacterium*); 2557604 (*Rhizobiales* genus *Devosia*); 4303249, 677165, 105470 (3 unique *Rhizobiales* genus *Methylobacterium*); 355774 (*Rhizobiales*); and 278226 (*Caulobacteriales*). Some of these have been identified in other studies as kit contaminants (Salter et al., 2014).

Results & Discussion

To understand how the skin acquires microorganisms from a variety of contexts, I performed a reciprocal transfer experiment using intact skin communities on human subject volunteers. 9 transfers were performed for every subject: dry-to-moist, dry-to-sebaceous, moist-to-dry, moist-to-sebaceous, sebaceous-to-dry, and sebaceous-to-moist, in addition to three same-site control transfers (moist-to-moist, dry-to-dry, and sebaceous-to-sebaceous). Non-destructive swab samples (samples taken from different places in the designated sampling area) of the recipient skin site were taken at 2-, 4-, and 8-hours post-transfer.

The Bray-Curtis similarity (1- Bray-Curtis Dissimilarity, also known as the Sorensen-Dice Index) was calculated between the recipient community at each time point sampled and the donor community transferred there. A generalized linear mixed model that uses a beta-distribution as the error term and a logit link function was fitted to this dataset because a) we were unable to obtain very well-behaved residuals, b) we wanted to better account for repeated measurements in the same subject over time, and c) the proportional data being fitted is naturally bounded from 0 to 1 (Appendix B). The purpose of fitting a model in this way was to estimate significant effects of time for my two hypotheses, among the six experimental transfer types (Figure 1.2). In this way, one can quantify whether and how much the recipient community shifts toward a state that more resembles the donor community after the transfer than before the transfer, and

whether this transfer persists across the 2-, 4-, and 8-hour sampling points. Of the six experimental transfer types, only the sebaceous-to-moist transfer showed any evidence of initial transfer followed by persistence ($\beta=1.139$, $p\text{-value}=0.0007$).

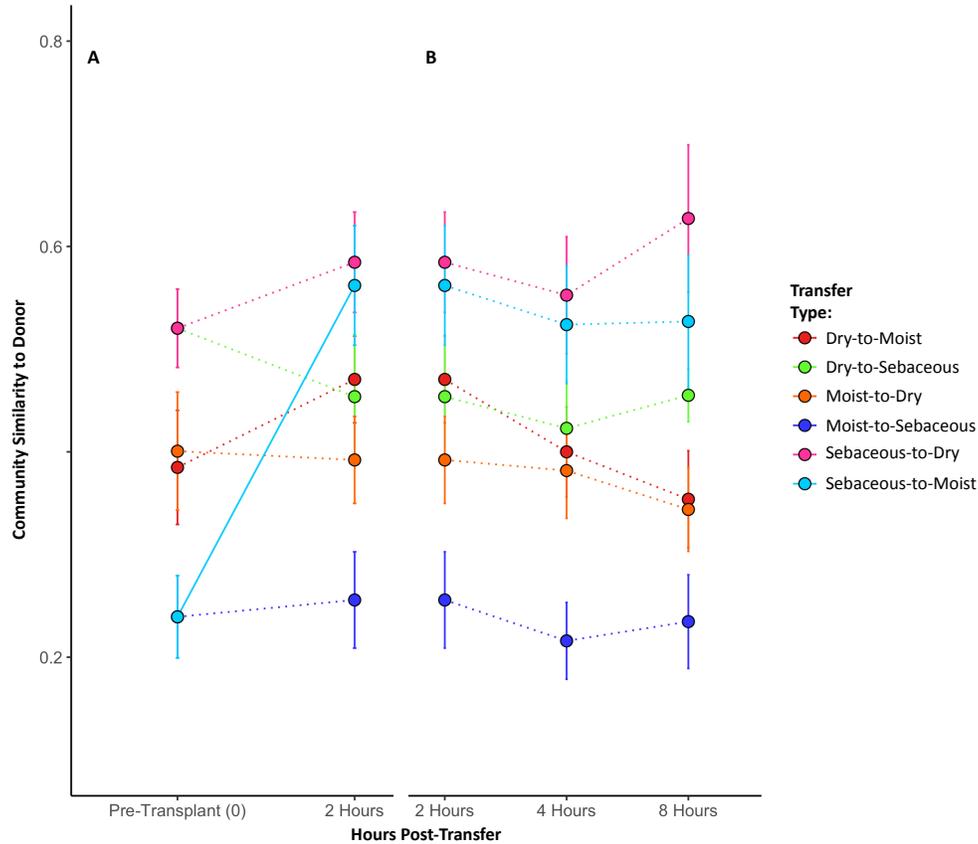


Figure 1.2: Modeling Transfer Effect and Persistence for Within-Subject Transfers. The average community similarity (1 - Bray-Curtis Similarity) of the recipient skin community (sebaceous, dry, or moist) to the skin donor community (sebaceous, dry, or moist) across the four time points sampled for 10 subjects who underwent each transfer type (dry-to-sebaceous, moist-to-sebaceous, sebaceous-to-dry, sebaceous-to-moist, dry-to-moist, and moist-to-dry). Panel A depicts the change in community similarity of the recipient community to donor community from before transfer (Time 0) to 2-hours post-transfer (Time 2). Panel B depicts the change in recipient community similarity to donor community from 2- to 4- to 8-hours (Time 2, 4, 8) post-transfer. The dotted lines are non-significant estimates of change in community similarity to donor and the one solid line represents a significant, positive estimate of change in community similarity to donor from before transplant (0) to 2-hours post-transfer ($\beta=1.139$; $p\text{-value}=0.0007$).

Given the significant effect of time for only one of the six transfer types, we wanted to take a closer look at the compositional changes of the bacterial community post-transfer for this transfer type (sebaceous-to-moist). The 10 subjects' sebaceous-to-moist transfers are shown in Appendix C. A representative selection (T & P) of subjects is shown in Figure 1.3.

The 10 subjects can be broadly divided into 2 groups: those with uneven sebaceous donor communities dominated by *Propionibacteriaceae* (8 of 10 subjects), and those with relatively diverse sebaceous donor communities inhabited by a larger proportion of rare species (<1%) and dominated by other taxonomic families, including *Staphylococcaeae*, *Streptococcaceae*, and to a lesser extent *Corynebacteriaceae*, *Micrococcaceae*, and *Tissierellaceae*. (2 of 10 subjects). In the first subject group dominated by *Propionibacteriaceae*, only one subject out of eight (Subject Q) demonstrated no evidence of community compositional shift. The remaining two subjects, O and P, showed varying degrees of compositional shift, with Subject P demonstrating the most community compositional shift of the two. Interestingly, in the case of Subject Q the moist recipient community was dominated by *Moraxellaceae* and *Micrococcaceae*, two taxonomic families either not present or at much lower relative abundances in the moist recipient communities of the other 9 subjects.

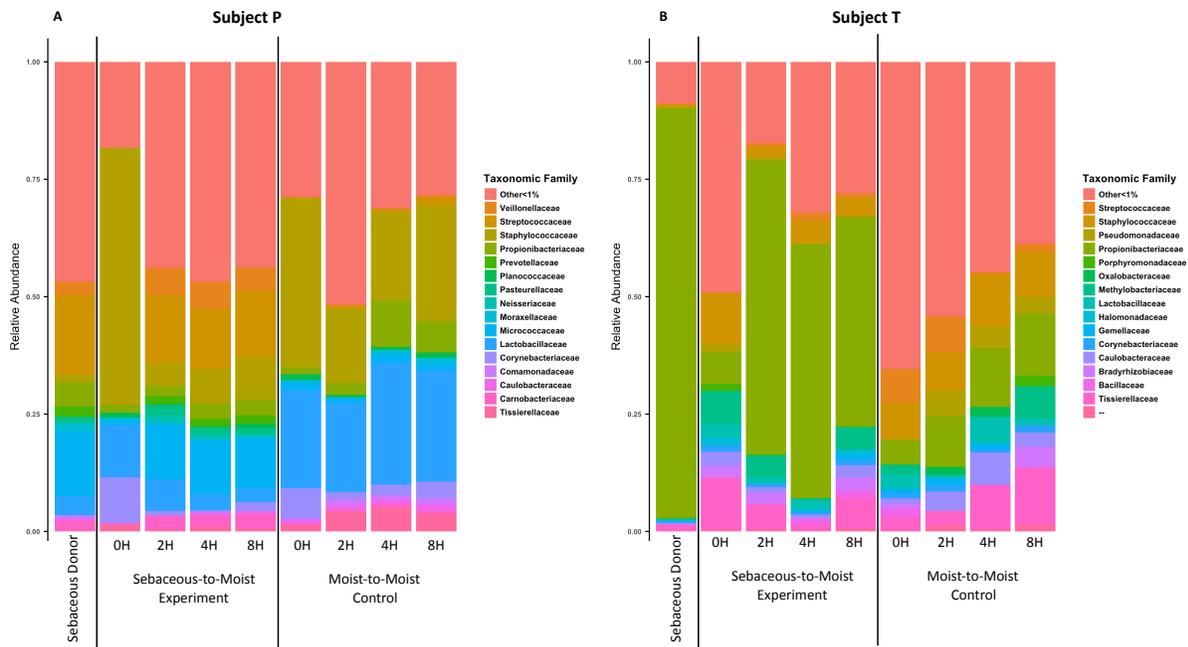


Figure 1.3: Sebaceous-to-Moist Transfers Within-Subjects: Subjects P and T. Panels A & B show two subjects, P & T, that are representative of compositional shifts observed across the 10 subjects’ sebaceous-to-moist transfer and the moist-to-moist control counterpart. The far left column in each panel represents a sebaceous donor community from the same subject at the baseline time point. The second through fifth columns in each panel represent the sebaceous to moist transplant at baseline (0), 2, 4, and 8 hours post-transplant. The last four columns in each panel represent the moist to moist control transplant at baseline (0), 2, 4, and 8 hours post-transplant. The taxa identified here are aggregated at the family level, and filtered to 1% abundance and present in at least 2 samples. The taxonomic families that do not meet these requirements are grouped together in the “Other < 1%” category.

One family in particular demonstrates the most significant increase, across subjects, in relative abundance in the sebaceous-to-moist transfer (Figure 1.4). The relative abundance of the family *Propionibacteriaceae* in the sebaceous-to-moist transfer increases significantly (on average) between 0 and 2-hours post-transfer as compared to the control moist-to-moist transfer in which the relative abundance of taxa of the family *Propionibacteriaceae* does not change significantly between 0 and 2-hours post-transfer. Moreover, the average relative abundance of *Propionibacteriaceae* does not differ significantly between every post-transfer sampling point (2-, 4-, and 8-hours post-transfer) in the sebaceous-to-moist transplant, demonstrating the ability of this specific taxonomic family group to persist at relatively high abundances in the moist skin site community. Notably, the sebaceous donor community has a particularly high relative

abundance, on average, of *Propionibacteriaceae* across subjects, which is apparently reflected in the moist recipient skin communities of subjects who receive a transfer from a sebaceous donor community.

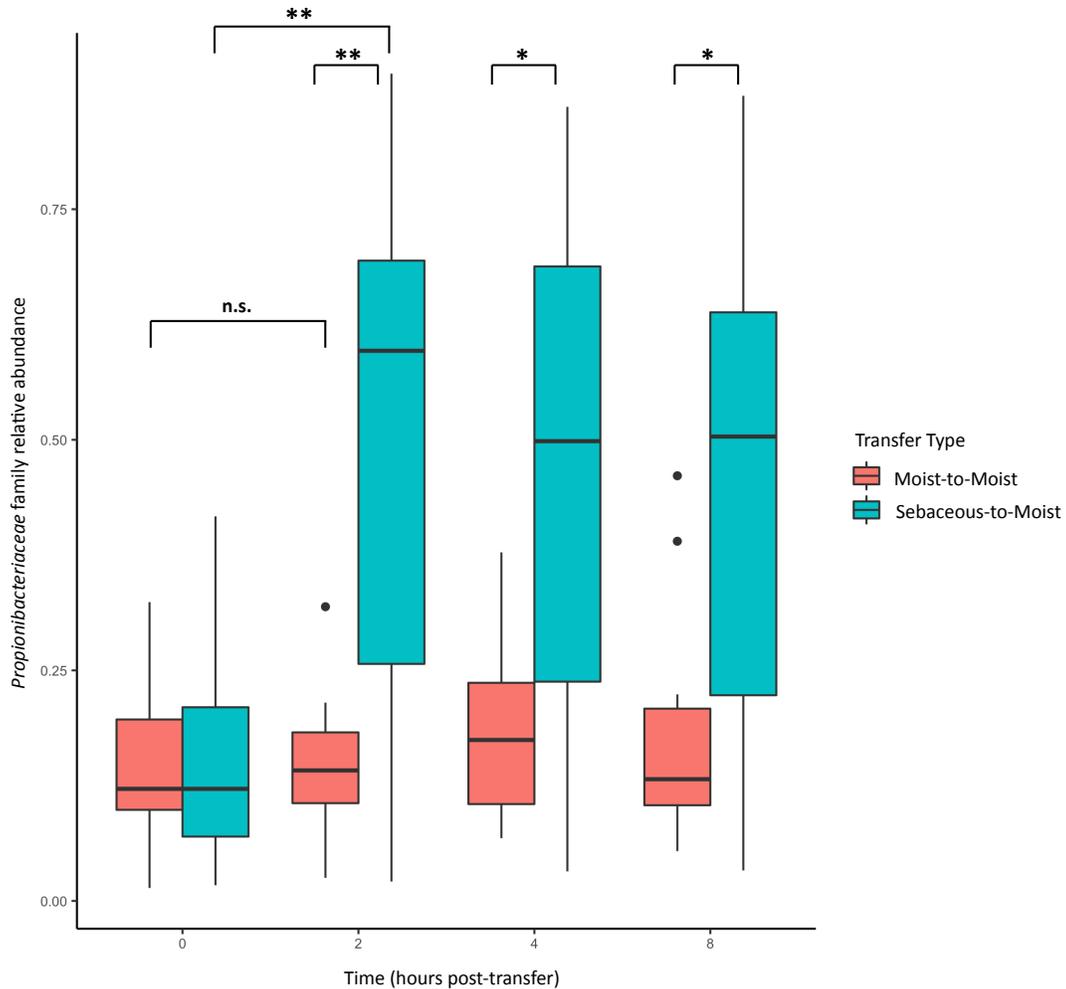


Figure 1.4: *Propionibacteriaceae* Increases Significantly After Transfer of Sebaceous Donor Community but not Moist. The mean relative abundance of *Propionibacteriaceae* family in the sebaceous-to-moist transplant and in the moist-to-moist control, before (0) & 2-, 4-, and 8-hours post-transplant was estimated (n=10). The solid lines are the medians for each sample group. The average relative abundance of *Propionibacteriaceae* family in the sebaceous-to-moist transplant (blue) increases significantly after transplant (0-2) and remains increased at 4 and 8 hours post-transplant while in the moist-to-moist control transplant (red), the average relative abundance of *Propionibacteriaceae* family does not significantly increase after transplant (0-2) and remains the same at 4 and 8 hours post-transplant. ANOVA followed by post-hoc Tukey comparison of means; adjusted p-values = 0.0067 and 1, respectively.

The bacterial composition of skin communities, including the ones sampled from sites utilized in the present study, have been well-characterized in several exploratory studies, including a recent metagenomic survey (Oh et al., 2014). The skin sites sampled herein - inner forearm (dry), back of the knee (moist), and the upper chest (sebaceous) - are indeed inhabited by the same broad bacterial phyla (mainly *Proteobacteria*, *Firmicutes*, *Actinobacteria*), as expected from previous work and illustrated in Figure 1.5. A differentiating feature of these communities is their observed level of species diversity, measured here by the Simpson's Index of Diversity (D-1) to assess both evenness and richness of the microbial community and fit by a linear model to account for subject variability as a random factor (Appendix B). Across subjects, moist sites were the most diverse (mean=0.92), while sebaceous sites were by far the least diverse (mean=0.42). Dry sites (mean=0.80) were significantly more diverse than sebaceous sites, and significantly less diverse than moist sites (t-values=17.08, 2.55, -8.625 & p-values=0, 0.0127, 0 for dry, moist, and sebaceous sites, respectively).

Inter-subject variability in regards to microbial composition at a specific site is significant, and likely contributed to dampening of a significant signal of time in the fitted GLM. When the data is fitted with NMDS and analyzed within an ANOVA framework, we observe that 16% of the variation in microbial community composition is explained by skin site, 27% is explained by subject membership, and together they explain 23% of the variation observed in the overall bacterial composition of the three skin sites pre-transfer (Figure 1.5). This result is in contrast to some studies that have shown greater similarity between the same site across individuals, rather than within a single individual across sites (Costello et al., 2009). Other studies, however, have shown that variation in skin bacterial communities are driven by both local biogeography (skin site) and strong individuality (subject specificity) (Oh et al., 2014). The results presented in this chapter support the latter hypothesis that bacterial composition on the skin can be driven strongly by subject specificity, in addition to an effect of skin environment. It may be less surprising, then, that the within-subject transfers of skin microbiota described in this chapter show a significant, overall effect of transfer on the recipient community's

composition and the relative abundance of specific taxonomic members in that community, and these effects persist for up to 8-hours after transfer.

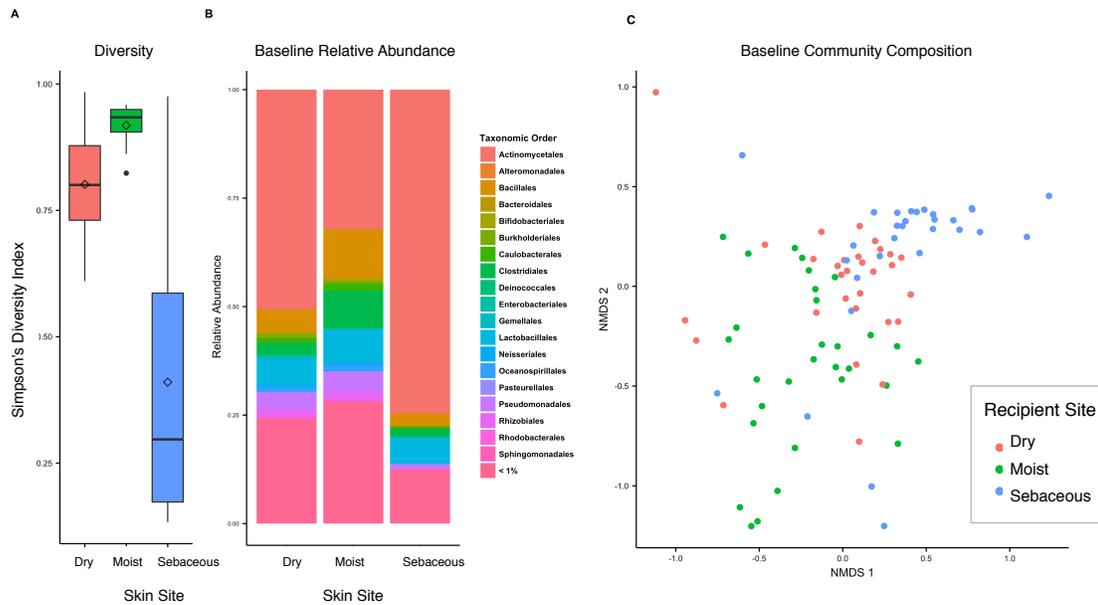


Figure 1.5: Baseline Community Diversity and Composition for Skin Sites. Each panel illustrates an aspect of observed bacterial community composition at the three skin sites before any transfer has taken place (baseline (T₀)). A) As measured by the Simpson's Index of Diversity (1-D), the mean alpha-diversities (diamond) were estimated for each site (sebaceous=0.416, moist=0.918, dry=0.804) and are significantly different between each skin site pair (sebaceous-dry: estimate=-0.39 & p<0.001; sebaceous-moist: estimate=-0.50 & p<0.001; and moist-dry: estimate=0.114 & p=0.029). The median alpha-diversity is shown as the black horizontal bar. B) The mean relative abundance of bacteria in skin samples at each skin site (dry, moist, and sebaceous) across 10 subjects' triplicate baseline samples (n=30). Only taxa present at more than 1% in at least 2 samples are identified; all other taxonomic orders present at a lower abundance and frequency are grouped together in the <1% category. C) Ordination of a NMDS applied to the Bray-Curtis distances between every triplicate skin sample from every subject K-T (n=30). Skin site contributes significantly (16%) to the variation observed in skin bacterial communities ($F_{(2,89)}=15.224$; $R^2=0.164$; $p=0.001$) when accounting for subject as the random factor.

Even after disinfection of the skin recipient site prior to transfer, the resulting effect of sebaceous transfer to a dry recipient site as in (Costello et al., 2009) appears to be qualitatively similar to the effect of a sebaceous donor transfer to a moist skin site. The results in this chapter, however, suggest that the sebaceous donor community transfers less effectively to the dry recipient skin community than it does to a moist

recipient skin community. In the case of the sebaceous-to-moist transfer, the significant change in community similarity appears to persist, on average, over the 8-hour sampling period. Given this observation, there must be forces that consistently act beyond the time scales measured here to re-configure the community composition of the skin to more “moist-like” community membership and structure. These factors likely include the selective conditions of the skin itself, as well as additional perturbations to the skin surface and bacterial community. Learning how long this “reversion” period takes, how and by whom the site is re-colonized, and what other factors may affect this process will be important future directions for this research. Additionally, it is possible that dispersal events between sebaceous donor communities and dry and moist recipient communities are insufficient in magnitude and/or in frequency to significantly transfer to the recipient communities in a noticeable fashion. Indeed, a combination of dispersal limitation or semi-limitation and selective forces that operate on a timescale longer than eight hours may be working in concert to maintain the characteristic composition of dry and moist skin microbial communities currently documented in the skin microbiome literature.

CHAPTER III

BETWEEN-SUBJECT MICROBIAL TRANSFERS

Introduction

With this second chapter, I expanded the set of donor microbial communities to include those from other host individuals instead of only from the same individual. Skin community transfers were performed both within and across host subject volunteers. The study by (Costello et al., 2009) also performed transfers between subjects, but did not report significant differences between the effects of transfer from their “within-subject” transfer set and their “between-subject” transfer set. Because the aforementioned study disinfected the recipient host skin before transferring within- or between-hosts, I hypothesize that we may observe significant differences between the effects of transfer in the “within-subject” transfer set and “between-subject” transfer set discussed in this chapter as a result of using intact, skin recipient sites. As discussed in the previous chapter, the bacteria already members of the intact skin microbiome at the recipient site are predicted to have significant effects on the colonization success of potential microbial invaders via both inter- and intra-specific interactions (Carriage et al., 2013; Christensen et al., 2016; Fitz-Gibbon et al., 2013; Iwase et al., 2010; Libberton et al., 2014; Rosenthal et al., 2011; van Rensburg et al., 2015). In addition, specific bacterial taxa (or even strains of those taxa) may transfer more readily across skin sites within a single subject than within skin sites but across subjects. As previously mentioned, a study by (Oh et al., 2014) found that certain bacterial strains (i.e. *Propionibacterium acnes*) were significantly more host-specific, varying significantly among individual hosts, while others were more site-specific (i.e. *Staphylococcus epidermidis*) and vary across body sites irrespective of individual host).

Given that we observe a significant change in relative abundance of the *Propionibacteriaceae* family (the family to which *P. acnes* belongs) after transfer of the sebaceous donor community to the moist recipient skin site I hypothesized that the effect of transfer may be weaker for the sebaceous donor community when transferred between individuals (and between sites) than across individuals and between skin sites.

Specifically, the hypotheses 1) whether an initial effect (between baseline and 2-hours post-transfer) of microbial transfer is observed in the intact, recipient skin community for both the “within-subject” transfer set and “between-subject” transfer set, and 2) whether this effect of transfer persists between 2-, 4-, and 8-hours post-transfer, will be tested for both “within-subject” and “between-subject” transfers across skin sites. Finally, I will compare the estimated effect of initial transfer of those transfer types that are identified as significant.

Materials & Methods

Experimental Design & Sample Collection

The study was performed on May 30, 2015 in a small, semi-private conference room at the University of Oregon from 07:00 to 20:30 hours. These experimental protocols were also approved under the same IRB Protocol Number: 06082013. 8 subjects (who met the same study requirements as described in the previous study) were ultimately included on the study day. In brief, these requirements necessitated that the healthy, adult human subject volunteers did not get their skin thoroughly wet (i.e. shower or bathe) or apply anything to the skin for 12 hours prior to the experiment. The same three skin sites (dry, moist, and sebaceous) were sampled for this experiment as were used in the previous chapter and similar sampling grids were drawn on each subject using ethanol-disinfected custom plastic vinyl stencils and thin-tipped permanent markers to denote equal “donor” and “recipient” areas of skin and to establish equal and distinct areas for sampling the skin before transfer and 2-, 4-, and 8-hours post-transplant (Figure 2.1). Initial baseline (T0) microbial community swab samples were obtained at each site for 8 subjects (each assigned a letter: A, B, C, D, E, F, G, H) who were each randomly assigned another person as a transfer “partner”, generating 4 pairs (AB, CD, EF, GH) for the between-subject transfer types.

After the baseline samples were collected, the microbial communities were swabbed at the designated donor area and immediately transferred within and between subjects as described in Figure 2.1. For both sample transfer and sample collection the same swabbing procedure was used as described in the previous chapter. In short, the

swab is dampened with swabbing solution and firmly rotated on the donor skin site for approximately 10-15 seconds. For transfer the swab is then applied firmly and rotated for 10-15 seconds on the recipient skin area. If the sample is for collection at the baseline (0), 2-, 4-, or 8-hour sampling time points the swab is immediately frozen at -20 C. Sterile gauze is lightly taped in place over the area and the subjects remained sedentary in a controlled, casual setting for 8-hours. As in the previous chapter, non-destructive swab samples (samples taken from separate, designated areas in the recipient skin site) are taken of each of the transfer types on each subject at the requisite time point 2-, 4-, and 8-hours post-transfer. The sterile gauze was replaced or re-taped if needed, and the location of the designated sampling area was randomized within the sampling grid to avoid spatial correlations.

11 transfers were performed per human subject volunteer in an experimental design illustrated in Figure 2.1. Essentially, reciprocal microbial transplants were performed between dry and sebaceous sites, and moist and sebaceous sites, for 4 subject pairs and the subjects individually. These four types of transfers were performed within a single subject and between subjects in a subject pair. Finally, same-site control transplants (moist-to-moist, dry-to-dry, and sebaceous-to-sebaceous) were performed between subjects in a subject pair, but not within a single subject. Dry-to-moist and moist-to-dry transfer types were also eliminated from this chapter's experiments to reduce the number of total samples and due to less likelihood of transfer and persistence based on the first chapter's results (dry-to-moist and moist-to-dry transfers showed no discernable effect of initial transfer or persistence).

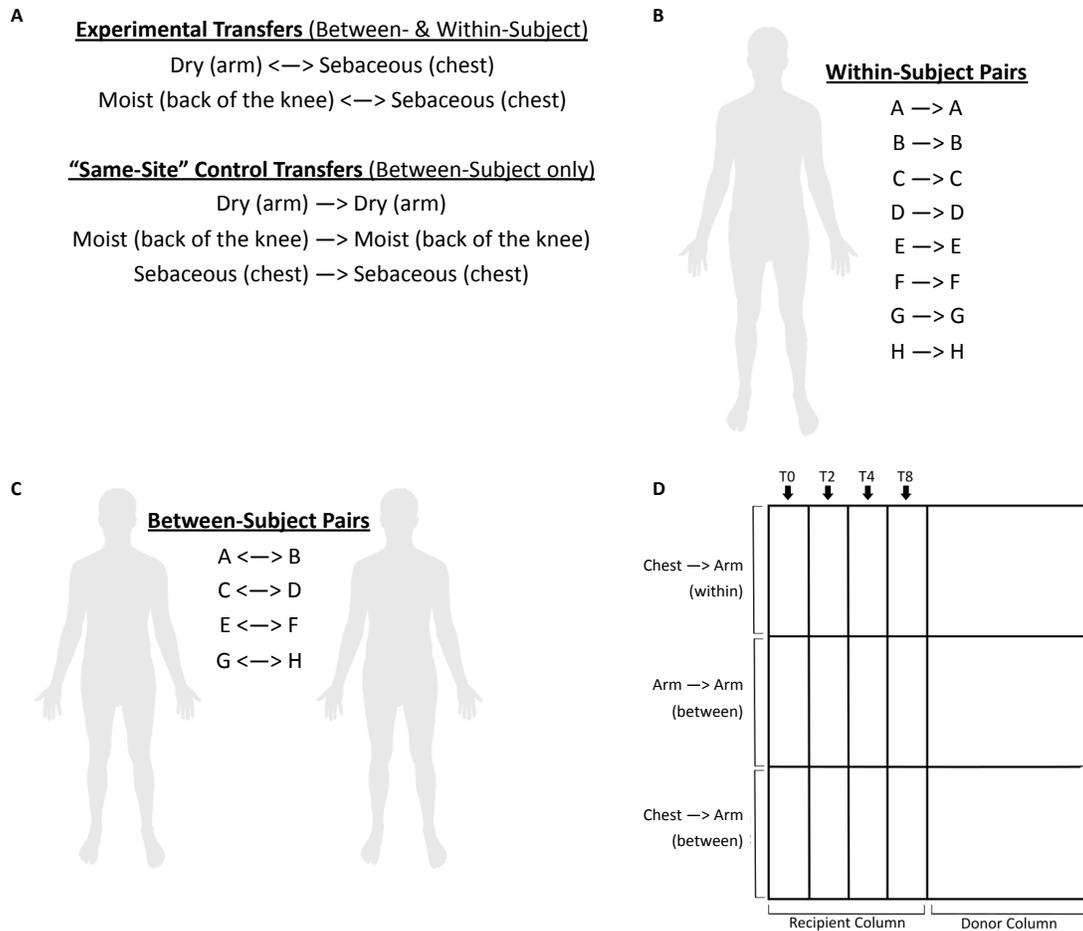


Figure 2.1: Sampling Regime for Between-Subject Microbial Transfers. Eight human subjects, de-identified as A-H, were recruited for this study portion. Panel A lists the four experimental transfer types (dry-to-sebaceous, sebaceous-to-dry, moist-to-sebaceous, sebaceous-to-moist) and the three control “same-site” transfer types (dry-to-dry, moist-to-moist, and sebaceous-to-sebaceous) performed in this part of the study. The experimental transfers are performed both within a single subject and reciprocally between subjects in a pair. In contrast, the control transfers are only performed between subjects in pair. Panel B lists the within-subject pairs and Panel C lists the between-subject pairs used in the transfer experiments. Panel D illustrates an example sampling grid at the dry arm site, where both “within-subject” and “between-subject” transfers are taking place from the chest to the arm, and a third transfer is donated from another subject’s arm to the recipient arm site. Swab samples are collected at T0 (baseline), T2, T4, and T8 (2-, 4-, and 8-hours post-transfer, respectively).

Illumina Library Preparation & 16S rRNA Gene Sequence Analysis

A total of 398 swab samples were processed and submitted for sequencing, including negative controls (swab, kit, and PCR) to identify possible sources of

contamination during library preparation. After single-sample DNA extraction with the MoBio PowerSoil DNA Isolation Kit amplicons of the V3V4 region (319-806) of the 16S rRNA gene were prepared in triplicate 25 uL PCR reactions with one PCR step using dual-barcoded primers constructed slightly differently than what was used in the first Chapter (Appendix A), cleaned with Ampure beads, quantified and pooled in equimolar volumes for subsequent paired-end 250bp next-generation sequencing on the Illumina MiSeq platform at the OSU sequencing facility.

Demultiplexed raw reads were received from the sequencing center. DADA2 was employed according to [<http://benjjneb.github.io/dada2/tutorial.html>] with non-default filtering parameters (trimLeft=10, truncLen=240, maxEE=2, trunQ=2, maxN=0) on the forward read only due to the poor quality of the reverse read (Callahan et al., 2016). The output is a filtered RSV (real sequence variants) table (counts) akin to an OTU (operational taxonomic unit) table with an average of 17121.27 reads/sample and a sister taxonomic table with 6191 unique taxa assigned with the RDP (Ribosomal Database Project) classifier (Wang, Garrity, Tiedje, & Cole, 2007).

Contaminant taxa were manually removed as described in the first chapter. The eight taxa removed are specific RSVs of bacteria all of phylum *Proteobacteria*: four are of the class *Alphaproteobacteria*, order *Rhizobiales*, family *Methylobacteriaceae*, genus *Methylobacterium*; one of class *Alphaproteobacteria*, order *Caulobacterales*, family *Caulobacteraceae*, genus *Brevundimonas*; one of class *Betaproteobacteria*, order *Burkholderiales*, family *Comamonadaceae*, genus *Delftia*; one of class *Betaproteobacteria*, order *Burkholderiales*, family *Alcaligenaceae*, genus *Achromobacter*; and one of class *Gammaproteobacteria*, order *Xanthomonadales*, family *Xanthomonadaceae*.

Results & Discussion

For each transfer type, the Bray-Curtis similarity (1- Bray Curtis dissimilarity, also known as the Sorensen-Dice Index) was calculated between the recipient skin community at each time point sampled and the donor skin community type used in that transfer. A generalized linear mixed model (Appendix B) that uses a beta-distribution as

the error term and a logit link function was fitted to estimate the effect of microbial transfer over time for each of the four experimental transfer types (Figure 2.2). With this model we can quantify whether and how much the recipient community shifts toward a state that more resembles the donor community after the transfer than before the transfer, and whether this transfer persists across the 2-, 4-, and 8-hour sampling points. In addition, we can compare the estimates of coefficients for the four transfers between “within-subject” and “between-subject” transfer sets to evaluate whether a given donor is more or less effective if transferred (between skin sites) within or across host individuals.

Transfers of the sebaceous donor community (sebaceous-to-moist and sebaceous-to-dry) have positive and significant estimates of transfer effect between 0 (baseline) and 2-hours post-transfer. Initial transfer effects were estimated for the sebaceous-to-moist transfer in both “within-subject” and “between-subject” transfer sets ((within: $\beta=0.94$, p-value=0.019) & (between: $\beta=1.15$, p-value=0.026)) while an initial transfer effect was estimated for the sebaceous-to-dry transfer in only the “between-subject” transfer set ((between: $\beta=0.986$, p-value=0.049)). No significant changes in community similarity were estimated at the 2-, 4-, and 8-hours post-transfer sampling points. Neither the dry-to-sebaceous or moist-to-sebaceous transfers have any significantly positive (or negative) time coefficient estimates from baseline (0) to 2-hours post-transfer, or across the 2-, 4-, and 8-hours post-transfer sampling points, and thus do not show any evidence that they experience a significant community shift in similarity to the donor community (Appendix B).

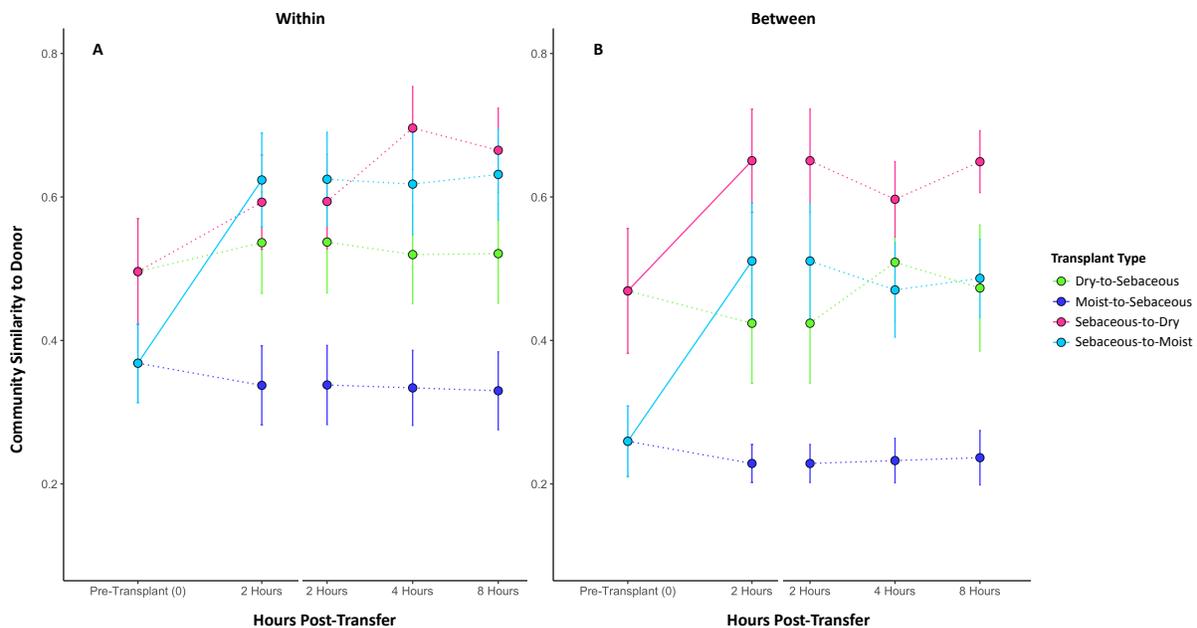


Figure 2.2: Modeling Transfer Effect and Persistence for Between-Subject Transfers. The average community similarity (1 - Bray-Curtis Similarity) of the recipient skin community (sebaceous, dry, or moist) to the skin donor community (sebaceous, dry, or moist) across the four time points sampled for 8 subjects who underwent each transplant type (dry-to-sebaceous, moist-to-sebaceous, sebaceous-to-dry, and sebaceous-to-moist) and participated in the “within-subject” transplants and also paired up in 4 pairs for the “between-subject” transplants. Panel A depicts the change in community similarity of the recipient community to donor community in the “within-subject” transplant set, from before transplant (Time 0) to 2-hours post-transplant (Time 2) on the left and from 2- to 4- to 8-hours (Time 2, 4, 8) post-transplant on the right. Panel B depicts the change in recipient community similarity to donor community in the “between-subject” transplant set, from before transplant (Time 0) to 2-hours post-transplant (Time 2) on the left and from 2- to 4- to 8-hours (Time 2, 4, 8) post-transplant on the right. The dotted lines are non-significant estimates of change in community similarity to donor and the solid lines represent significant, positive estimates of change in community similarity to donor.

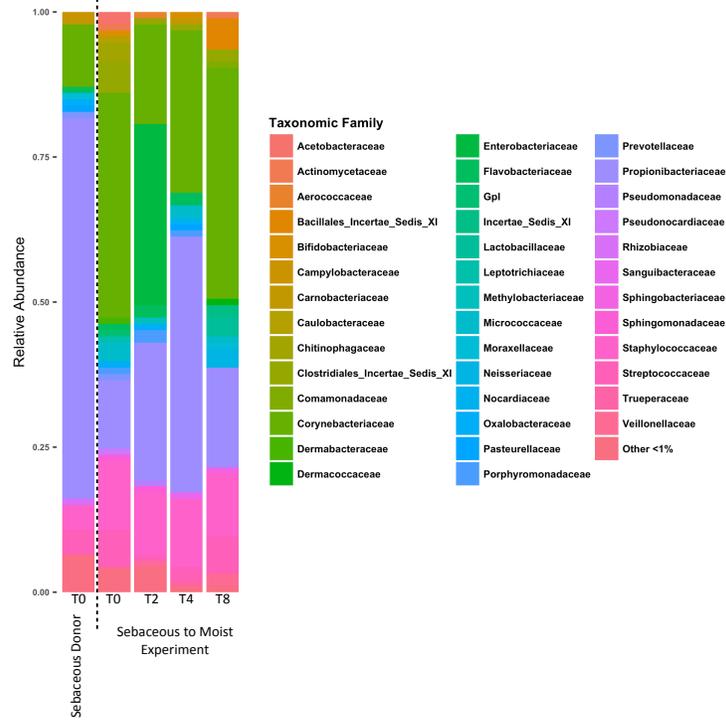
Because the sebaceous-to-moist transfers were the only transfers identified with significant estimates of change in similarity toward the sebaceous donor community, these have the most directly comparable model estimates across the two transfer sets, “within-subject” and “between-subject”. The “between-subject” sebaceous-to-moist transfer has a somewhat larger estimate for initial transfer effect (baseline (0) to 2-hours post-transfer) than the “within-subject” sebaceous-to-moist transfer (1.15 compared to 0.94, respectively). Although not as directly comparable, the sebaceous-to-dry transfer

shares a sebaceous donor in common with the sebaceous-to-moist transfer. The estimates for initial transfer effect (baseline (0) to 2-hours post-transfer) of the sebaceous-to-dry transfer and the sebaceous-to-moist transfer in the “between-subject” transfer set show a somewhat greater effect for the sebaceous-to-moist transfer than the sebaceous-to-dry transfer (1.15 compared to 0.98). The sebaceous-to-dry transfer did not show a significant effect of initial transfer in the “within-subject” transfer set; however, it does display a positive trend.

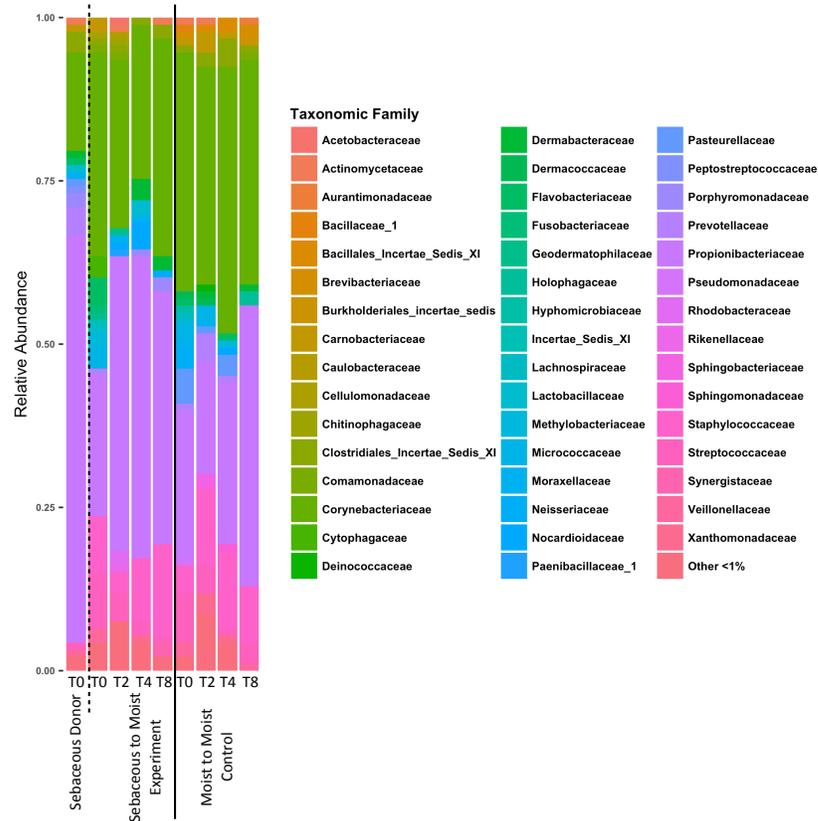
The compositional change over time in the sebaceous to moist transfers for both “within-subject” and “between-subject” transfer sets for all eight subjects are shown in Appendix E. As an example, this information for Subject A is plotted in Figure 2.3. The most notable pattern across the eight subjects and across the “within-subject” and “between-subject” transfer sets is the apparent increase in the bacterial family *Propionibacteriaceae* following transfer of communities primarily dominated by this taxonomic group and the persistence of this transfer effect across the time points sampled.

Figure 2.3: Sebaceous-to-Moist Transfers Between-Subjects: Subject A. Panels A & B represent two transfer “sets” undergone by Subject A that are representative of compositional shifts observed across the 8 subjects’ sebaceous-to-moist transfers both within-host (Panel A) and between-hosts (Panel B). The far left column in each panel represents a sebaceous donor community from either the same subject or a different subject, sampled at the baseline sampling point (T0). The second through fifth columns in each panel (within-subject and between-subject) represent the sebaceous-to-moist transfer at baseline (T0), 2-, 4-, and 8-hours post-transfers. The last four columns in each panel (within-subject and between-subject) represent the moist-to-moist control transfer at baseline (T0), 2-, 4-, and 8-hours post-transfer. The taxa identified here are aggregated at the family level and filtered to 1% abundance and present in at least two samples shown here. The taxonomic families that do not meet these requirements are grouped together in the “Other <1% category”.

A
Subject A



B
Subject Pair AB



The family *Propionibacteriaceae* does indeed significantly increase post-transfer in the sebaceous-to-moist transfer in both “within-subject” and “between-subject” transfer sets (Figure 2.4).

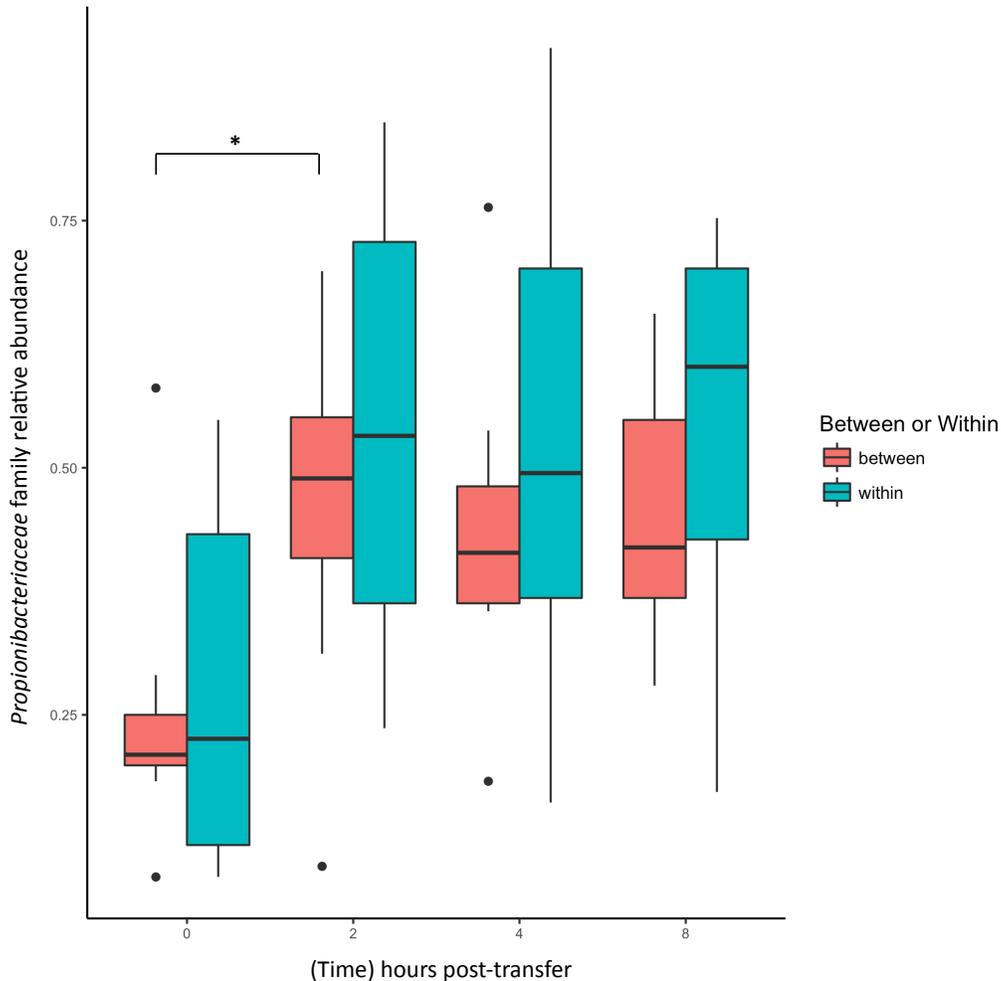


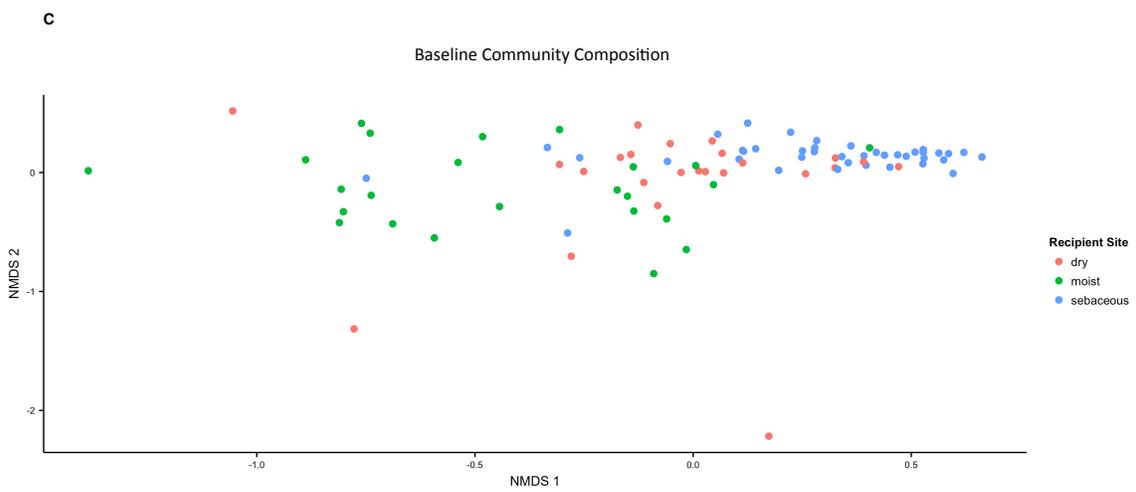
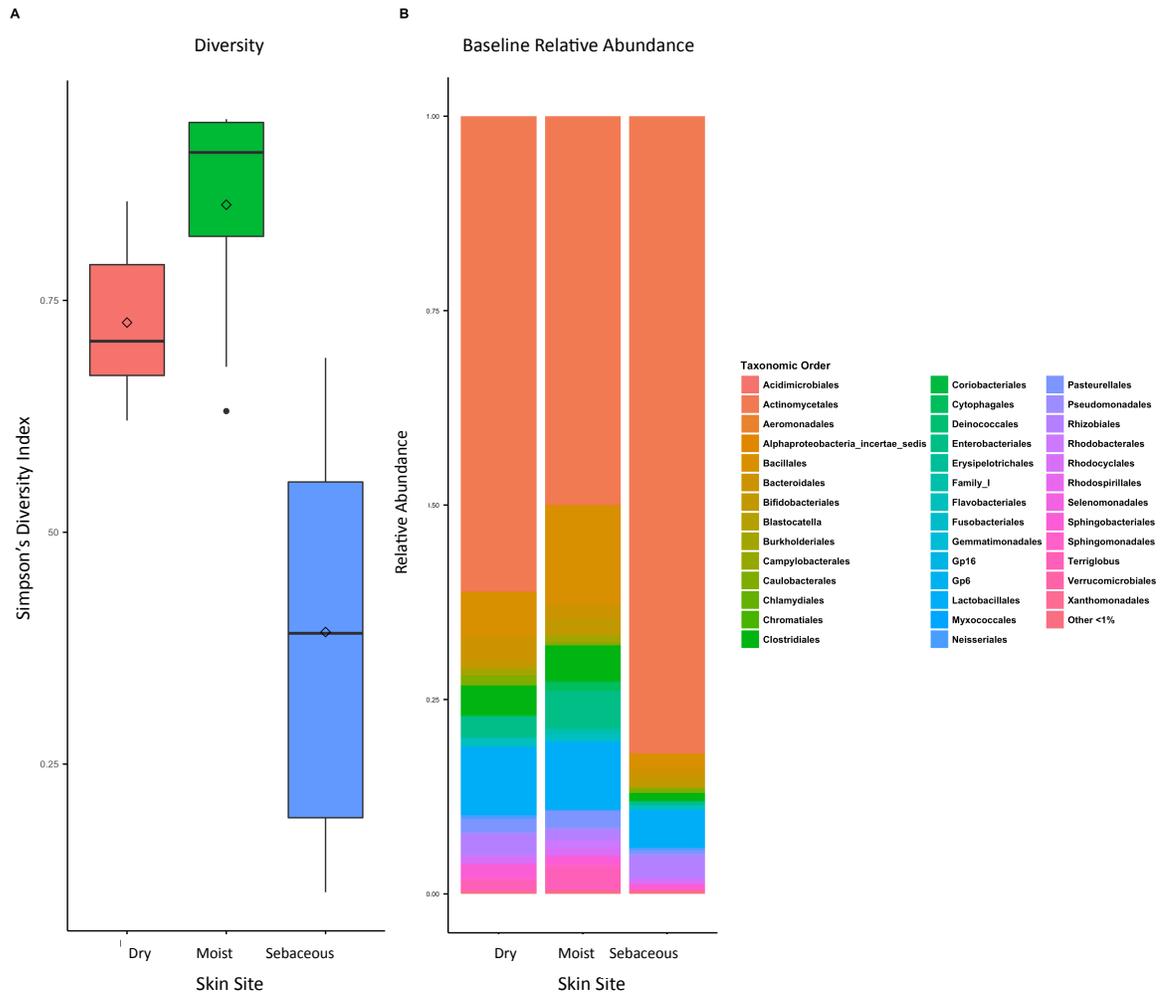
Figure 2.4: *Propionibacteriaceae* Increases Significantly After Transfer of Sebaceous Donor Community Both Within and Between Subjects. The mean relative abundance of taxa assigned to the *Propionibacteriaceae* family in the sebaceous-to-moist transplant, both in the transplants within hosts (colored green) and between hosts (colored red) is shown at the four time points sampled: 0 (baseline), 2-, 4-, and 8-hours post-transplant. The mean relative abundance of *Propionibacteriaceae* is significantly different from baseline (0) to post-transfer at 2-hours post-transfer overall (adjusted p-value = 0.0046). The mean relative abundance of *Propionibacteriaceae* is not significantly different between the “between-subject” and “within-subject” transfer sets (adjusted p-value<0.16). (ANOVA followed by post-hoc Tukey’s contrasts and multiple comparison correction).

While the relative abundance of taxa belonging to the family *Propionibacteriaceae* is significantly different between baseline (0) and 2-hours post-transfer for both “within-subject” and “between-subject” sebaceous-to-moist transfer sets (ANOVA followed by post-hoc Tukey comparison of means: adjusted p-value=0.0046), the relative abundance of *Propionibacteriaceae* does not significantly differ between “within-subject” and “between-subject” sebaceous-to-moist transfer sets (adjusted p-value=0.165), although the 95% confidence intervals around the medians of the relative abundance of *Propionibacteriaceae* appear substantially larger in the “within-subject” set compared to the “between-subject” set, and the median values also appear somewhat greater throughout (Figure 2.4). In addition, *Propionibacteriaceae* appear to persist in the recipient moist community without significant change in relative abundance at 2-, 4-, and 8-hour post-transfer sampling points (adjusted p-values = 2-hours-4-hours: 0.999; 4-hours-8-hours: 0.999).

The consistency of inter-subject and inter-site skin microbial community variability between the baseline sites sampled in the previous chapter and in this chapter (total subjects: n = 18) is remarkable. Again, across subjects, moist sites were on average the most diverse (estimated mean = 0.84) as measured by the Simpson’s Index of Diversity (D-1) and fit by a linear model to account for subject variability as a random factor (Appendix D). Sebaceous sites were by far the least diverse on average (estimated mean=0.39) and dry sites were again on average more similar in diversity to the moist sites (estimated mean=0.72).

When the data is fitted with NMDS and analyzed with an ANOVA framework we observe that 19% of the variation in community similarity is explained by skin site, 29% is explained by subject membership, and together their interaction explains 19% of the variation observed in the overall bacterial composition of the three skin sites pre-transfer (Figure 2.5). Again, this result is strikingly consistent to that found in the previous chapter, and is in contrast to studies that find more variation in community similarity explained by site rather than subject (Costello et al., 2009). Collectively, my results support the hypothesis that bacterial composition on the skin can be driven strongly by host-specificity, in addition to an effect of skin environment. This could either indicate host-specific environmental conditions that permit the growth of taxa not found in other subjects, or unique microbial exposures from which the host acquires microbiota not found in other subjects.

Figure 2.5: Baseline Community Diversity and Composition for Skin Sites Recapitulated. Each panel A-C illustrates aspects of observed bacterial community composition at the three skin sites before any transplantation had taken place (baseline). A) As measured by the Simpson's Index of Diversity (1-D), alpha-diversity was significantly different at each skin site (dry, moist, and sebaceous). B) The relative abundance of bacteria in baseline skin samples averaged across 8 subjects' replicate baseline samples. Only taxa present at more than 1% and in at least two samples were identified; all other taxonomic orders present at a lower abundance and frequency are grouped together in the <1% category. C) Ordination of a NMDS applied to the Bray-Curtis distances between every baseline skin sample from every subject A-H, colored here by skin site. Skin site contributes significantly (18%) to the variation observed in skin bacterial communities ($F_{(2,87)}=17.4092$; $R^2=0.1856$; $p=0.001$) when accounting for subject as the random factor.



No significant difference in *Propionibacteriaceae* relative abundance was found between the post-transfer samples in the “within-subject” transfer set compared to the “between-subject” transfer set. This result is in contrast to my hypothesis that “between-subject” transfers of *Propionibacteriaceae*-dominated sebaceous skin communities would not result in a significant transfer effect or would result in a decreased transfer effect because of *Propionibacteriaceae*'s reported subject-specificity as opposed to site-specificity. It is possible that if any biological difference between a “within-subject” transfer and a “between-subject” transfer of a sebaceous community exists as a result of competition between *Propionibacteriaceae* strains, it may only be observed beyond the time scales measured here.

The study in this chapter identified one transfer type, sebaceous-to-dry, that showed a significant initial effect of transfer in one transfer set, “between-subject”, and not the other “within-subject” transfer set. This is in contrast to the (Costello et al., 2009) study that reported no significant differences between “within-subject” and “between-subject” transfer types. Overall, the difference in magnitude between the initial effects of transfer among the two transfers types identified as significant (sebaceous-to-moist and sebaceous-to-dry) were not large; additional subjects and more transfers would help to better resolve the differences in the initial transfer effects estimated among transfer types with more confidence. In all cases where an initial effect of transfer is observed, (sebaceous-to-moist (within), sebaceous-to-moist (between), and sebaceous-to-dry (between)) the significant change in community similarity appears to persist, on average, over the 8-hour sampling period. Given this observation, we must again conclude that there are forces consistently acting beyond time scales measured here to re-configure the community composition of the recipient skin to its original community membership and structure. As discussed in the previous chapter, a combination of dispersal limitation and selective forces that operate on a timescale longer than eight hours may be working together to maintain the characteristic composition of dry and moist skin microbial communities currently documented in the skin microbiome literature. Nevertheless, these experiments demonstrate that commensal microbial taxa (sebaceous donor communities, in particular) can transfer and persist within and between hosts, and between skin site types.

CHAPTER IV

ENVIRONMENTAL MICROBIAL TRANSFERS

Introduction

With this final and third Chapter, I expanded the set of donor microbial communities to include those from non-human donors. Specifically, I asked if environmental microbiota (indoor houseplant leaves and farm soil) would transfer and persist on the intact, dry skin of human volunteer subjects. These donor sources were chosen for their ubiquity in the lives of human beings, their likelihood of exposure to the skin while indoors and outside, and because of their potential positive association with human health outcomes (Azad et al., 2013; Lehtimäki et al., 2017; G. A. Rook, 2013; Ruokolainen et al., 2015). Plants, in particular, have been suggested as important sources of microbial taxa to the built environment (Mahnert et al., 2015; Prussin et al., 2015). Soil, too, has been suggested as an important component of the indoor built environment, likely brought in with hosts moving in and out of the indoors (people, pets, etc). (Kembel et al., 2012; Lax et al., 2015; Meadow et al., 2014).

For the transfer experiments in this final chapter, three types of transfer experiments will be performed: soil-to-skin, leaf-to-skin, and skin-to-skin. Only the dry, inner forearm is used as the recipient skin community site, because it was the easiest site to accommodate the transplants in the experiment and was a skin site that we felt would be likely to interact with the environmental donor community sources (plants and soil) on a daily basis. Using only one skin site type limited the number of samples in the study to a tractable number for processing and sequencing, permitted the addition of extra sampling points, and allowed space for additional subjects to increase statistical power in downstream analysis. The subject group was split in half after the 8-hour sampling point for two treatments: one half immediately washed and were then subsequently sampled, and the other half did not wash but instead returned to the sampling facility 24-hours post-transfer for a final sampling point. These additional treatments after the 8-hour sampling point permitted us to better assess the persistence of the environmental transplants. Washing is a commonplace part of skin maintenance, and assessing its

impact on the transference and persistence of foreign microbial taxa is required to understand how to best cultivate or maintain particular microbial communities on the skin while also maintaining regular hygiene practices. The 24-hour sampling point, in particular, allowed sampling of the skin after the recipient skin site was free of the gauze covering and could therefore experience regular interactions with the external environment before being re-sampled.

Given the frequency (if not abundance) with which bacterial taxa associated with environmental sources are found on the skin and inside our homes, I hypothesized that both soil and leaf donor microbial communities would significantly transfer to the dry skin recipient community, and that this initial transfer effect would likely persist over the 8-hours sampled. Persistence at 24-hours is not expected; the recipient skin site is likely exposed to microbiota in the subject's home before returning to the lab for the 24-hour sampling point. Similarly, washing is expected to be a significant perturbation and remove the signature of initial transfer effect, and thus not show evidence of persistence at the "wash" sampling point.

In this final chapter, I will specifically test the following hypotheses: 1) whether an initial effect (between baseline and 2-hours post-transfer) of microbial transfer is observed in the intact, recipient (dry) skin community for the three transfer types tested, 2) whether this effect of transfer persists between 2-, 4- and 8-hours post-transfer, and c) whether this effect will persist either i) returning after 24-hours post-transfer or ii) after washing the skin site. To test these hypotheses transfer experiments were performed using a) houseplant leaves b) farm soil.

Materials & Methods

Experimental Design & Sample Collection

The study was performed on June 20th-21st, 2016; June 27th-28th, 2016; and July 13th-14th, 2016 at the ESBL (Environmental Studies in Buildings Laboratory) facility in Portland, Oregon from approximately 9:00am to 7:00pm on the study days listed. These experimental protocols were also approved under the same IRB Protocol Number: 06082013. 16 subjects (who met the same study requirements as described in the

previous two chapters) were included across the three study periods. In brief, these requirements necessitated that the healthy, adult human subject volunteers did not get their skin thoroughly wet (i.e. shower or bathe) or apply anything to the skin for 12 hours prior to the experiment. A representative dry skin site, the inner forearm, was selected for this part of the experiment given both the experimental and practical considerations discussed in the Introduction. Sampling grids similar to those used in the first two chapters' experiments were drawn on each subject using ethanol-disinfected custom plastic vinyl stencils and thin-tipped permanent markers to denote "recipient" and "donor" area(s) of skin for the three transfer types. For this experiment, five equal and distinct areas were designated for sampling the skin at five time points: before transfer (0); 2-, 4-, and 8-hours post-transfer; and a spot for either a 24-hours post-transfer or post-wash sampling time point (Figure 3.1).

Initial baseline (T0) microbial community swab samples were obtained at the inner forearm skin site for 16 subjects each assigned a number: #01-#16, and for the donor microbial communities of leaves and soil. Specifically, leaf top surface samples were obtained for three common, indoor plants: *Spathiphyllum wallisii* (Peace Lily), genus *Dieffenbachia* (Mother In-Law's Tongue), and genus *Calathea* (Prayer Plant). The plants used in this study were purchased at nurseries local to Eugene, OR. Soil samples were aliquoted from a colleague's collection of Mohawk River farm soil that was 2mm sieved and passively air-dried. This soil was chosen because it was predicted to be representative of the type of soil people would directly contact when farming.

After the baseline samples of donor and recipient communities were collected, microbial communities were immediately transferred from a donor plant leaf and from an aliquot of farm soil to the dry skin of an individual human recipient subject. Dry-to-dry control transfers were performed using a designated area of dry skin on the arm to inoculate an adjacent area of skin. For transfer of the donor leaf community with the swab we followed exactly the same protocol as for the transfer of a skin donor community, swabbing a different donor leaf for each human subject volunteer. Subjects 01-06 received a leaf donor community from *Spathiphyllum wallisii*, Subjects S07-S12 received a leaf donor community from *Dieffenbachia*, and Subjects S13-S16 received a leaf donor community from *Calathea*. For transfer of the soil donor community, the

protocol is identical except that after the swab is dampened it is briefly dipped into a ~20 mL aliquot of farm soil to collect soil particulate and microbiota. The swab is then applied firmly and rotated for 10-15 seconds on the recipient skin area to deposit the microbial community (and some of the particulate matter).

For sample collection, the same swabbing procedure and gauze covering was used as described in the previous chapters except for the additional sampling point and treatments (“wash” & 24-hour post-transfer). Specifically, half the subjects wash the recipient skin area with Dr. Bronner’s Castille soap for approximately 15-30 seconds and pat dry with sterile paper towels just after the 8-hour sampling time point, and are again immediately sampled after washing for a post-wash (TW) sampling time point. The remaining eight subjects do not wash the recipient skin area but had the gauze removed from the recipient area and returned home. They were asked not to wash or apply anything to their skin, although they could resume normal activities of daily life. These subjects were sampled the following day for a 24-hour post-transplant sampling time point. All swab samples were immediately frozen at -20 C at the research facility and were eventually transferred to the Eugene campus of the University of Oregon for further processing.

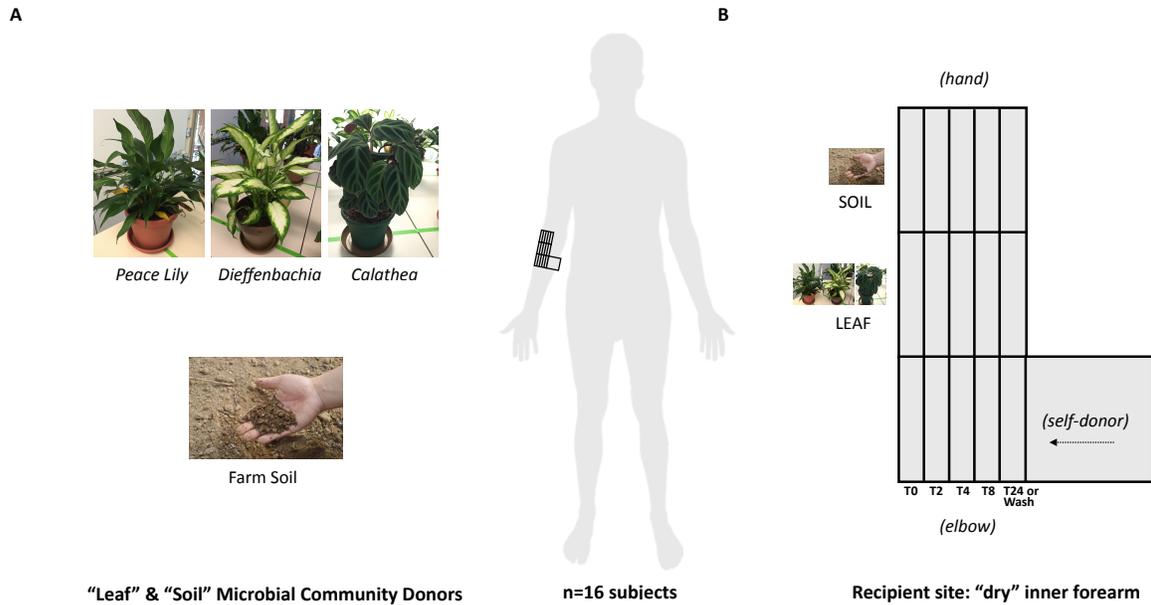


Figure 3.1: Sampling Regime for Environmental Microbial Transfers. Panel A depicts the two types of donor microbial communities used in this set of transfers: plant leaf top (the *Peace Lily* donated to subjects 1-6, the *Dieffenbachia* donated to subjects 7-12, and the *Calathea* donated to subjects 13-16) and farm soil from the Mohawk Valley, OR. Microbial communities from these donors were transferred to the dry, inner forearm of 16 human subject participants. Panel B depicts the sampling grid used for this set of transfers that was drawn onto the skin of the inner forearm. In addition to the “leaf” and “soil” transfers, a “control” transfer was performed with an adjacent skin microbial community from the self-same human subject. The recipient skin site is sampled at 5 time points: T0 (pre-transfer), and T2, T4, T8 (2-, 4-, and 8-hours post-transfer). Half of the subjects washed immediately after the 8-hour sampling and were sampled thereafter (TW), and the other half of the subjects did not wash but were sampled at 24-hours post-transfer (T24). As in the previous transfer sets, the location of each time point sampled on the recipient skin site was randomized among subjects.

Illumina Library Preparation & 16S rRNA Gene Sequence Analysis

A total of 623 swab samples were processed and submitted for sequencing across two, paired-end 250bp Illumina MiSeq runs. These two sampling libraries included not only experimental samples but also the appropriate negative controls (swab, kit, and PCR) to identify possible sources of contamination during library preparation. After single-sample DNA extraction with the MoBio PowerSoil DNA Isolation Kit, amplicons of the V3V4 region (319-806) of the 16S rRNA gene were prepared in 50 uL PCR reactions with one PCR step using dual-barcoded primers (Appendix A) cleaned with

Ampure beads, quantified and pooled in equimolar volumes for subsequent paired-end 250bp sequencing on the Illumina MiSeq platform at the OSU sequencing facility. Demultiplexed raw reads were received from the sequencing center and DADA2 was employed according to [<http://benjjneb.github.io/dada2/tutorial.html>] with custom filtering parameters (trimLeft=10, truncLen=240, maxEE=2, truncQ=2, maxN=0) run on the forward read only due to the poor quality of the reverse read (Callahan et al., 2016). The final output is a filtered RSV (real sequence variants) table (counts) akin to an OTU (operational taxonomic unit) table with an average of 22460 reads/sample and a taxonomic table with 35304 unique taxa assigned with RDP (Ribosomal Database Project) classifier (Wang et al., 2007). This number is much higher than in the first two chapters, probably due to the presence of many environmental samples that are *a priori* higher in biodiversity than skin (i.e. soil). Unlike in the first two chapters, no obvious contaminants were readily detectable using the same methods; therefore, none were removed from the dataset.

Results & Discussion

For each experimental transplant type, the Bray-Curtis similarity (1- Bray Curtis dissimilarity also known as the Sorensen-Dice Index) was calculated between the skin recipient community at each time point sampled and the donor community used in that transfer. A generalized linear mixed model (Appendix B) that uses a beta-distribution as the error term and a logit link function was fitted to estimate the effect of microbial transfer over time for each of the three experimental transfer types (Figure 3.2). With this model we can quantify whether and how much the recipient community shifts toward a state that more resembles the donor community after the transfer than before the transfer, and whether this transfer persists across the 2-, 4-, and 8-hour sampling points. In addition, we can quantify whether the transfer effect continues to persist past 8-hours at the 24-hour sampling point, and whether washing of the recipient skin area significantly reduces any effect of persistence.

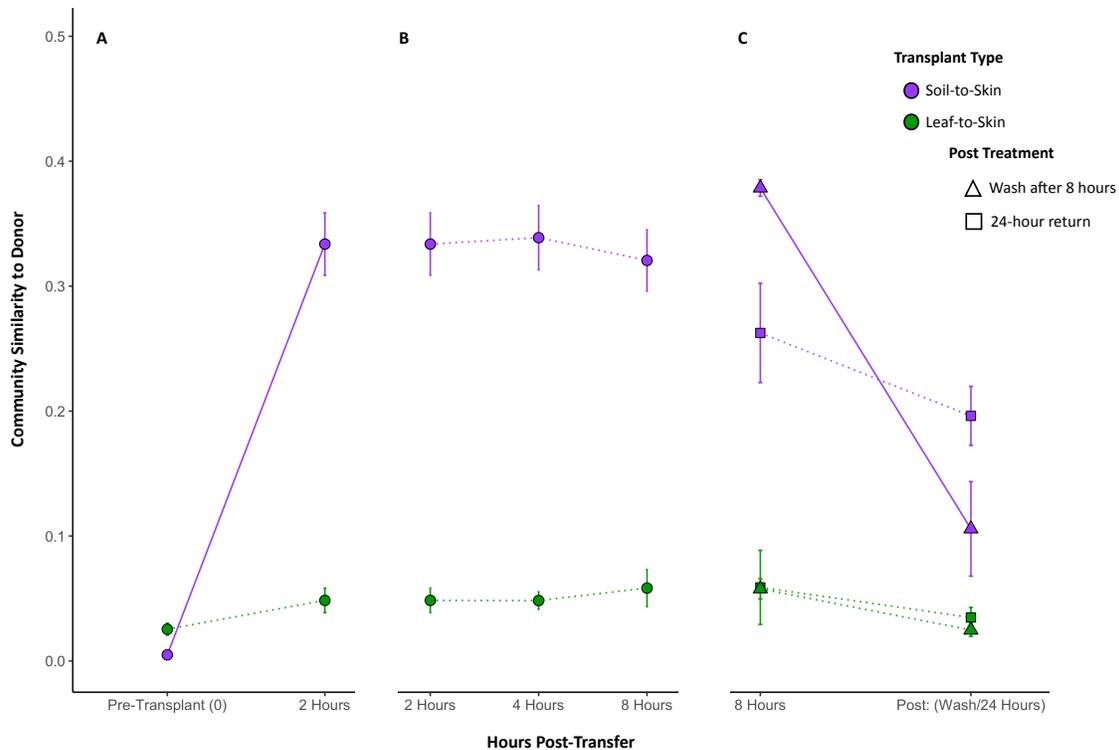


Figure 3.2: Modeling Transfer Effect and Persistence for Environmental Microbial Transfers. Panels A-C show the average community similarity (1 - Bray-Curtis Similarity) of the recipient skin community to the environmental donor community (soil or leaf) across the five time points sampled in 16 subjects that underwent each transfer type (soil-to-skin shown in purple & leaf-to-skin shown in green). 8 subjects washed immediately after the 8-hour sampling time point and were subsequently re-sampled for the post-“wash” time point while the other 8 subjects were sampled for the “post” 24-hour sampling time point. Panel A depicts the change in community similarity to donor community from before transfer (Time 0) to 2-hours post-transfer (Time 2). Panel B depicts the change in community similarity to donor community from 2- to 4- to 8-hours (Time 2, 4, 8) post-transfer. Panel C depicts the change in community similarity in two treatment groups: 1) from 8-hours post-transfer to 24-hours post-transfer and 2) from 8-hours post-transfer to the “wash” treatment immediately thereafter. The dotted lines are non-significant estimates of change in community similarity to donor and the solid lines represent significant, positive or negative estimates of change in community similarity to donor. The soil-to-skin transfer showed a very positive effect of transfer on community similarity to donor ($\beta=3.293$, $p\text{-value}=<2e^{-16}$) and a negative effect after washing ($\beta=-1.5883$, $p\text{-value}=4.12e^{-05}$).

Among the three transfer types tested, only the soil-to-skin transfer showed a significant and positive effect of initial transfer between baseline (T0) and 2-hours post-transfer ($\beta=3.29$, $p\text{-value}=<2e^{-16}$). No significant changes in community similarity were

estimated over the 2-, 4-, and 8-hour sampling points for any of the three transplant types, including the soil-to-skin transfer, indicating persistence of the initial transfer effect. The leaf-to-skin transfer type had a trending, positive estimate at 4-hours post-transfer but was not quite significant ($\beta = 0.723$, $p\text{-value} = 0.0595$). After 24-hours the recipient skin community is significantly less similar to the soil donor community than it was at 8-hours, however, washing of the recipient skin area reduces the effect of initial microbial transfer and persistence even more ($\beta = -1.5883$, $p\text{-value} = 4.12e^{-05}$).

The compositional change over time in the soil to skin transplants for all 16 subjects are shown in Appendix F. As an example, this information for Subjects 07 and 15 are plotted in Figure 3.3. The most notable pattern across all 16 subjects is the preponderance of rare taxa (Other <1%) in the donor soil community that apparently successfully transfers to the recipient skin community. In some cases (including S07 and S15), the transfer of the rare soil taxa appears to persist after 24-hours and even after washing (Figure 3.3). In other cases, the persistence of rare soil taxa is less pronounced but still visible (Appendix F).

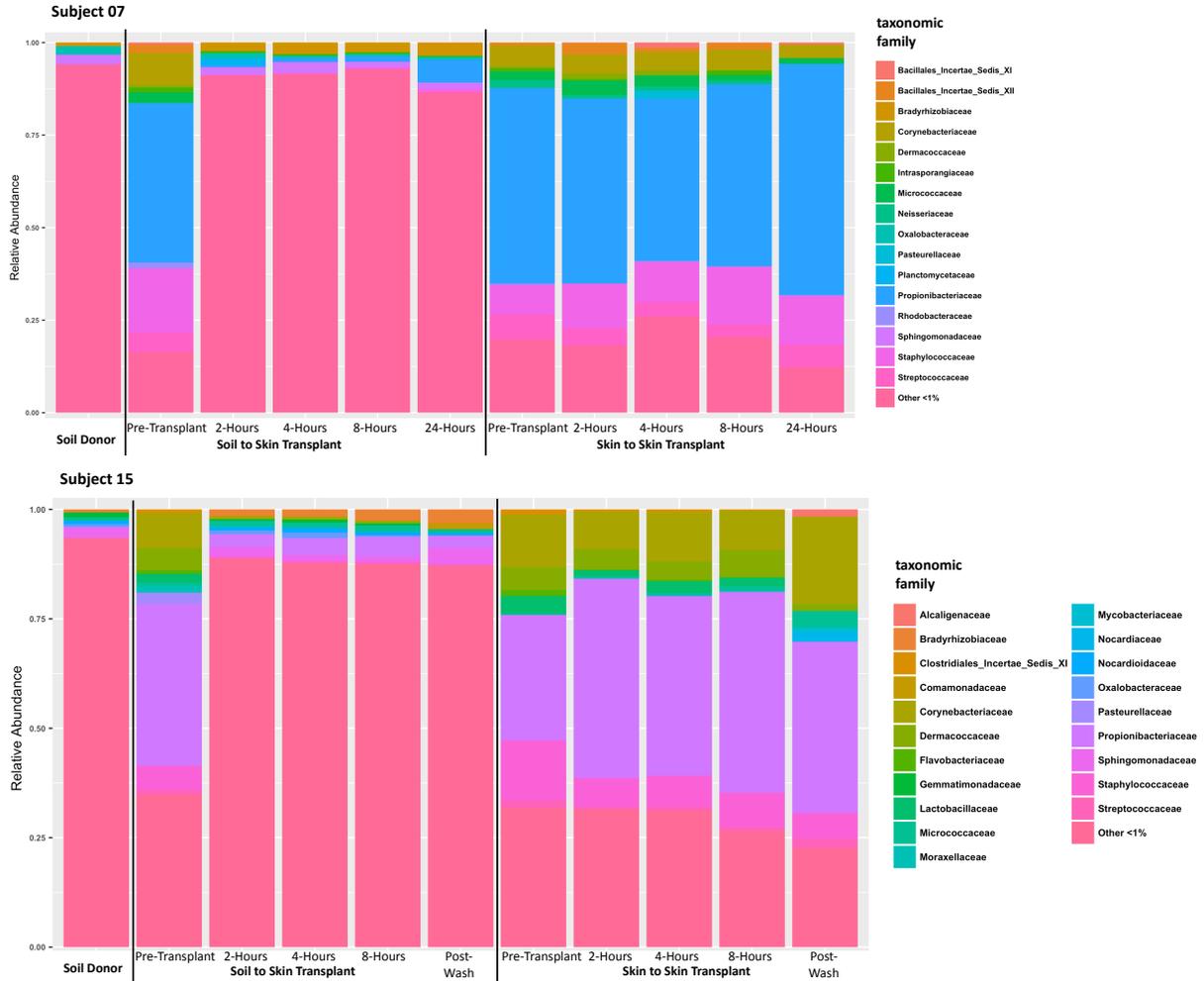


Figure 3.3: Soil-to-Skin Transfer: Subject S07 and S15. The far left column in each panel (top and bottom) represents a soil donor community sampled at the baseline time point. The second through fifth columns in each panel represent the soil-to-soil transfers at baseline, 2-, 4-, and 8-hours post-transfer. Subject 07 was sampled at 24-hours (the 6th column in the top panel) and Subject 15 was sampled after washing (the 6th column in the bottom panel) The last four columns in each panel represent the skin-to-skin control transfer at baseline, 2-, 4-, and 8-hours post-transfer, with the 6th column representing the 24-hour time point or the post-wash time point for Subject 07 and 15, respectively. The taxa identified here are aggregated at the family level and filtered to 1% abundance and present in at least two samples shown here. The taxonomic families that do not meet these requirements are grouped together in the “Other <1% category”.

In order to better resolve which taxa significantly increase in relative abundance post-transfer, especially rare taxa, we can apply DESeq analysis (Love, Huber, & Anders, 2014). DESeq tests for differential abundance in count data (in this case, sequence data)

using the negative binomial distribution and outputs the list of taxa that differ significantly in relative abundance in one sample group versus another. When pairwise comparisons are made between the skin at each post-transfer sampling point and the skin prior to soil transplant, we can order this group of enriched taxa and visualize their relative abundances using a heatmap, as is shown for the soil-to-skin transfer type in (Figure 3.4).

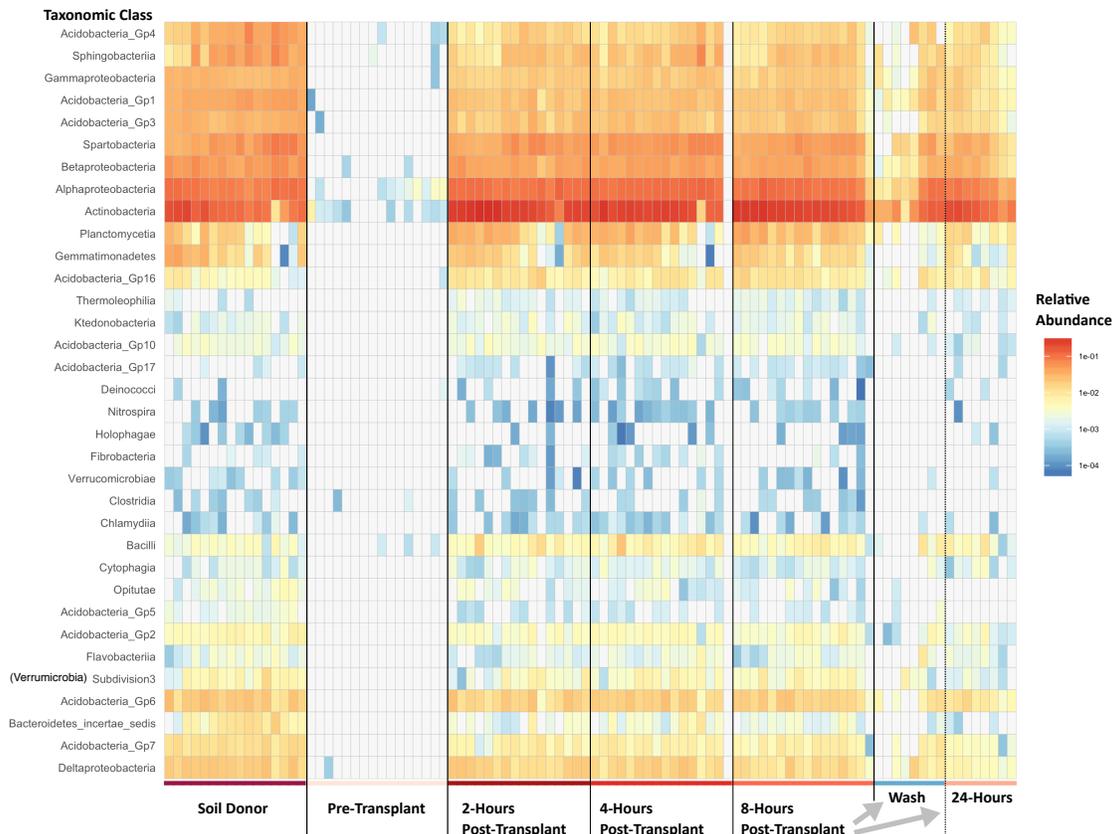


Figure 3.4: TMM-normalized relative abundance of taxa identified by DESeq2 as enriched in skin (differentially abundant) after soil transplant compared to the relative abundance of those same taxa in the soil donor community and the recipient skin community pre-transplant. From left to right: Soil Donor, Skin Pre-Transplant, Skin 2-Hours Post-Transplant, Skin 4-Hours Post-Transplant, and Skin 8-Hours Post-Transplant (n=16); Wash & 24-Hours (n=8). Enriched taxa were aggregated at the Class level and summed.

In agreement with our model fit of overall community similarity to the soil donor community, specific taxonomic classes appear on skin between 0 and 2 hours post-transfer, remain on the skin at 2, 4-, and 8-hours post-transfer, and are not completely removed after washing or after 24-hours post-transfer has passed. Even more strikingly,

when visualized alongside the relative abundances of those same taxa in the soil donor community one can clearly see the “microbial fingerprint” of the soil donor community as it is initially transferred and as it persists over time and through perturbation.

For better clarity, Figure 3.5 shows a schematic of a subset of DESeq comparisons and the specific bacteria that were identified as enriched in these comparisons. The most abundant soil bacteria in the donor soil are also shown for comparison. 707 taxa were enriched in the skin at 2-hours post-transfer compared to the skin before transplant, and 132 taxa were enriched on the skin at 24-hours post-transfer compared to the skin before transplant. No taxa were identified as enriched when the skin at 2- and 4-hours post-transfer was compared, or when comparing the skin at 4- and 8-hours-post transplant. On average, the relative abundance of taxa in the skin at 2-hours post-transplant is correlated to the relative abundance of those same taxa in the soil donor (Spearman’s rank correlation $\rho = 0.6244$). For many taxa, then, transfer will occur proportionally to the relative abundance in the soil donor community, consistent with a mass effects theoretical framework of meta-community dynamics.

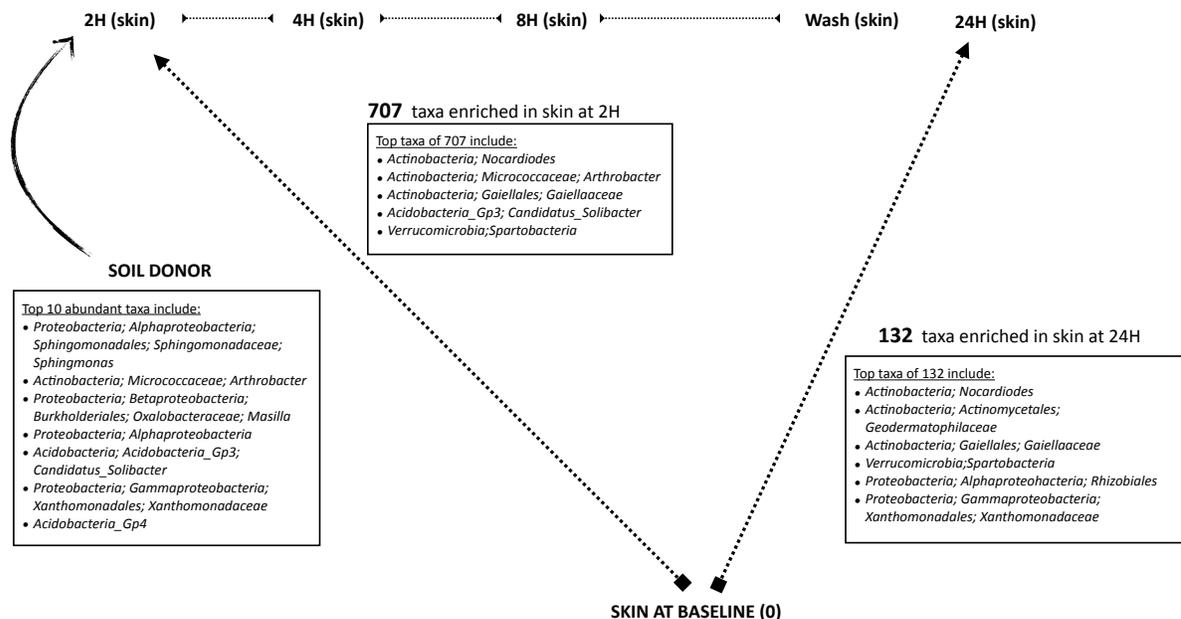


Figure 3.5: Pairwise “site” comparisons for DESeq analysis are shown here with dotted arrows. The site at the end of the arrowhead indicates the site in which the taxa listed is enriched (significantly, differentially abundant) and the site at the tail of the arrow indicate the site against which the enrichment comparison was made. Taxa included in the top ten most abundant in the soil donor community are listed here for comparison against the lists of enriched taxa.

A comparison that tells us which soil taxa might be especially able to transfer and persist on the skin is to compare the relative abundance of taxa in the soil donor community to the relative abundance of taxa in the skin at 2-hours post-transfer (Table 3.1). The taxa that are enriched in the skin post-transfer compared to their relative abundance in the soil donor taxa should help to elucidate which soil taxa preferentially transferred to the skin compared to other members of the soil community. There will also be skin taxa that appear enriched because they do not decrease substantially in relative abundance post-transfer compared to baseline. Indeed, we see that *Propionibacteriaceae* is enriched in the skin recipient community at 2-hours post-transfer compared to the soil donor community. Moreover, *Propionibacteriaceae* is enriched at 24-hours post-transfer compared to the skin recipient community at 2-hours post-transfer, suggesting the ability of *Propionibacteriaceae* to maintain a high relative abundance compared to other skin taxa in the skin recipient site after microbial transfer of the soil donor. Other taxa that were among the most enriched in the skin at 2-hours post-transfer in the skin compared to the soil donor community include class *Actinomycetales*; family *Geodermatophilaceae*; *Blastococcus* and class *Actinomycetales*; family *Pseudonocardia*. Taxa identified as *Geodermatophiaceae* have been isolated from soils and rock surfaces and are organisms with a complex life cycle similar to the animal pathogen *Dermatophilus congolensis* which causes dermatitis in animals and humans (Luedemann, 1968; Urzõ Á et al., 2001).

Table 3.1: The taxa listed here were identified using DESeq as significantly enriched in the recipient skin community 2-hours after soil-transplant compared to the soil donor community, and are listed in order of confidence in this enrichment (adjusted p-values not shown). The log2FoldChange indicates the amount by which the taxon increased in relative abundance in the skin 2-hours post-transplant compared to the soil donor.

DADA2/RDP taxonomic assignment	Closest NCBI isolate & accession #	log2FoldChange	Isolate source environment	Similarity to NCBI isolate
<i>Actinobacteria; Actinomycetales; Propionibacteriaceae; Propionibacterium</i>	<i>Propionibacterium acnes</i> strain ATCC 6919: #NR_040847	3.915335621	<i>Cutibacterium acnes</i> (<i>Propionibacterium acnes</i>) culture collection	100%
<i>Actinobacteria; Actinomycetales; Geodermatophilaceae; Blastococcus</i>	<i>Blastococcus jejuensis</i> strain KST3-10: #NR_043633	8.648246107	South Korea: Jeju coast marine sediment	98%
<i>Planctomycetes; Planctomycetia; Planctomycetales; Planctomycetaceae; Aquisphaera</i>	<i>Aquisphaera giovannonii</i> strain OJF2: #NR_122081	8.081069728	isolated from the sediments of a freshwater aquarium	95%
<i>Actinobacteria; Actinomycetales; Pseudonocardiaceae; Pseudonocardia</i>	<i>Pseudonocardia sediminis</i> strain YIM M13141: #NR_118632	7.833547104	marine sediments of South China Sea	97%

Table 3.1 (Continued.)

DADA2/RDP taxonomic assignment	Closest NCBI isolate & accession #	log2FoldChange	Isolate source environment	Similarity to NCBI isolate
<i>Actinobacteria; Solirubrobacterales</i>	<i>Solirubrobacter ginsenosidimitans</i> strain BXN5-15: #NR_108192	7.770068148	isolated from the soil of a ginseng field on Baekdu Mountain in China	97%
<i>Planctomycetes; Planctomycetia; Planctomycetales; Planctomycetaceae</i>	<i>Singulisphaera rosea</i> strain S26: #NR_116969	7.162216599	sphagnum peat from Russia (Tver region)	91%
<i>Actinobacteria; Actinomycetales</i>	<i>Cryptosporangium cibodasense</i> strain LIP111-2-Ac046: #NR_145891	6.527348635	leaf litter from Indonesia	98%
<i>Actinobacteria</i>	<i>Aciditerrimonas ferrireducens</i> strain IC-180: #NR_112972	6.355503508	sofataric soil from Japan (Kanagawa, Hakone)	92%
<i>Actinobacteria</i>	<i>Aciditerrimonas ferrireducens</i> strain IC-180: #NR_112972	6.178140481	sofataric soil from Japan (Kanagawa, Hakone)	94%
<i>Actinobacteria</i>	<i>Gaiella occulta</i> strain F2-233: #NR_118138	5.814040165	deep mineral water aquifer in Portugal	96%
<i>Actinobacteria; Acidimicrobiales</i>	<i>Aquihabitans daechungensis</i> strain CH22-21: #NR_132289	5.220975426	isolated from a water sample taken from Daechung Reservoir, Republic of Korea	96%
<i>Actinobacteria; Acidimicrobiales</i>	<i>Iamia majanohamensis</i> strain NBRC 102561: #NR_041634.1	4.876658671	isolated from the abdominal epidermis of a sea cucumber, <i>Holothuria edulis</i> , collected from seawater off the coast of Japan	93%
<i>Verrucomicrobia; Spartobacteria</i>	<i>Chthoniobacter flavus</i> strain Ellin428: #NR_115225	4.735020499	environmental soils	91%
<i>Actinobacteria; Actinomycetales</i>	<i>Lysinimicrobium subtropicum</i> strain HI12-128: #NR_145858	3.916774197	isolated from various samples collected from mangrove forests in Japan	97%
<i>Actinobacteria; Solirubrobacterales</i>	<i>Solirubrobacter ginsenosidimitans</i> strain BXN5-15: #NR_108192	3.984404357	isolated from the soil of a ginseng field on Baekdu Mountain in China	97%

Soil and skin are definitively distinct microbial communities, and this is statistically and visually illustrated when the data is fitted with an NMDS (Figure 3.6, panel C). Leaves and skin are also distinct microbial communities, however, and all three communities are significantly compositionally distinct from one another (Figure 3.6). All three microbial community types (dry skin, soil, and leaves) are also highly diverse as measured by the Simpson's Diversity Index, and as illustrated by their compositional makeup (Figure 3.6, panels A & B; Appendix D).

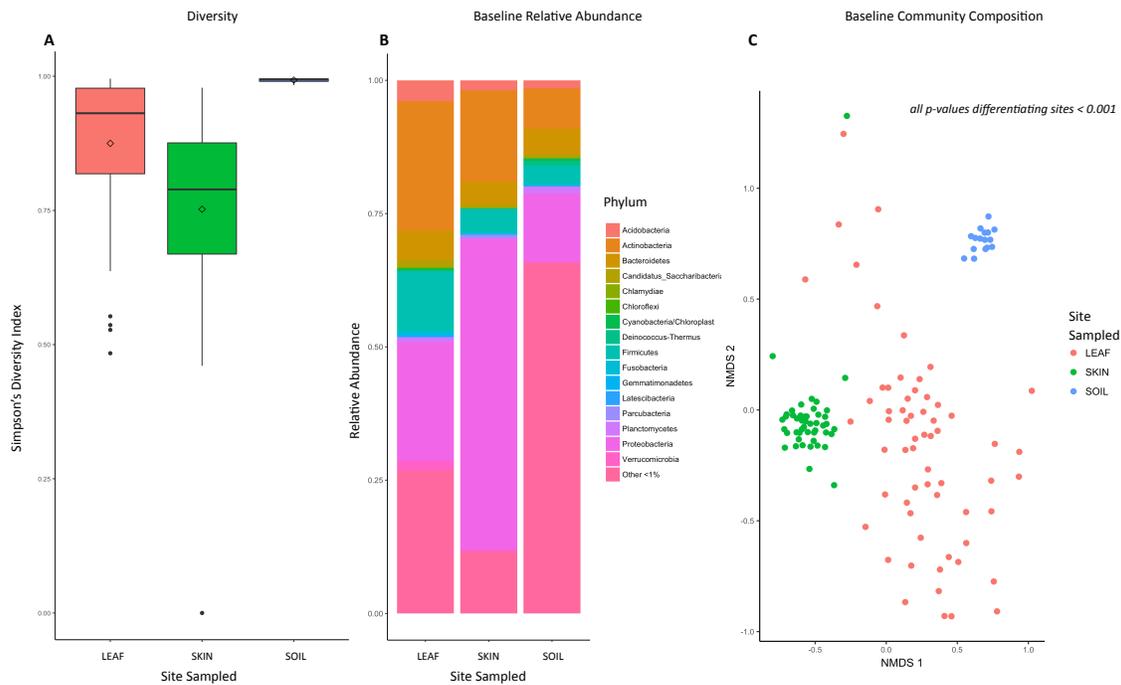


Figure 3.6: Baseline Community Diversity and Composition for Leaves, Soil, and Skin. Each panel A-C illustrates aspects of observed bacterial community composition at the three sites sampled: leaf surfaces, soil, and dry skin before any transplantation had taken place (baseline). A) As measured by the Simpson's Index of Diversity (1-D), alpha-diversity was significantly different at each site sampled, with all sites demonstrating relatively high levels of alpha-diversity. B) The average relative abundance of bacteria in the sites sampled (n=63, 48, and 16 for the leaf, skin, and soil samples, respectively). Only taxa present at more than 1% and in at least two samples were identified; all other taxonomic orders present at a lower abundance and frequency ("rare" taxa) are grouped together in the <1% category. C) Ordination of a NMDS applied to the Bray-Curtis distances between and among the three site types sampled, colored here by those sites (leaf, skin, and soil). Sample type contributes significantly (18%) to the variation observed overall in environmental bacterial communities ($F_{(2,329)}=22.314$; $R^2=0.12$; $p=0.001$).

While the soil-to-skin transfer type shows an effect of initial transfer and of persistence through time, the leaf-to-skin transfer type does not. Soil and leaf communities are compositionally distinct, so it is possible that taxa that are members of soil microbial communities are better at transfer and persistence on dry skin than the taxa that are members of leaf microbial communities. It is also possible that the leaf donor community did not have sufficient biomass for the microbial dispersal event to

overwhelm selection at the dry recipient skin site. Further experiments that vary biomass while holding donor community source constant should help to determine if there exists a threshold at which the biomass of a particular donor community is insufficient to overwhelm selection at a given skin site. It is also plausible that the presence of residual particulate matter on the skin helped to increase the effect of initial transfer for the soil-to-skin transfer.

For some people and not others, 24 hours appeared to be enough time to recover the dry skin community profile that they began with prior to soil-transfer; however, on average across subjects the recipient skin community at 24 hours was still enriched for soil microbial taxa not present at the skin before microbial transfer. *Propionibacteriaceae* was the only group that was significantly enriched in skin sites at 24 hours post-transplant compared to the skin site at 2-, 4-, and 8-hours post-transfer, indicating the potential importance of this taxa for the resistance of skin communities in the face of constant external microbial exposure. Ultimately, washing of the recipient area proved to be the best way to remove the effect of initial transfer. A study by (Noah Fierer et al., 2008) showed that washing had a significant effect on skin community composition but not on overall levels of diversity, leading the authors to suggest that the hand bacterial populations quickly re-establish themselves with environmental microbiota. Unfortunately, we did not follow-up at 24-hours with the subjects who had washed after the 8-hour sampling point. Interestingly, order *Lactobacilales* family *Streptococcaceae* was identified in this chapter's study and in the aforementioned study as relatively more abundant on the skin after washing. More studies are needed that explore the ecological consequences for the associated microbiota of bathing or not bathing the skin. This knowledge will be required to most effectively harness the therapeutic potential of microbial transfers to the skin by understanding the frequency with which they need be performed in the face of modern hygiene rituals.

CONCLUSION

These experiments were intended to mimic and monitor the transient dynamics of microbial transfers that human skin and its associated bacterial communities experience in daily life. We used a sterile swab designed to pick up and deposit a concentrated number of bacterial cells in a consistent, repeatable fashion between and across human subjects. It has been previously estimated from qPCR assays that these swabs capture c.a. 10,000 cells per cm² skin sampled (E A Grice et al., 2008). Although the total and viable bacterial biomass may vary across donor sources, we embraced this as “natural” variation in alignment with our mission to mimic natural microbial dynamics as closely as possible. The variation in live and total bacterial biomass in skin communities appears to be large (unpublished Honors thesis data by Maria Sarao). We also acknowledge that a non-negligible fraction of transferred bacterial biomass comprised relic DNA from dead microorganisms. However, even dead bacterial cells participate in important ecological processes such as nutrient cycling, providing habitat for other microbes, or preventing colonization of new microbes (Jones & Lennon, 2010). Future experiments should be designed to disentangle the dynamics of living, metabolically active and dead or dormant microorganisms during microbial transfer to the skin. Finally, while these results were produced with measures of relative abundance rather than absolute abundance, the hypotheses I wished to test did not require the use of difficult-to-estimate absolute abundance measurements. Instead, we used overall measures of similarity to the microbial donor in order to study temporal changes at the scale of the entire community. Regardless, I cannot differentiate between growth of a particular taxon and simply “not dying while others around you are”. For this reason, we regard persistence as simply maintaining a consistent, measurable presence in the community over time, similar to the definition of persistence described by (Caporaso et al., 2011).

My first two sets of experiments demonstrate that it is possible to transfer skin microbiota within sites on a single subject and across subjects, even without prior disinfection of recipient skin sites. I observed successful transfer, however, only when transferring the sebaceous donor community generally dominated by the

Propionibacteriaceae bacterial family. This is similar to what was reported for the forehead transplant to disinfected dry forearms by (Costello et al., 2009). I observed that the sebaceous skin site is the most resistant to microbial transfer, possibly due to the more severe abiotic conditions at these sites. The moist skin site was most hospitable to microbial transfer in my experiments, despite it being the most diverse and likely the most abundant (Gao et al., 2010). Interestingly, within-community turnover in skin communities over time has been shown to be weakly anti-correlated with community diversity (Oh et al., 2016), mirroring the result found here that the most diverse community (moist) was the least resistant to microbial transfer. Furthermore, the effect of transfer by the sebaceous donor community appears to persist when the moist (and in some cases dry) recipient skin community is sampled at 2-, 4-, and 8- hours post-transplant. Understanding the timescales at which skin communities can be significantly and predictably altered through the transfer of microbial inoculum is critical to furthering our ability to apply manipulative biotic interventions to the treatments of skin disorders.

My third experiment demonstrated that it is possible to transfer non-human associated, environmental microbiota to the dry forearm, namely farm soil microbiota. In fact, this microbial transfer had the largest estimated effect (β coefficient) across all of the transfer types tested. Moreover, this transfer showed no evidence of decay away from the donor community when sampled over the 2-, 4- and 8-hours post-transplant sampling points. Even after 24-hours post-transplant, the skin retained significant community similarity to the soil donor community. Although washing reduces this similarity even further, we still detected a signal of transferred soil. Thus, direct environmental microbial exposure can result in a significant microbial transfer that persists over 8-hours, has decayed significantly at 24-hours, yet maintains some effect of transplant after this initial transient period. Both a diverse soil donor community and a relatively depauperate sebaceous skin donor community, largely dominated by members of the family *Propionibacteriaceae*, were able to be successfully transferred to both moist and dry skin sites, and showed evidence of persistence over the timescale considered herein. Longitudinal studies that extend this kind of experiment beyond 24-hours are necessary to determine exactly how and why skin bacterial communities eventually tend to exclude most environmental taxa.

At least one key avenue for future research will be to sample additional skin microbial communities across a wider variety of demographic groups comprising people who experience significantly different microbial exposures and thus colonization of the skin microbiome. The microbiome literature is replete with samples from predominantly Caucasian, college-aged students (including the present studies), highlighting the need for more diverse human subject bases for microbiome analyses. One particular study provides an instructive example for the research potential when we go beyond the average American sampling populations. When the forearm microbiota of American and Venezuelan Amerindian subjects are compared, substantial differences in bacterial taxonomic composition are found (Blaser et al., 2012). In the case of one cluster of Venezuelan subjects, their skin was not dominated by a single taxon such as *Propionibacterium*, but instead by a broad diversity of *Proteobacteria*, including *Pseudomonas*, *Xanthomonadaceae*, and *Methylophilus*. This cluster of Amerindian subjects also had significantly higher α -diversity on their skin than the other subject clusters (that included both Amerindians and Americans). *Xanthomonadaceae*, moreover, was found to be one of the top 10 most abundant taxa in the soil donor community used in my experiment, and was one of the top 10 significantly enriched family groups at 24-hours after transfer compared to the skin before transfer. Furthermore, many of the taxa binned as persistent and transient in the (Caporaso et al., 2011) study were identified as belonging to phylum *Acidobacteria* with classes of *Acidobacteria* and *Solibacteres*, phylum *Verrucomicrobia* of class *Spartobacteria*, and several classes of *Proteobacteria* including *Alphaproteobacteria*, *Betaproteobacteria*, and *Gammaproteobacteria*. All of these taxa were among the most abundant in the soil donor community used in my transfer experiment, suggesting soil as a potentially major source of bacterial transfer to the skin of these individuals at some point during their sampling trajectory. The relatively strong and persistent effect of microbial transfer demonstrated in my experiment also raise the intriguing possibility that transient microbial colonists can invade and establish populations in skin communities if microbial exposures are frequent or large enough through dispersal-mediated coexistence (Fahimipour & Anderson, 2015; Leibold et al., 2004). Future experiments should vary the

frequency and timing of microbial exposures to the skin to assess the success of repeated transfer and persistence over time.

My research shows that it is possible to acquire bacterial taxa from environmental and human microbial sources, and this significant effect of transfer persists (in one case) for up to 24 hours and after washing. (Oh et al., 2016) suggests that very little environmental microbiota will be acquired by the skin based on an assessment of transient versus core microbiota sampled at relatively distant time points (monthly & yearly). The present research suggests that assessment of transient microbiota may owe to the disparate sampling points that result in the filtering out of environmental microbiota that subjects may acquire between sampling points, and that could persist on the skin for hours, days, or weeks at a time. Indirect effects of so-called “transient” microorganisms could occur in several ways, either by briefly competing with the established skin microbiota to alter the bacterial community structure or by indirectly or directly modulating the immune system, which also then leads to changes in the structure of the bacterial community. Indeed, effects of transient colonists on longer-term community dynamics have been documented in macroscopic ecosystems (Fahimipour & Anderson, 2015).

An altered community structure has the capacity to directly influence host physiology. When the immune system of a host is genetically modified or impaired we may observe a corresponding response in the host bacterial community; when this altered bacterial community is experimentally transferred between hosts, the negative phenotypic effect of the original impaired host can be transmitted to the recipient host via the microbiota ((Elinav et al., 2011; Wen et al., 2008) demonstrating the effect of the microbiota as a significant mediator of host physiology. In at least one case of an environmental saprophyte, *Mycobacterium vaccae*, it can cause immunoregulatory effects that suppress allergic responses without needing to colonize and without even being a living organism (Hunt, Martinelli, Adams, Rook, & Brunet, 2005; Zuany-Amorim et al., 2002).

Rather than changing our definition of what constitutes a transient taxon in a community, we should instead focus on increasing our understanding of the direct and indirect impacts of these transient bacteria for the host. Bacterial taxa that are binned into

core, persistent, and transient categories will vary based on both intrinsic (genetics) and extrinsic (lifestyle and activities) host factors. Rather, future studies should aim to determine how these factors interact to facilitate the transfer of transient or environmental bacterial taxa and determine their persistence.

The “hygiene hypothesis” or “old friends” hypothesis suggest humans (especially in the developed world) are missing critical microbial exposures, especially in childhood. The transient or persistent taxa that tend to be ignored could actually be our missing, “old friends”. A study by (Lehtimäki et al., 2017) found an age-specific effect of environment on the skin microbiota of children, where the effect of living around green space was most prominent in the skin microbiota of younger children (strongest in toddlers) and not present at all in teenagers. The researchers conclude that the amount of time spent outdoors and interacting with the environment is directly related to the amount of influence that the environment has on the skin microbiota. Given that the signal of environment is most detectable in the skin of children, and that early childhood is the most important time for education of the immune system (Tamburini, Shen, Wu, & Clemente, 2016), an important enterprise for future research will be to determine the potential impact of environmental microbial sources on the skin microbiome composition of children and future allergy development. This “window of opportunity” in early childhood is likely to be the most useful time to amend or modulate the microbiome to influence desirable health outcomes.

Any medical practitioner will tell you that a healthy diet is essential to maintain human health. Personalized diets have become popular since we’ve learned more about genetic predispositions to different dietary inputs. In the future a “microbial diet”, perhaps designed to complement the unique aspects of your human and microbial genetic content, could also be recommended to achieve improvement of host health. To get to this future therapeutic goal, we must continue experiments such as those conducted for this dissertation to learn how our interactions with our immediate environment result in microbial dispersal from those environments, the ways in which the skin community is altered as a result, and ultimately the exciting potential impacts for human health.

APPENDIX A

ILLUMINA SEQUENCING PRIMERS

Chapter II:

With the assistance of the staff at the University of Oregon Genomics Core Facility, custom “spacer” dual-barcoded primers were designed as described in (Fadrosh et al., 2014) to create 319F-806R primers for the microbial 16S rRNA gene for sequencing on the Illumina MiSeq platform that required, at the time of sequencing, additional complexity to be added to amplicon libraries in order to sequence successfully. These primers were designed for use with two separate PCR amplification steps.

319F:

Sequencing Primer	“Spacer”	Gene Primer
5'-TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG		ACTCCTACGGGAGGCAGCAG
5'-TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG T		ACTCCTACGGGAGGCAGCAG
5'-TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG CT		ACTCCTACGGGAGGCAGCAG
5'-TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG GGT		ACTCCTACGGGAGGCAGCAG
5'-TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG AACG		ACTCCTACGGGAGGCAGCAG
5'-TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG TTGTT		ACTCCTACGGGAGGCAGCAG

806R:

Sequencing Primer	“Spacer”	Gene Primer
5'-GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG		GGACTACHVGGGTWTCTAAT
5'-GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG A		GGACTACHVGGGTWTCTAAT
5'-GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG TA		GGACTACHVGGGTWTCTAAT
5'-GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG CCC		GGACTACHVGGGTWTCTAAT
5'-GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG ATTT		GGACTACHVGGGTWTCTAAT
5'-GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG GCACA		GGACTACHVGGGTWTCTAAT

Chapter III & IV:

319-806R primers for the microbial 16S rRNA gene were designed for sequencing on the Illumina MiSeq platform that no longer required “spacer” sequences to be added for successful sequencing of low-complexity amplicon libraries. Again, the staff at the University of Oregon Genomics Core Facility designed the complete primer sequences to be compatible with the current Illumina sequencing platforms.

319F:

Illumina “P5” sequence	example barcode 2	“primer pad”	319F sequence
5'AATGATACGGCGACCACCGAGATCTACAC	TAGATCGC	TATGGTAATTGT	ACTCCTACGGGAGGCAGCAG 3'

806R:

Illumina “P7” sequence	example barcode 1	“primer pad”	806R sequence
5' CAAGCAGAAGACGGCATACGAGAT	TCACCTAG	AGTCAGTCAGCC	GGACTACHVGGGTWTCTAAT 3'

APPENDIX B

GENERALIZED LINEAR MIXED MODELS

For each GLMM table, selected transfer types are shown. Those transfers for which a significant change in similarity to the donor community is estimated for a given timeframe are highlighted in bold. In all models, subjects were specified as a random factor.

Within-Subject Transfers (Chapter II)

Transfer Type	Timeframe	β Estimate	Std. Error	z-value	P-value
Sebaceous-to-Dry	T0-T2	-0.00065	0.333294	-0.002	0.998444
Sebaceous-to-Moist	T0-T2	1.139186	0.336448	3.386	0.000709
Sebaceous-to-Dry	T2-T4	0.08731	0.38201	0.229	0.819204
Sebaceous-to-Moist	T2-T4	0.06782	0.3717	0.182	0.855215
Sebaceous-to-Dry	T4-T8	0.68638	0.37763	1.818	0.069124
Sebaceous-to-Moist	T4-T8	0.37475	0.36491	1.027	0.30443

Within-Subject Transfers (Chapter III)

Transfer Type	Timeframe	β Estimate	Std. Error	z-value	P-value
Sebaceous-to-Dry	T0-T2	0.270000	3.71E-01	0.729	0.4661
Sebaceous-to-Moist	T0-T2	0.941000	3.78E-01	2.49	0.0128
Sebaceous-to-Dry	T2-T4	0.52305	0.40418	1.294	0.19563
Sebaceous-to-Moist	T2-T4	0.05308	0.40171	0.132	0.89487
Sebaceous-to-Dry	T4-T8	0.36801	0.40243	0.914	0.36047
Sebaceous-to-Moist	T4-T8	0.10292	0.40301	0.255	0.79843

Between-Subject Transfers (Chapter III)

Transfer Type	Timeframe	β Estimate	Std. Error	z-value	P-value
Sebaceous-to-Dry	T0-T2	0.9857	0.50069	1.969	0.049
Sebaceous-to-Moist	T0-T2	1.15073	0.51619	2.229	0.0258
Sebaceous-to-Dry	T2-T4	-0.4681	0.4262	-1.098	0.27211
Sebaceous-to-Moist	T2-T4	-0.3724	0.4235	-0.879	0.37923
Sebaceous-to-Dry	T4-T8	-0.1761	0.4318	-0.408	0.68346
Sebaceous-to-Moist	T4-T8	-0.219	0.4286	-0.511	0.60939

Environmental Transfers (Chapter IV)

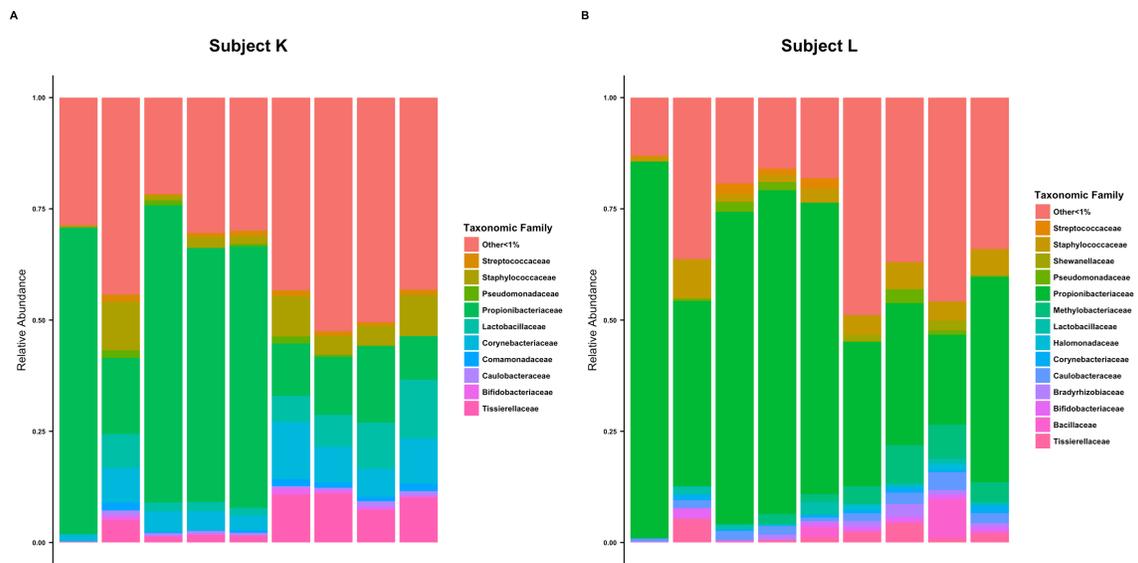
Transfer Type	Timeframe	β Estimate	Std. Error	z-value	P-value
Leaf-to-Skin	T0-T2	0.45549	0.33818	1.347	0.178
Soil-to-Skin	T0-T2	3.29324	0.33636	9.791	<2e-16
Leaf-to-Skin	T2-T4	0.7279	0.3863	1.884	0.05955
Leaf-to-Skin	T4-T8	-0.152	0.377	-0.403	0.6869
Soil-to-Skin	T2-T4	0.5862	0.3202	1.831	0.06716
Soil-to-Skin	T4-T8	-0.2668	0.3101	-0.86	0.38952
Soil-to-Skin	T8-T24	1.5883	0.3873	4.101	4.12e-05
Leaf-to-Skin	T8-T24	0.3433	0.3233	1.062	0.288352

APPENDIX C

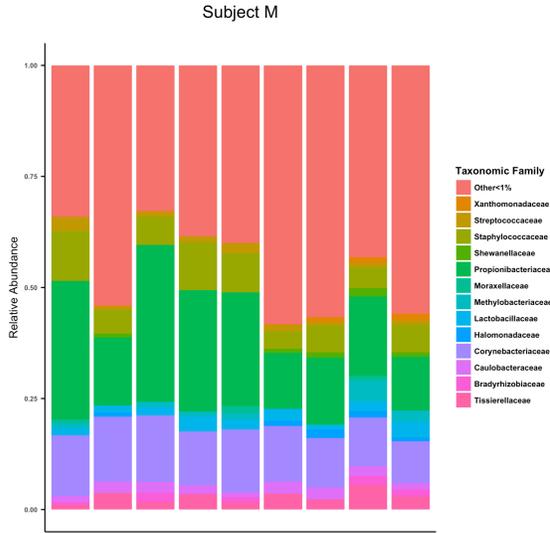
COMPOSITIONAL BAR PLOTS: WITHIN-SUBJECT MICROBIAL TRANSFERS

Bar plots A-J illustrate the 10 subjects sampled in this experiment (K, L, M, N, O, P, Q, R, S, T). Each bar plot shows the bacterial composition of the sebaceous to moist transplant (columns 2-5) and the moist to moist control (columns 6-9), pre-transplant at baseline and 2-, 4-, and 8-hours post-transplant. The composition of the sebaceous donor is shown on the far left. The taxa identified here are aggregated at the family level and filtered to 1% abundance and present in at least 2 samples. The taxonomic families that do not meet these requirements are grouped together in the “Other <1% category”.

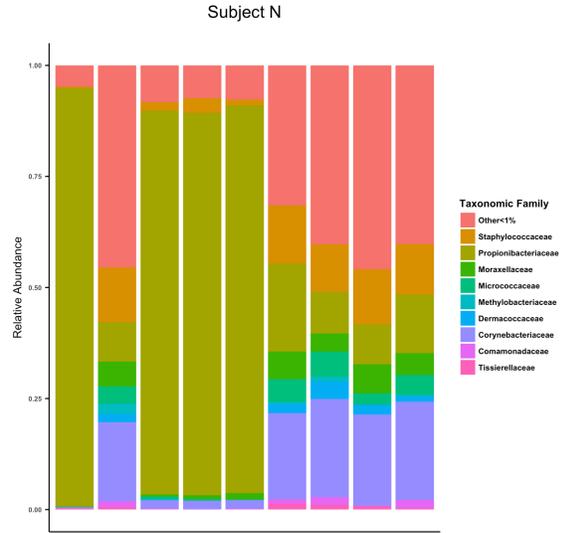
For each bar plot, the columns are as follows: sebaceous donor, moist skin at T0 before sebaceous transfer, moist skin at T2 after sebaceous transfer, moist skin at T4 after sebaceous transfer, moist skin at T8 after sebaceous transfer, moist donor, moist skin at T0 before moist transfer, moist skin at T2 after moist transfer, moist skin at T4 after moist transfer, moist skin at T8 after moist transfer.



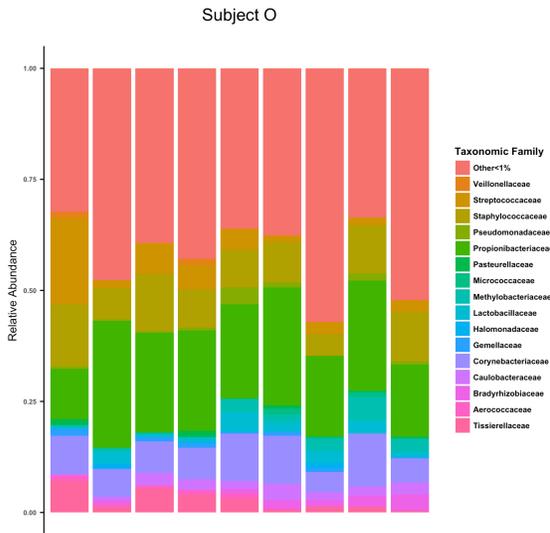
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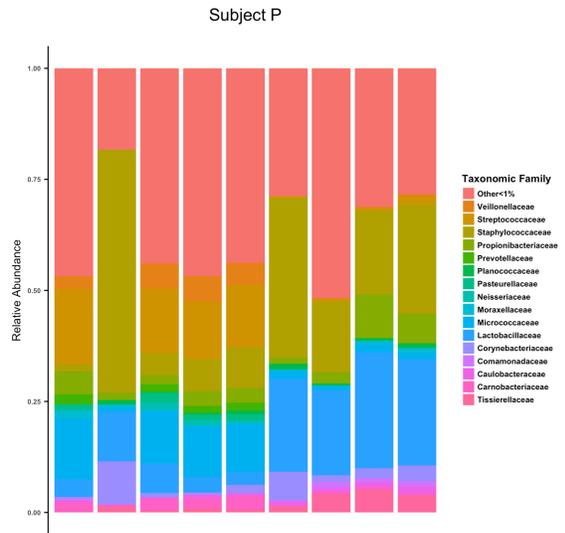
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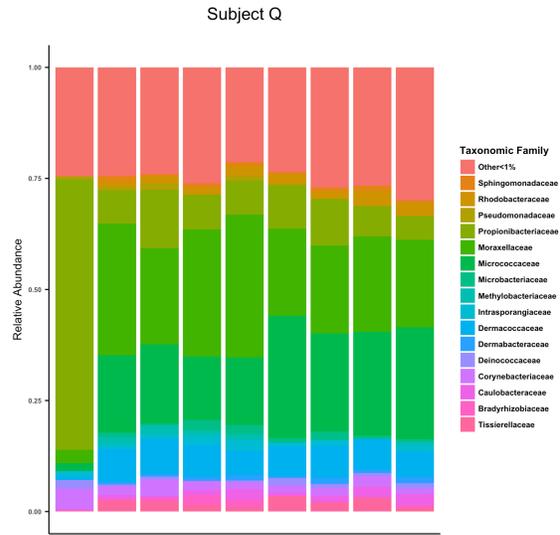
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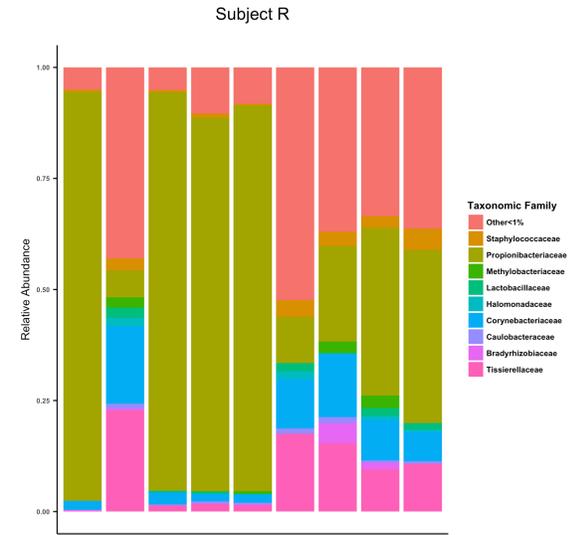
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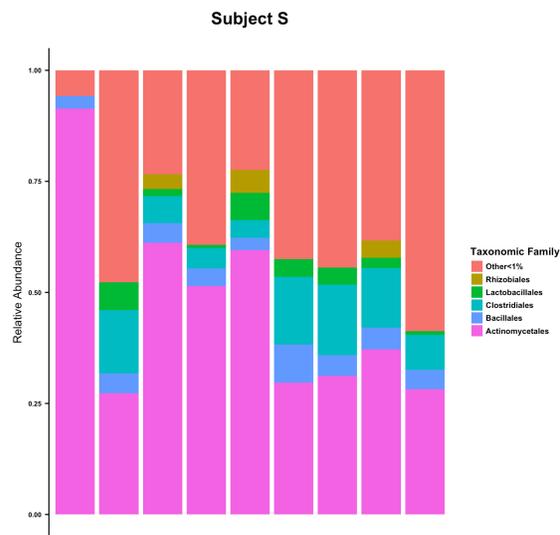
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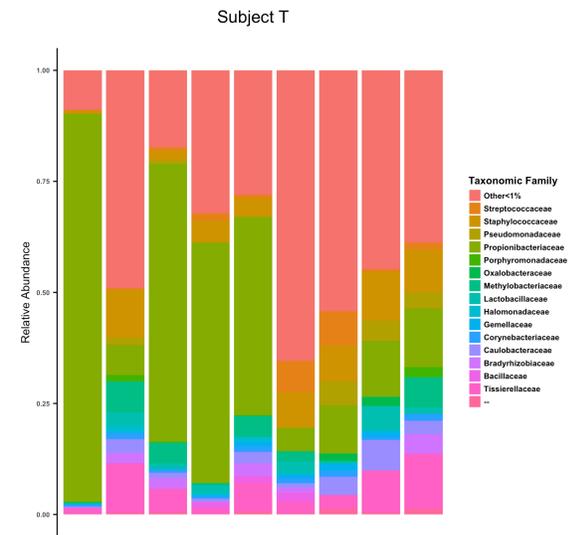
H



I



J



APPENDIX D

DIVERSITY

The tables below from Chapter II and Chapter III compare the diversity estimates (Simpson's Diversity) among skin sites using a linear mixed effects model controlling for subject as a random factor. The table from Chapter IV compares the diversity estimates among environmental sample types using an ANOVA model ($F_{(2,135)}=28.43$, p -value= $4.9e^{-11}$). In every table, the pairwise comparisons for which we can reject the null hypothesis after correcting for multiple comparisons are highlighted in bold.

Skin Site Diversity (Chapter II)

Skin Site Comparisons	Estimate	Std. Error	t-value	p-value
Moist-Dry == 0	0.11381	0.04461	2.551	0.029
Sebaceous-Dry == 0	-0.38835	0.04503	-8.625	<0.001
Sebaceous-Moist == 0	-0.50216	0.04461	-11.257	<0.001

Skin Site Diversity (Chapter III)

Skin Site Comparisons	Estimate	Std. Error	t-value	p-value
Moist-Dry == 0	0.12704	0.04056	3.132	0.00485
Sebaceous-Dry == 0	-0.33371	0.03628	-9.198	<0.0001
Sebaceous-Moist == 0	-0.46075	0.03628	-12.7	<0.0001

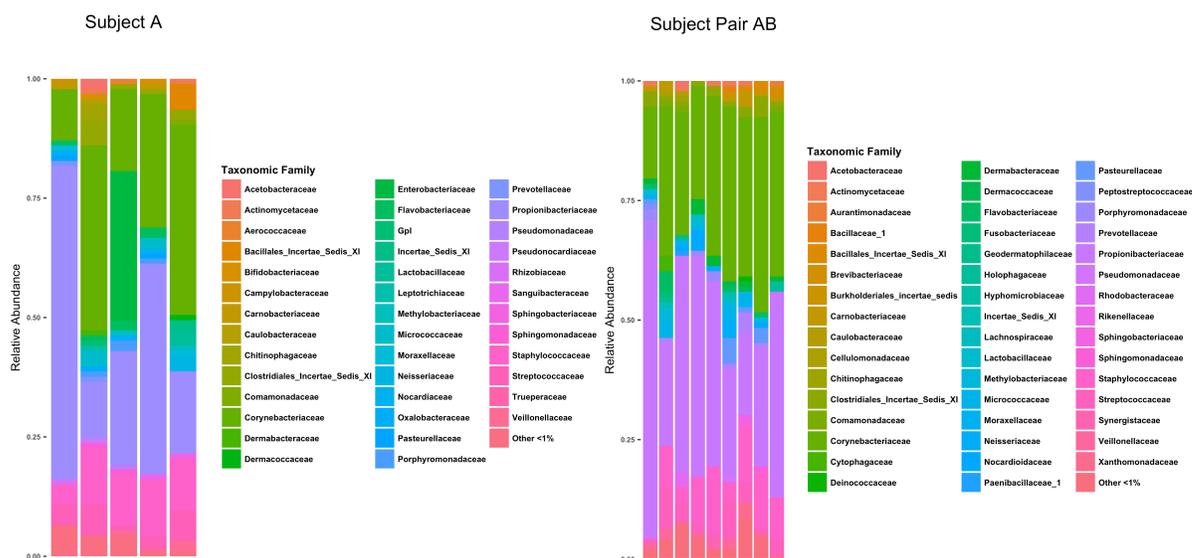
Environmental Sample Diversity (Chapter IV)

Sample Comparisons	Estimate	Std. Error	t-value	p-value
Skin-Leaf == 0	-0.12273	0.02629	-4.668	<0.0001
Soil-Leaf == 0	0.11789	0.03114	3.786	0.00063
Soil-Skin == 0	0.24062	0.03252	7.399	<0.0001

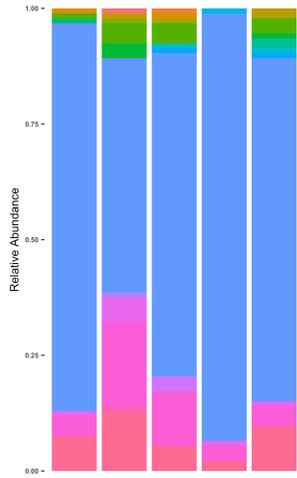
APPENDIX E

COMPOSITIONAL BAR PLOTS: WITHIN-SUBJECT & BETWEEN-SUBJECT MICROBIAL TRANSFERS

The following bar plots display the compositional change that was observed during a sebaceous-to-moist transfer for each of eight subjects (A, B, C, D, E, F, G, H) that underwent both “within-subject” and “between-subject” transfer sets. The control moist-to-moist transfer was performed for the between-subject transfer but not the within-subject transfer. For better clarity we describe the bar plot axes here: The far left column in each bar plot represents a sebaceous donor community from either the same subject or a different subject, sampled at the baseline sampling point (T0). The second through fifth columns in each bar plot (both “within-subject” and “between-subject”) represent the sebaceous-to-moist transfer at baseline (T0), 2-, 4-, and 8-hours post-transfers. The last four columns in the “between-subject” bar plots only represent the moist-to-moist control transfer at baseline (T0), 2-, 4-, and 8-hours post-transfer. The taxa identified here are aggregated at the family level and filtered to 1% abundance and present in at least two samples shown here. The taxonomic families that do not meet these requirements are grouped together in the “Other <1% category”.

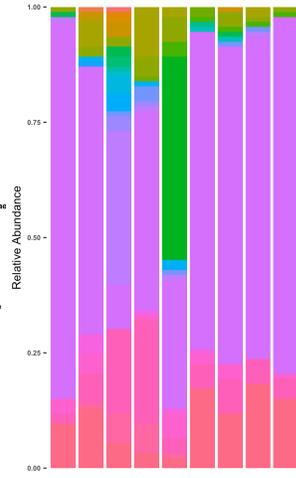


Subject B



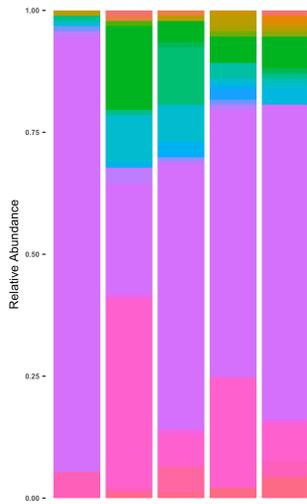
- Taxonomic Family**
- Actinomycetaceae
 - Aeromonadaceae
 - Bacillales_Incertae_Sedis_XI
 - Carnobacteriaceae
 - Clostridiales_Incertae_Sedis_XI
 - Comamonadaceae
 - Corynebacteriaceae
 - Lactobacillaceae
 - Leuconostocaceae
 - Micrococcaceae
 - Moraxellaceae
 - Nocardiodiaceae
 - Oxalobacteraceae
 - Prevotellaceae
 - Propionibacteriaceae
 - Rhodocyclaceae
 - Ruminococcaceae
 - Staphylococcaceae
 - Streptococcaceae
 - Xanthomonadaceae
 - Other <1%

Subject Pair BA



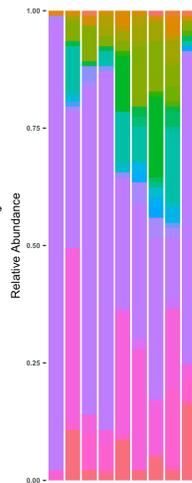
- Taxonomic Family**
- Acetobacteraceae
 - Actinomycetaceae
 - Bacillales_Incertae_Sedis_XI
 - Campylobacteraceae
 - Carnobacteriaceae
 - Chitinophagaceae
 - Clostridiales_Incertae_Sedis_XI
 - Corynebacteriaceae
 - Cytophagaceae
 - Dermaococcaceae
 - Enterobacteriaceae
 - Flavobacteriaceae
 - Fusobacteriaceae
 - Gpl
 - Lachnospiraceae
 - Lactobacillaceae
 - Leptotrichiaceae
 - Micrococcaceae
 - Moraxellaceae
 - Neisseriaceae
 - Nocardiaceae
 - Pasteurellaceae
 - Porphyromonadaceae
 - Prevotellaceae
 - Propionibacteriaceae
 - Rhodobacteraceae
 - Sphingomonadaceae
 - Staphylococcaceae
 - Streptococcaceae
 - Verruonellaceae
 - Other <1%

Subject C



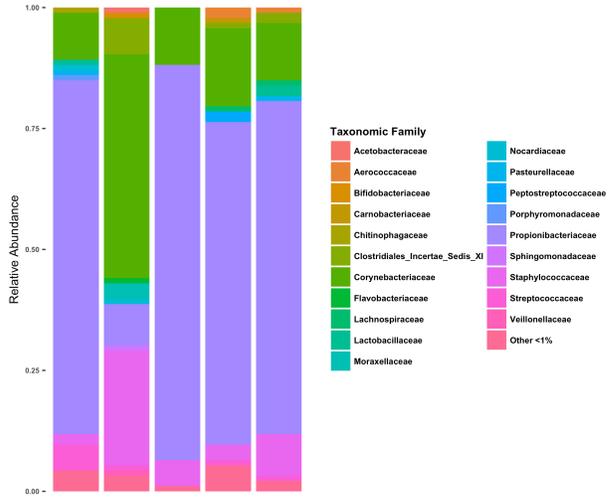
- Taxonomic Family**
- Actinomycetaceae
 - Aerococcaceae
 - Aeromonadaceae
 - Alcaligenaceae
 - Bacillales_Incertae_Sedis_XI
 - Bifidobacteriaceae
 - Carnobacteriaceae
 - Caulobacteraceae
 - Clostridiales_Incertae_Sedis_XI
 - Comamonadaceae
 - Corynebacteriaceae
 - Cytophagaceae
 - Enterobacteriaceae
 - Flavobacteriaceae
 - Geodermatophilaceae
 - Lachnospiraceae
 - Lactobacillaceae
 - Microbacteriaceae
 - Micrococcaceae
 - Moraxellaceae
 - Oxalobacteraceae
 - Pasteurellaceae
 - Porphyromonadaceae
 - Prevotellaceae
 - Propionibacteriaceae
 - Rhodobacteraceae
 - Sphingomonadaceae
 - Staphylococcaceae
 - Streptococcaceae
 - Xanthomonadaceae
 - Other <1%

Subject Pair CD

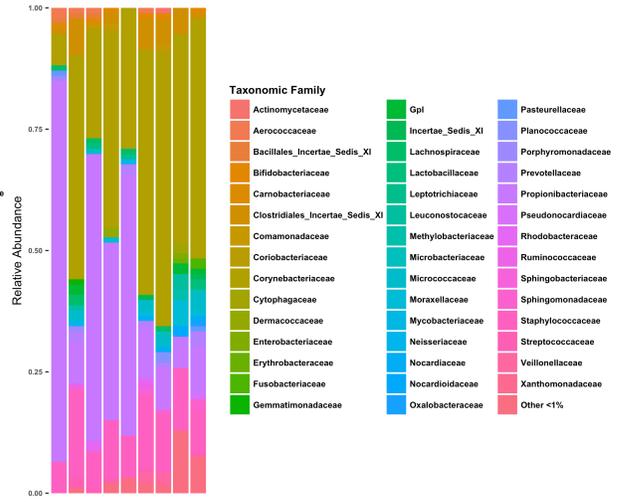


- Taxonomic Family**
- Acetobacteraceae
 - Aerococcaceae
 - Aeromonadaceae
 - Bacillales_Incertae_Sedis_XI
 - Bifidobacteriaceae
 - Carnobacteriaceae
 - Caulobacteraceae
 - Cellulomonadaceae
 - Chitinophagaceae
 - Clostridiales_Incertae_Sedis_XI
 - Comamonadaceae
 - Corynebacteriaceae
 - Cytophagaceae
 - Dermaobacteriaceae
 - Dermaococcaceae
 - Enterobacteriaceae
 - Flavobacteriaceae
 - Gpl
 - Hyphomicrobiaceae
 - Intrasporangiaceae
 - Kineosporiaceae
 - Lactobacillaceae
 - Leptotrichiaceae
 - Microbacteriaceae
 - Micrococcaceae
 - Micromonosporaceae
 - Moraxellaceae
 - Neisseriaceae
 - Nocardiaceae
 - Nocardiodiaceae
 - Parachlamydiaceae
 - Pasteurellaceae
 - Prevotellaceae
 - Propionibacteriaceae
 - Rhodobacteraceae
 - Rubrobacteraceae
 - Ruminococcaceae
 - Sphingomonadaceae
 - Staphylococcaceae
 - Streptococcaceae
 - Trueperaceae
 - Verruonellaceae
 - Vibrionaceae
 - Xanthomonadaceae
 - Other <1%

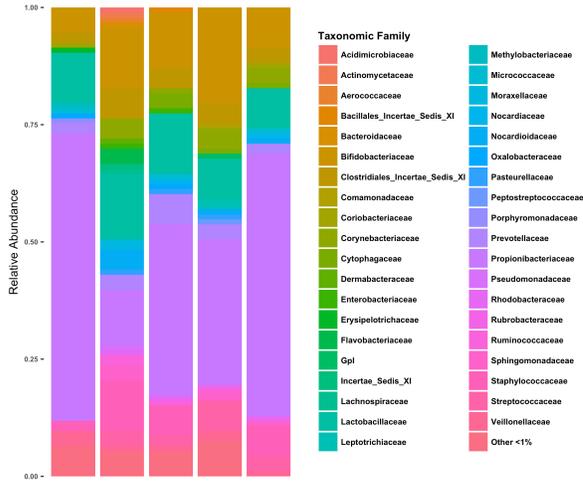
Subject D



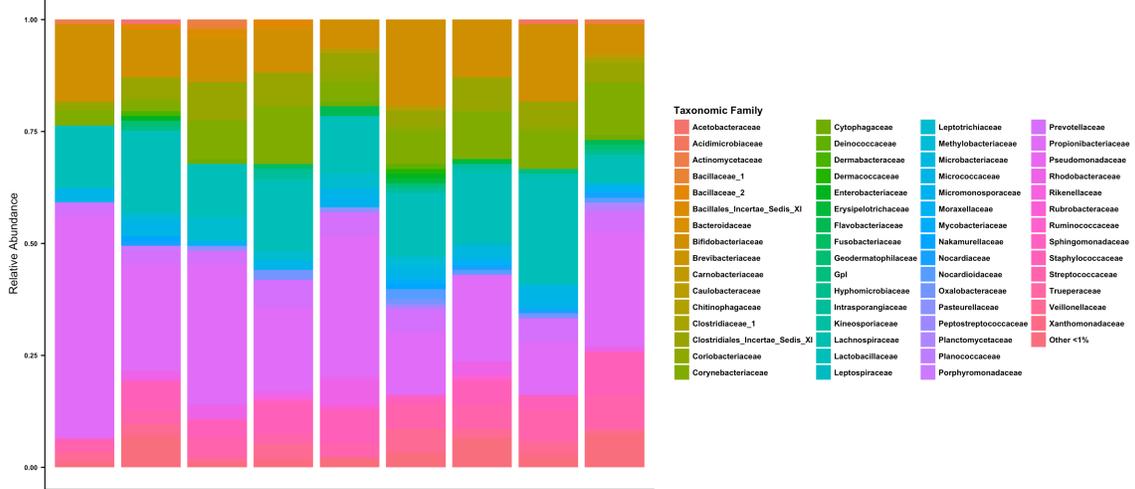
Subject Pair DC



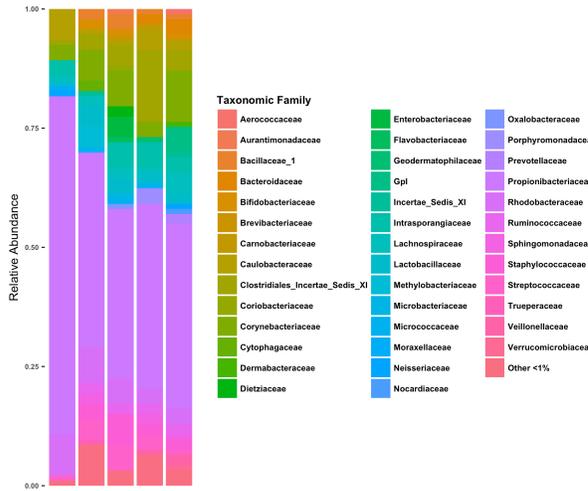
Subject E



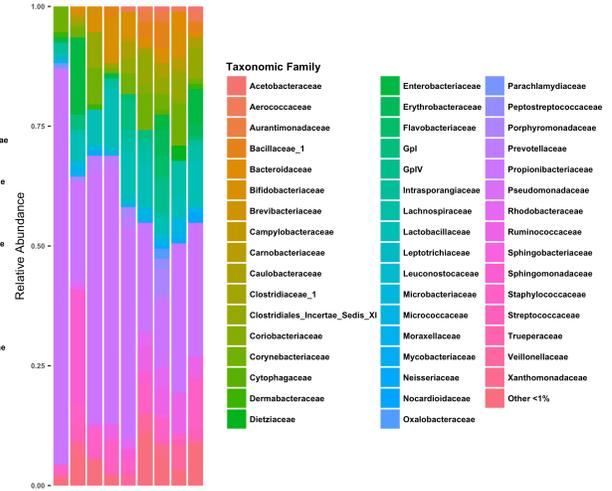
Subject Pair EF



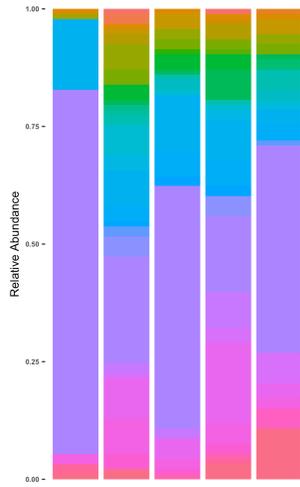
Subject F



Subject Pair FE



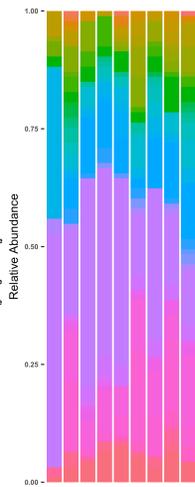
Subject G



Taxonomic Family

- Bacillaceae_1
- Bacillales_Incertae_Sedis_XII
- Bifidobacteriaceae
- Caulobacteraceae
- Chitinophagaceae
- Clostridiales_Incertae_Sedis_XI
- Comamonadaceae
- Coriobacteriaceae
- Corynebacteriaceae
- Cytophagaceae
- Dermacoccaceae
- Dietziaceae
- Enterobacteriaceae
- Flavobacteriaceae
- Gpl
- Hyphomicrobiaceae
- Kineosporiaceae
- Lactobacillaceae
- Methylobacteriaceae
- Microbacteriaceae
- Micrococcaceae
- Moraxellaceae
- Nocardiaceae
- Nocardioidaceae
- Oxalobacteraceae
- Prevotellaceae
- Propionibacteriaceae
- Pseudomonadaceae
- Sphingobacteriaceae
- Sphingomonadaceae
- Staphylococcaceae
- Streptococcaceae
- Trueperaceae
- Veillonellaceae
- Xanthomonadaceae
- Other <1%

Subject Pair GH



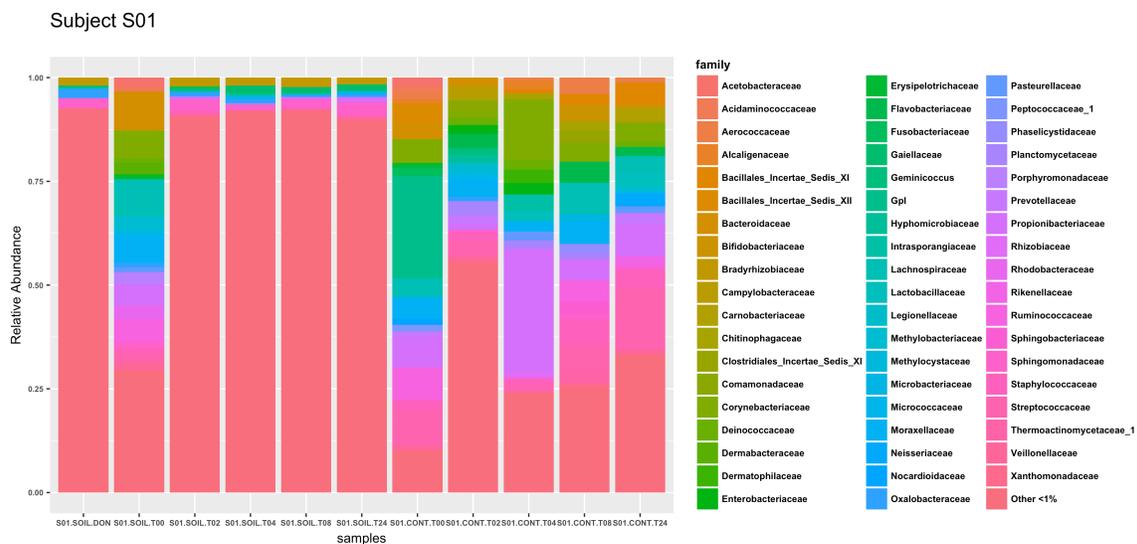
Taxonomic Family

- Actinomycetaceae
- Aurantimonadaceae
- Bacillaceae_1
- Bdellovibrionaceae
- Bradyrhizobaceae
- Carnobacteriaceae
- Caulobacteraceae
- Chitinophagaceae
- Clostridiales_Incertae_Sedis_XI
- Comamonadaceae
- Coriobacteriaceae
- Corynebacteriaceae
- Cytophagaceae
- Dermacoccaceae
- Dietziaceae
- Enterobacteriaceae
- Flavobacteriaceae
- Fusobacteriaceae
- Gpl
- Halomonadaceae
- Hyphomicrobiaceae
- Kineosporiaceae
- Lachnospiraceae
- Lactobacillaceae
- Leptotrichiaceae
- Methylobacteriaceae
- Methylocystaceae
- Microbacteriaceae
- Micrococcaceae
- Moraxellaceae
- Mycobacteriaceae
- Nakamurellaceae
- Nocardiaceae
- Nocardioidaceae
- Oxalobacteraceae
- Paenibacillaceae_1
- Porphyromonadaceae
- Prevotellaceae
- Propionibacteriaceae
- Pseudomonadaceae
- Rhizobiaceae
- Rhodobacteraceae
- Rhodocyclaceae
- Sphingobacteriaceae
- Sphingomonadaceae
- Staphylococcaceae
- Streptococcaceae
- Trueperaceae
- Tsukamurellaceae
- Veillonellaceae
- Xanthomonadaceae
- Other <1%

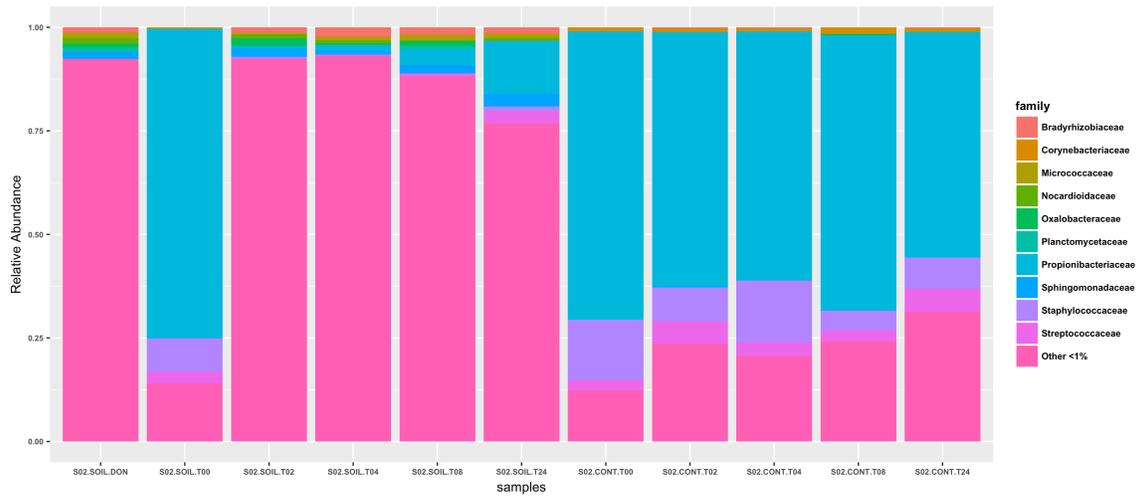
APPENDIX F

COMPOSITIONAL BAR PLOTS: ENVIRONMENTAL MICROBIAL TRANSFERS

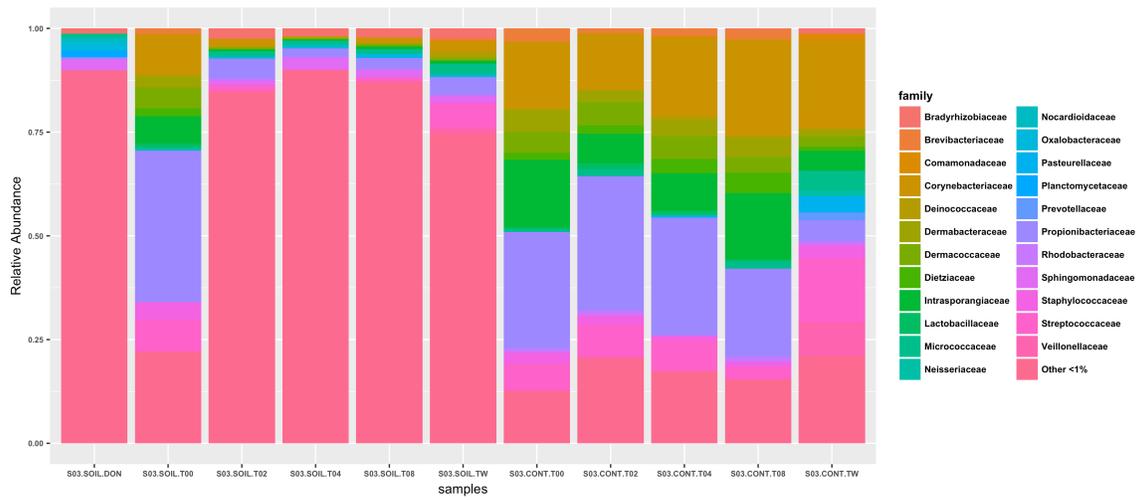
The following bar plots illustrate the compositional change that was observed during a soil-to-skin transfer for each of 16 subjects (S01-S16). The far left column for each subject's bar plot represents a soil donor community sampled at the baseline time point. The second through fifth columns in each panel represent the skin-to-skin transfers at baseline, 2-, 4-, and 8-hours post-transfer. Depending on whether the subject was sampled at 24-hours or after washing, the 6th column will reflect this time point. The last five columns in each panel represent the skin-to-skin control transfer at baseline, 2-, 4-, and 8-hours post-transfer, with the 6th column representing the 24-hour time point or the post-wash time point depending. The taxa identified here are aggregated at the family level and filtered to 1% abundance and present in at least two samples shown here. The taxonomic families that do not meet these requirements are grouped together in the "Other <1% category".



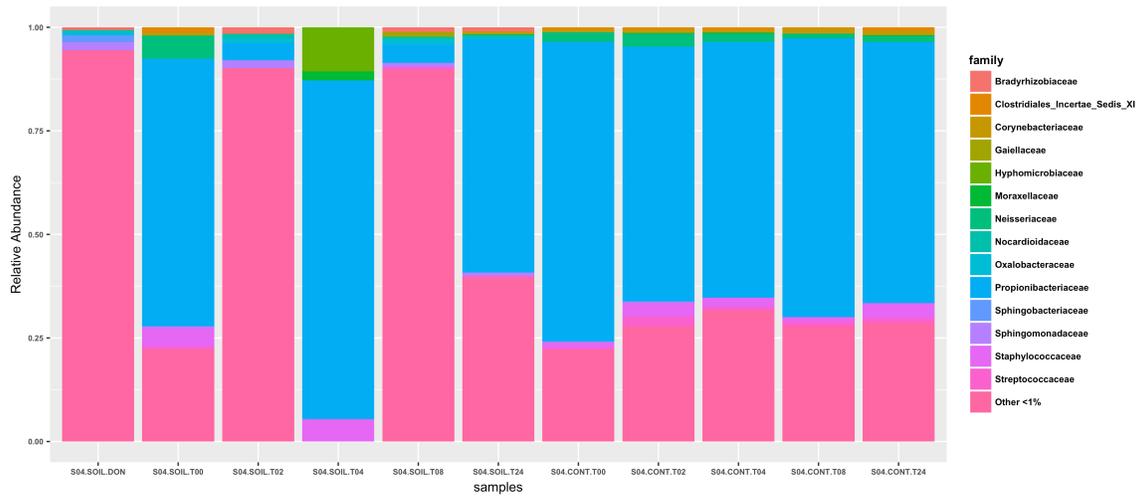
Subject S02



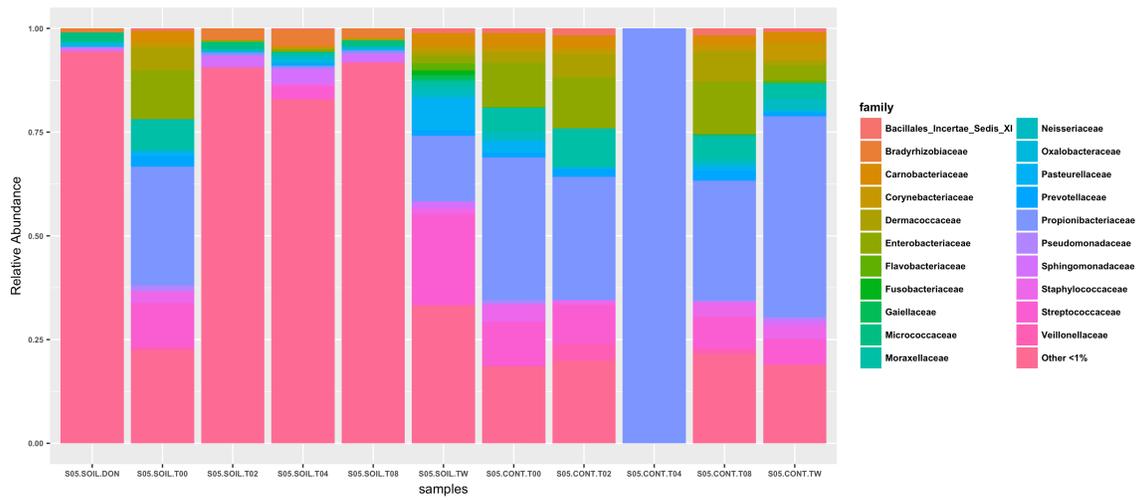
Subject S03



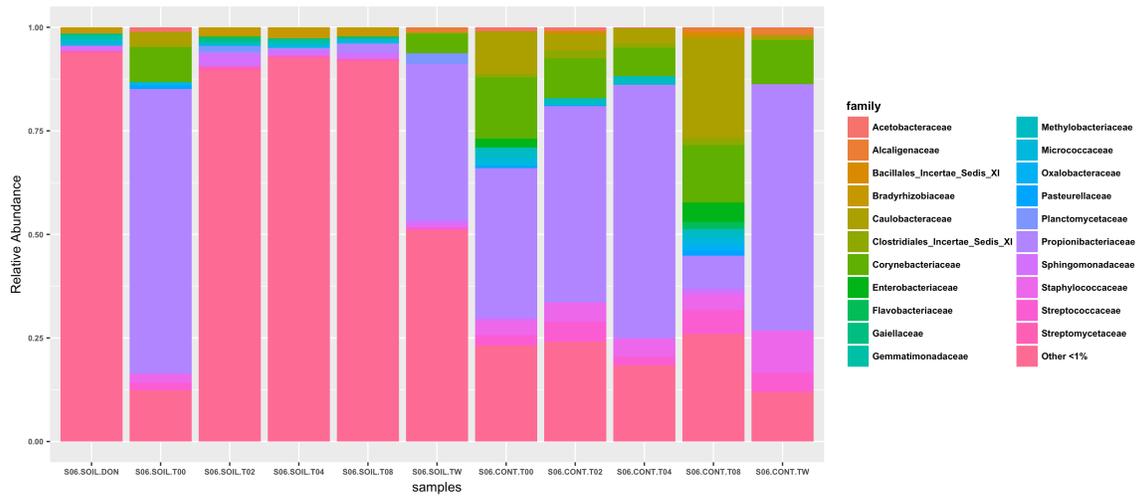
Subject S04



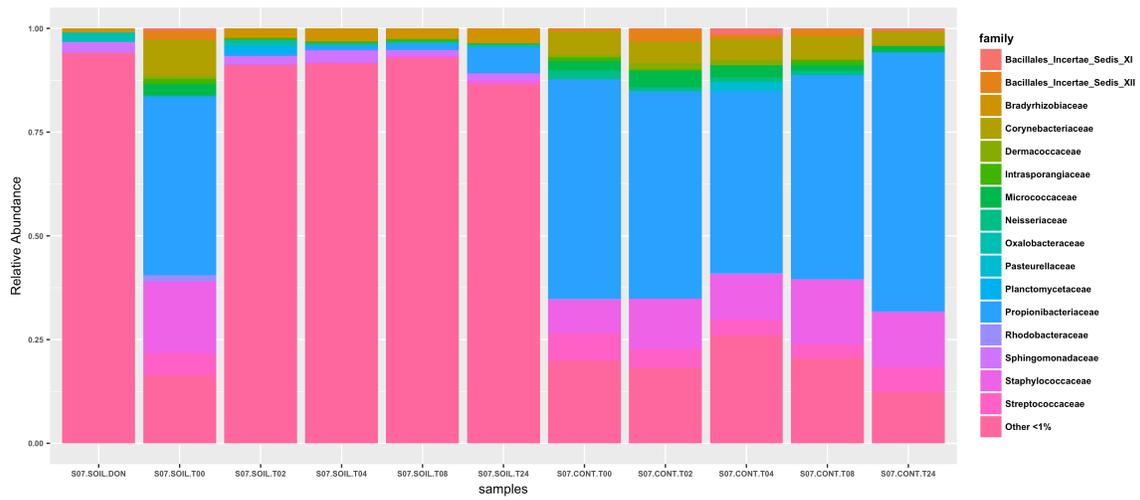
Subject S05



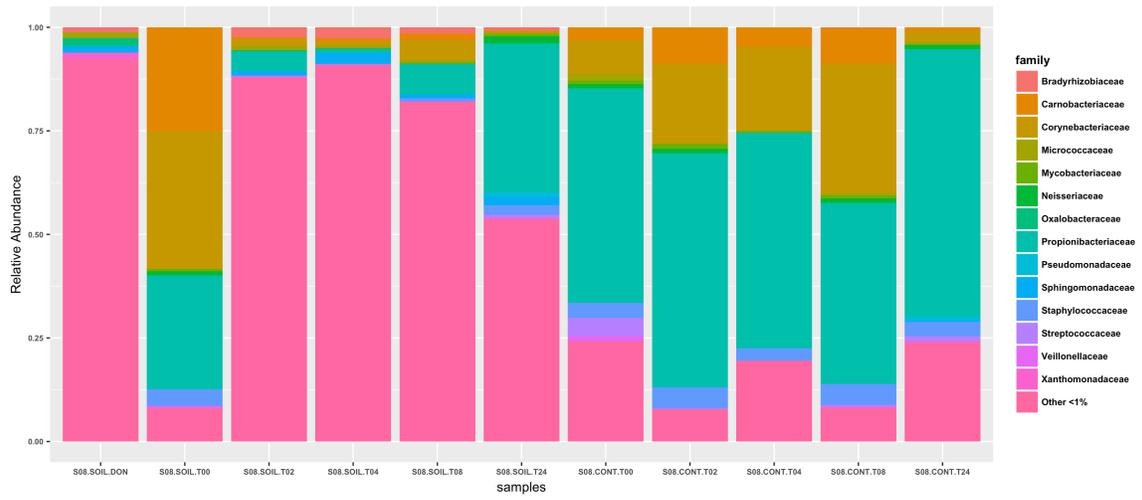
Subject S06



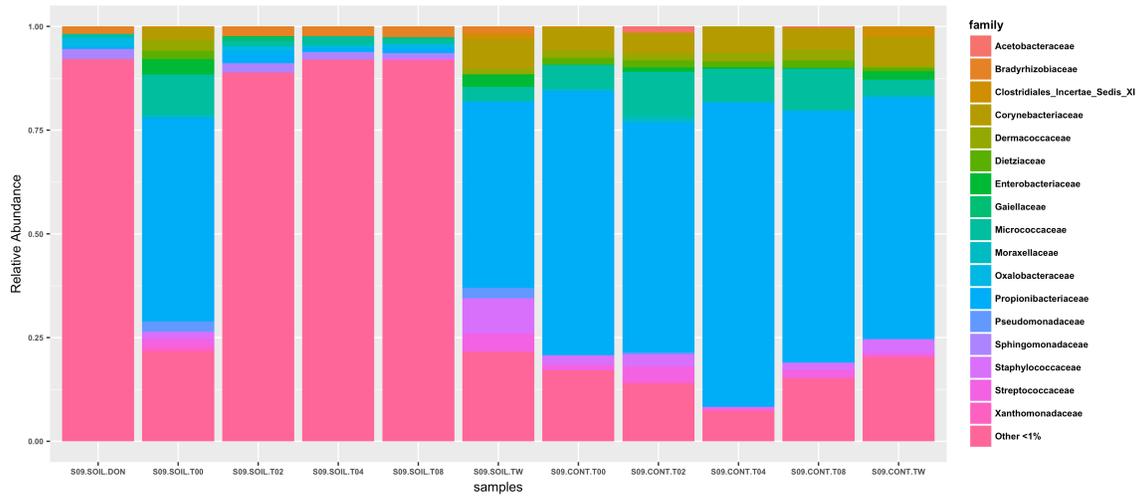
Subject S07



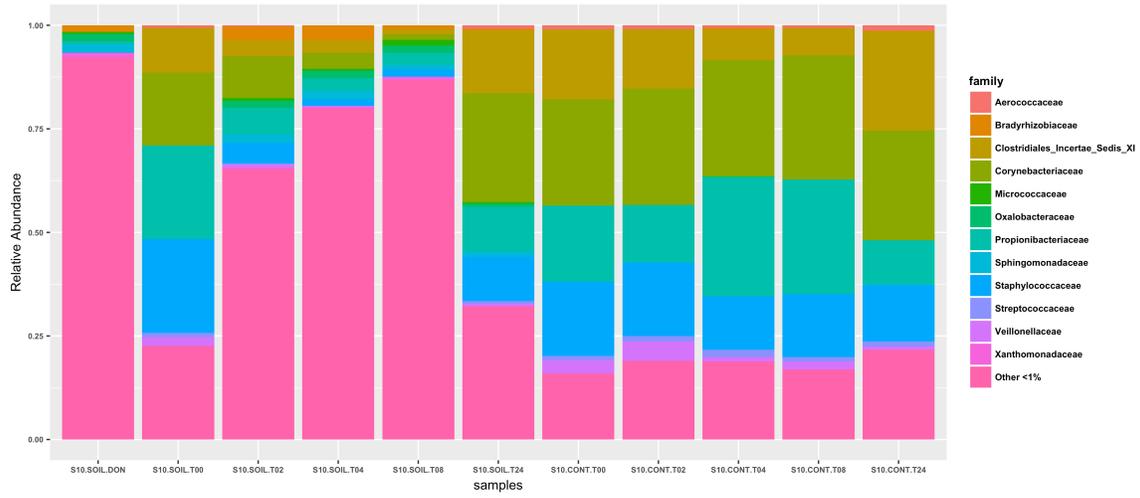
Subject S08



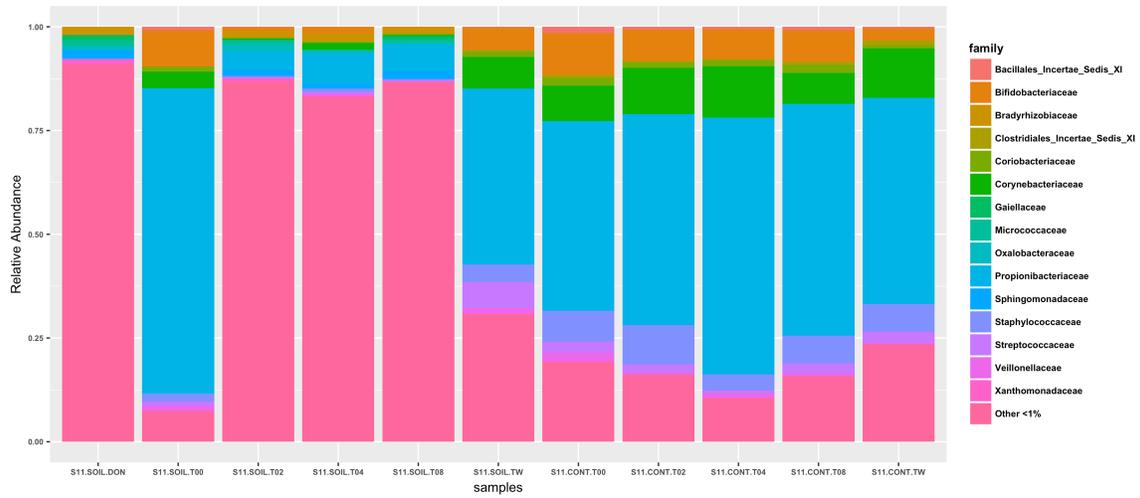
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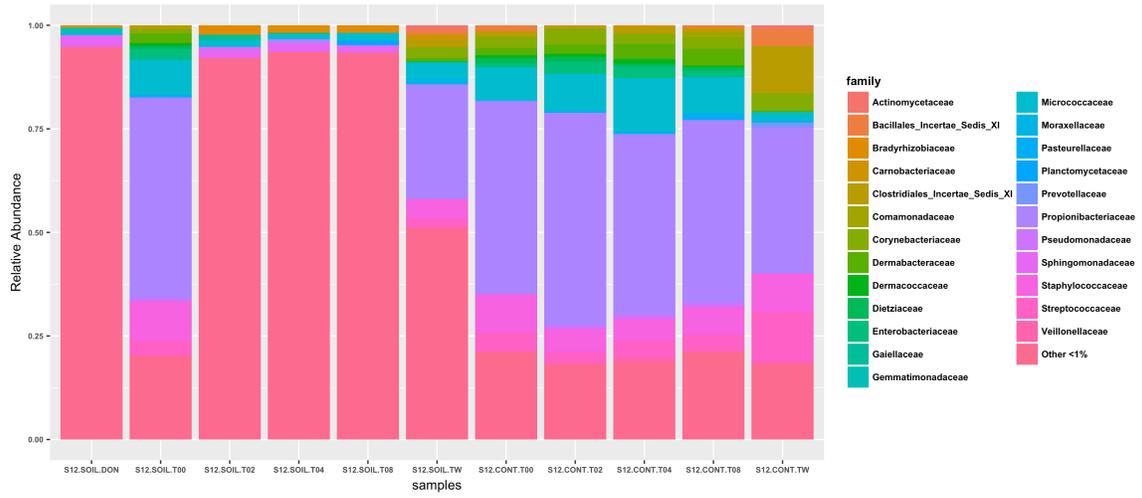
Subject S10



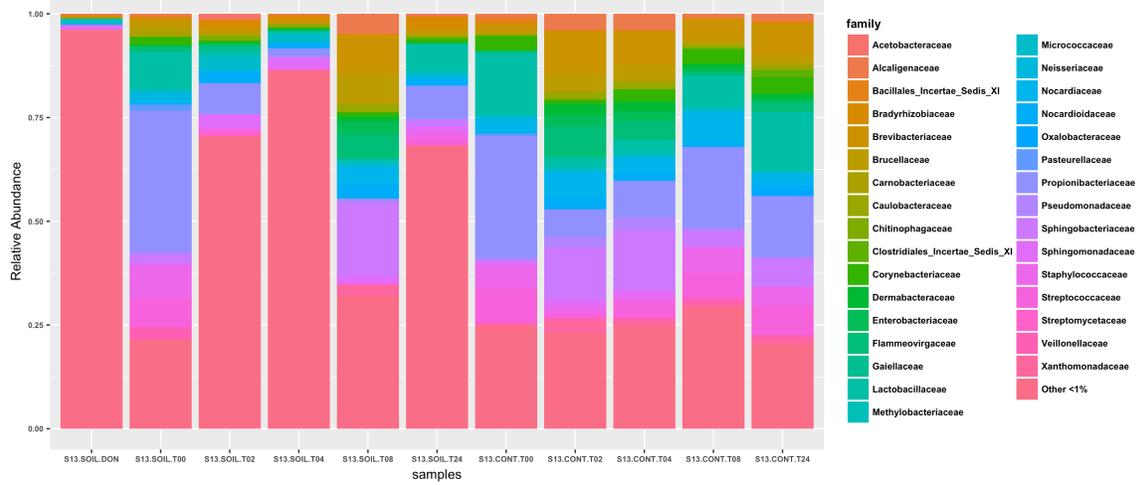
Subject S11



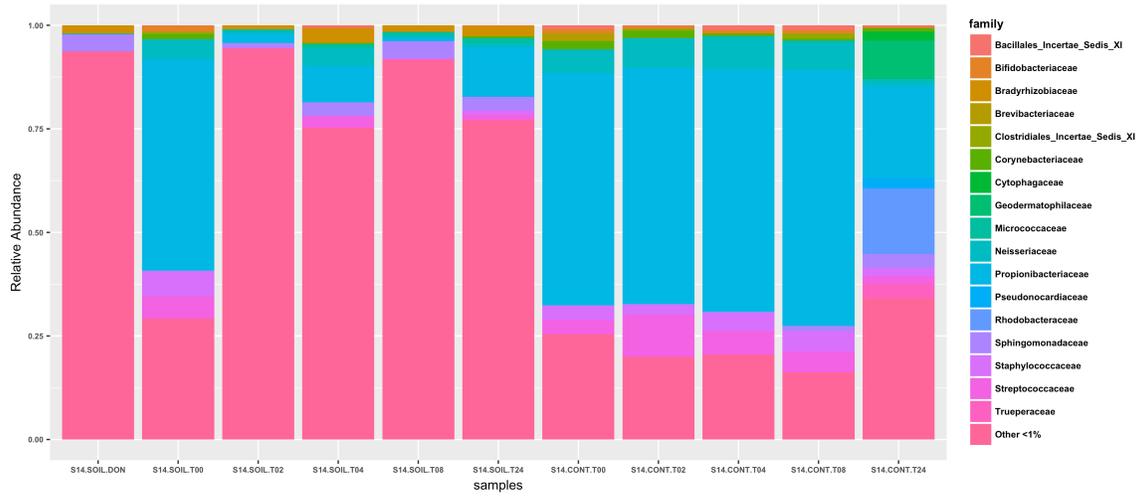
Subject S12



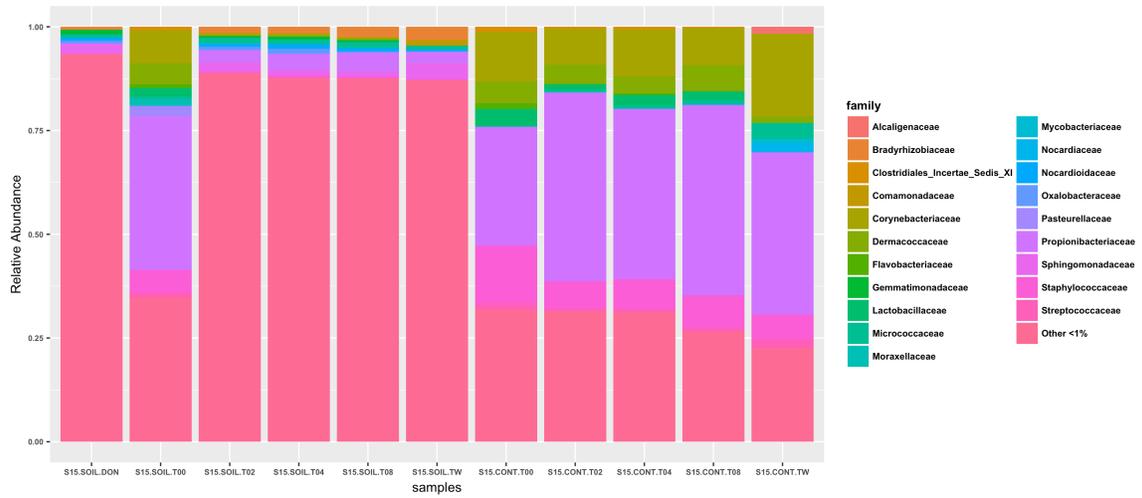
Subject S13



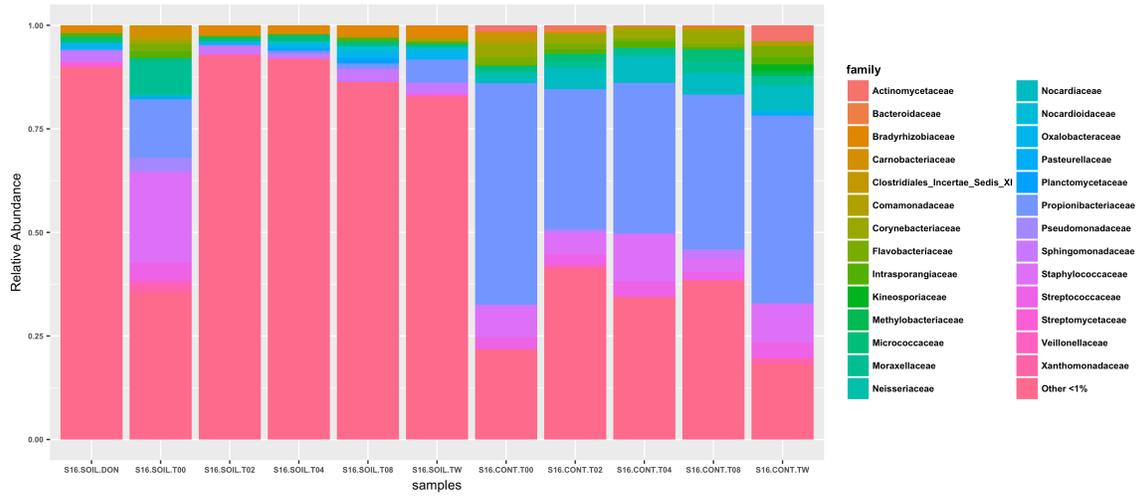
Subject S14



Subject S15



Subject S16



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