

## ABSTRACT

Title of Thesis: THE ECOLOGY OF URBANIZATION: A  
STUDY OF SOIL MICROBIAL COMMUNITY  
RESPONSE

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Urbanization is associated with global biodiversity loss of macrofauna and flora through direct and indirect mechanisms, but to date few studies have examined urban soil microbes. Although there are numerous studies on the influence of agricultural management on soil microbial community composition, there has been no global-scale study of human control over urban soil microbial communities. This thesis extends the literature of urban ecology to include soil microbial communities by analyzing soils that are part of the Global Urban Soil Ecology and Education Network (GLUSEEN). Chapter 1 sets the context for urban ecology; Chapter 2 addresses patterns of community assembly, biodiversity loss, and the phylogenetic relationships among community members; Chapter 3 addresses the metabolic pathways that characterize microbial communities existing under different land-uses across varying

geographic scales; and Chapter 4 relates Chapter 2 and 3 to one another and to evolutionary theory, tackling assumptions that are particular to microbial ecology.

THE ECOLOGY OF URBANIZATION:  
A STUDY OF SOIL MICROBIAL COMMUNITY RESPONSE

by

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## Dedication

I dedicate this thesis to my grandparents, Robert and Amelia Epp, and Melvin and Charlotte Schmidt, who have each committed substantial portions of their lives towards caring for society's vulnerable people, and protecting the environment. I am proud of the ethos of stewardship that they have cultivated, and I am honored to be a part of their legacies.

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# Chapter 1: Humans as Ecosystem Engineers and the Convergence Paradigm

## *A Brief History*

Humans are supremely effective at altering the environment to fit our needs; our species dominates global ecological processes<sup>1</sup>. In the last ten-thousand years we have given rise to entirely new ecosystems, which we called cities. Cities developed concomitantly with an agricultural system that was simultaneously intensive<sup>2</sup> and extensive in nature<sup>3,4</sup>. These cities, and their agricultural support system, exert new controls and represent a new ecological regime across the landscape<sup>5</sup>. In the last two hundred years, cities have transformed in scale, in structure, and in chemical signature, with the effect of vastly increasing their regional impact<sup>6</sup>. The move towards globalization of our economy has had direct impacts on the dispersal of plant and animal material, and has connected the human behavioral controls on ecological function across the globe, in what has been referred to as the tele-coupling of ecosystem services<sup>7</sup>.

Our function as a keystone species and ecological engineer has evolved; we began with a propensity to encourage plants that provisioned us. But our ecological manipulations grew as we domesticated plant and animal varieties, harnessed water and shaped its flow across the landscape, and demolished entire mountains and laid down vast impervious surfaces. We have even exerted our control over the very chemical composition of the atmosphere and perturbed our planet's long-term climactic cycles<sup>8,9</sup>.

Western tradition has traditionally held human activity apart from the natural world, as the “unnatural” yang to the yin of nature. As such, ecologists have at times struggled to place human ecology as a part of the natural landscape in ecological framework. This cultural phenomena is covered extensively in the conservation biology literature<sup>10</sup> as conservation biologists and ecologists have been forced to wrestled with our widespread cultural value system. But we have made great strides as we have embraced both the role of keystone species and ecological engineers in ecosystem processes, and our particular efficacy in this role.

In this philosophy, we have created various frameworks for human control in ecological processes. In one instance, this has been conceptualized as the biotic homogenization hypothesis<sup>11</sup>. This hypothesis posits that human behavior is a dominant control on ecosystem process across a human-influenced landscape by intentionally and unintentionally promoting organisms better adapted to human disturbance over those sensitive to human impact<sup>12</sup>. This construct has been particularly applied in urban ecology<sup>13</sup>, in agricultural landscapes<sup>14</sup>, and to forests<sup>15</sup> in order to connect local management behavior with global ecological patterns. A shortcoming of the biotic homogenization hypothesis is its implicit focus on active transport and promotion, of species as the driving force behind community ecology, and the de-emphasis of other indirect human impacts and interactions. Work in urban ecology has led to the reformulation of the biotic homogenization hypothesis as the urban convergence hypothesis. This approach focuses on the diverse set of human behaviors that structure the interaction between physicochemical alterations imposed on the landscape by urbanization and the adaptive responses of individual organisms<sup>16</sup>. These include such

properties as human transport and cultivation, but are not limited by them. According to the urban convergence hypothesis, the aggregate of these behavior-environment feedback interactions across the landscape lead to a convergence of urban community ecology and ecosystem process towards similar states across the globe.

### *Mechanisms and Outcomes of Cultural Control*

Urban centers represent only about 2 percent of the human-occupied land area<sup>5</sup>, but have far reaching implications on global biogeochemical cycles and biodiversity<sup>6</sup>. Urban centers control land-use via their pattern of consumption. Some resources, such as drinking water, must be captured at local-regional scales. On the other hand, food may be traded globally. In such a way, local environmental services such as water provisioning become tele-coupled at regional and global scales by urban living and global trade<sup>7</sup>. A fundamental feature of the human managed landscape is patchiness<sup>17</sup>; driven by land-use partitioning<sup>18</sup>. Humans meet the urban resource demands by partitioning the landscape at local, regional, and global scales, to maximize the production of the required resources to support cities<sup>19</sup>. Physical infrastructure, such as road and rail systems, have direct impacts on local topography, and also catalyze global trade in resource-use, effectively coupling local land-use decisions with global-scale resource partitioning. These multi-scalar interactions are fundamental to the understanding of human ecology. For example, crop diversity hotspots (regions in the United States that maintain unusually high crop diversity, particularly heirloom varieties) such as Appalachia, maintain a deep cultural heritage celebrating local crop varieties and are generally isolated from market opportunities<sup>20</sup>. To fully understand human impacted ecology, we must acknowledge and

incorporate cultural and economic knowledge so as to understand the complex landscapes that humans create and to also make practical and feasible management decisions.

### GLUSEEN

The Global Urban Soil Ecology Education Network (GLUSEEN) is an international collaboration among urban ecologists to use a common methodology to understand soil ecosystem process in urban centers. GLUSEEN primarily seeks to test the urban convergence hypothesis, which posits that urbanization creates a habitat suitable for humans, and thus urban habitats tend to converge towards one another relative to their historic state. GLUSEEN has treated urbanization as an intensification of human impact in two categories: management, and disturbance. The framework of urban centers as a patchwork of land-use decisions is central to the experiments of GLUSEEN. Within this experiment, seven land-use categories are defined with the intention to capture a range of human impacts along management-disturbance spectrums; however this pilot study uses only four of the GLUSEEN categories (Table 1.1). The categories used in this study are defined as follows: reference sites are unmanaged sites that represent the historic state of the ecosystem. Remnant sites are similarly unmanaged sites reflecting the historic state of the ecosystem, but are located within the urban matrix. They are intended to capture the influences of anthropologically influenced floral and faunal communities (including human use), and to capture the array of chemical influences of urbanization (atmospheric N deposition, elevated CO<sub>2</sub> concentrations, heavy metal deposition etc.). The turf site category is intended to represent a characteristically human ecosystem: lawns. This is our management category, and is intended to capture the array of behaviors that humans leverage to keep their lawns presentable by cultural standards. The ruderal site category is



the disturbance category, representing substantial disruption of the soil profile through demolition or development work, and also representing a shorter time since disturbance.

Table 1.1: A summary of the four site categories defined along a disturbance management spectrum from low disturbance and low management to high disturbance or high management.

Site	Within City	Disturbed soil	Unmanaged
Reference			✓
Remnant	✓		✓
Turf	✓	✓	
Ruderal	✓	✓	✓

In my definition of management, I do not treat human intervention per se as a disturbance event; rather we treat human management as imposing a new homeostatic condition that persists through time. It is, however, important to acknowledge that much of the urban landscape is significantly disturbed early in the urbanization process, and therefore most management conditions within urbanization are applied after a disturbance event. Thus, greater time since disturbance is an implied feature of the management category.

At each sampling location, GLUSEEN protocols stipulate the collection of edaphic data, including data on physiochemical properties of soils (texture, pH, SOM; available N, P, and K etc.), and biological features (decomposition rates and microbial community composition). These sampling protocols were replicated across five urban centers that were distributed across the globe: Baltimore, USA; Helsinki and Lahti, Finland; Budapest, Hungary; and Potchefstroom, South Africa. Each site category was

replicated five times within each city. Data on physicochemical properties of urban soils have been reported separately<sup>16</sup>, and are used as explanatory variables for interpreting the microbial community data reported in this thesis. A key objective of GLUSEEN is to provide a proof of concept for including citizen science in soil ecology. In view of that objective, the microbial dataset presented in this thesis is a supporting dataset that will be used in interpreting the decomposition data that is part of a related citizen science project

### *Unique Energetic Controls on Soil Microbial Ecology*

Carbon (C) availability, soil pH and redox potential (eH) contribute to determining which metabolic strategies provide positive energetic returns for microbes. The factors affecting microbial community assembly are thoroughly reviewed by Nemergut et al., (2013)<sup>21</sup>. Among the typical cadre of factors that are studied in community ecology (such as dispersal and life history), the unusual diversity of metabolic strategies among microbes is a prominent feature that defines our understanding of microbial community assembly. In brief: microbial energy capture can be broadly divided into two strategies: autotrophy and heterotrophy. Although autotrophy linked to photosynthesis is common in plants, microbes engage in a wider range of autotrophic strategies that include the oxidation of ammonium, hydrogen, iron, and sulfur. Heterotrophy is dominant in most microbial communities, however, and relies on sufficient carbon (C) inputs. Under anaerobic conditions, some microbes can carry out fermentation reactions and others use alternative electron donor-acceptor pairs. Landscape features, such as water saturation or the mineralogy of the soil thus influence community composition and metabolic activity.

## Urban Soils

A recent publication by the Global Urban Soil Ecology Network (GLUSEEN) have drawn attention to some “universal” impacts of urbanization on soils. Particularly, GLUSEEN documented a convergence of soil pH, soil organic matter (SOM) concentration, and total soil nitrogen (N)<sup>16</sup>. SOM and pH were inversely related, with highest pH and lowest SOM generally occurring in turf and ruderal sites across cities, and the greatest similarity across cities (convergence) in soil pH and SOM occurred in ruderal sites. However, they also found that available potassium and phosphorous diverged within land-use categories among cities, with the greatest divergence occurring in turf sites. It was not clear whether this was due to an interaction with parent material or fertilization; however, it is worth noting the phosphorous and potassium are each common in fertilizers.

An assumption of this research is that similar ecological processes across urban environments leads to the similar outcomes. If we consider the five factors of soil formation (climate, organisms, time, topography, parent materials), urbanization either directly or indirectly impacts each factor. Aspects of climate such as temperature (via the heat island effect<sup>22</sup>), and moisture regime (through irrigation and drainage<sup>17</sup>) are influenced by urbanization. The macrofloral and faunal communities are certainly altered by urbanization<sup>12,13,23</sup>, and this thesis will show that the microbes are as well. Microbes in particular have been shown to contribute to mineral transformations, contributing to soil weathering<sup>24-26</sup>. The grading and drainage that occurs in development and the installation of new roads can alter local topography, effectively changing the landscape position of soils. Particularly at early stages in soil development, parent material is an

important factor in soil formation; urbanization introduces entirely new parent material (concrete, asphalt, gravel etc.) onto the landscape<sup>27</sup>. It has been argued that in the cases where we bury soils under anthropogenic material (as opposed to transported soil material)<sup>27</sup> that we reset the clock for pedogenesis and thereby also impact the time factor of soil formation<sup>28</sup>. Logically, if urbanization impacts each of the five important factors in pedogenesis, then it must be true that the processes of pedogenesis are altered by urbanization.

These well-known anthropogenic changes to local hydrology, pH, and carbon inputs<sup>23,29,30</sup> impact the energetic potential on which microbial life is dependent. For example, while elevated N inputs in the urban setting may prime the microbial community for greater dominance by N-cycling organisms, the transformations that take place depend on the form of N input, C availability, and redox potential of the system. While ammonium might be oxidized by ammonium oxidizing archaea, if the inputs are primarily oxygenated N species (such as nitrate), ammonium oxidation is not available as a metabolic pathway. Denitrification may occur, but only if soils become sufficiently anaerobic and sufficient C substrate exists to fuel the reaction. Therefore, the convergence of various landscape features will shape the microbial community.

We may expect that if humans are imposing a consistent environmental filter via our various impacts, then ecological communities, ecological function, and ecosystem processes should converge in accordance with the type of impact. There is a modest literature<sup>13</sup> documenting the phylogenetic convergence of plant communities<sup>31</sup>, insect communities<sup>32</sup>, and bird communities<sup>33</sup> in urban environments. However, I am only aware of one study that explicitly considers land-use on the convergence of microbial

community composition, and that on bacterial community structure in a single agricultural system in Brazil<sup>14</sup>.

### *The Questions Considered*

Microbial ecologists have been challenged to justify the study of soil microbial communities with arguments which claim that microbial community composition is not a necessary factor in predicting soil ecosystem function<sup>34</sup>. Yet soil microbes are important: loss in soil microbial diversity has been shown to significantly impact N cycling<sup>35</sup>, and there is a general recognition that large portions of the plant community (including some important agricultural cultivars) depend on fungal symbionts for nutrient capture<sup>36</sup>. Meanwhile, certain groups of microbes, such as fungal root symbionts, are more likely to act as keystone species (e.g. the presence or absence of ectomycorrhizal fungal networks may impact seedling success and thereby control plant succession<sup>37</sup>). In recognition of their importance, microbial ecologists have studied microbial community dynamics; the literature available on microbial community stability would suggest that structural disruption of soils significantly increases the vulnerability of the microbial community to other forms of disturbance<sup>38</sup>. The richness of a community (or biodiversity), which is often incorporated into beta diversity metrics in microbial ecology, may not always be a good predictor of ecosystem response because dominant, minor, and keystone species each play different roles in determining a given stability domain<sup>39-41</sup>. In an urban center, however, where soils are regularly and significantly disturbed, a sharp decrease in species richness may relate to the loss of specific functions<sup>42</sup>.

Both direct and indirect human management may influence local ecology; the four GLUSEEN site categories are intended to capture a range of effects including direct

disturbance effects (ruderal), direct management effects (turf), indirect effects of anthropogenic dispersal and atmospheric deposition (remnant), and also includes minimally affected sites (reference). This thesis seeks to answer the broad question: **Do defined urban land uses have a characteristic impact on soil microbial community composition and functional potential?** If so do these common land use features lead to convergence among urban microbial communities (i.e. what is the interaction between urbanization and microbial biogeography on a global scale)?

Chapters 2 and Chapter 3 represent two different molecular approaches to understanding microbial community response to land-use regimes. Chapter 2 uses amplicon sequencing to ask: do the microbial communities in all cities experience a phylogenetic convergence, leading to a uniquely urban soil microbial community? I hypothesize that land use will cause microbial communities to converge relative to the “historic” proxy category (e.g. “reference”), and that the convergence will be driven by a unique interaction between land-use and the ecology of the specific microbes making up the community.

Chapter 3 uses shotgun sequencing to generate a microbial metagenome. This approach allowed me to identify metabolic pathways that reflect adaptations to the new environmental conditions. Microbial metagenomic functional annotations have been shown to be sensitive enough to detect biome-scale differences in community function pathways using the same techniques employed in chapter 3<sup>43</sup>. I address three major questions: 1) Are the distribution of soil microbial functions driven by geographic differences among cities? 2) Do land-use categories structure microbial metabolic pathways? 3) Does land-use drive a convergence of functional profiles on a global scale?

I hypothesize that there will be detectable differences in the abundance and composition of functional DNA pathways according to city (geography) and to land use. Furthermore, there will be a convergence of functional pathways under globally similar management schemes. In my concluding chapter, I compare the metagenomics approach to the amplicon sequencing approach, and discuss some biases associated with annotating the shotgun library sequences.

## Chapter 2: Urbanization Leads to a loss of Ectomycorrhizal Fungal Diversity and the Convergence of Archaeal and Fungal Soil Communities

### Introduction

We are in an era of unprecedented human impact<sup>5</sup>, as modified landscapes alter ecosystem function<sup>44,45</sup> and shift the local physical, chemical, and biotic environments<sup>46,47,23</sup>. Urban ecosystems favor species that are well-adapted to a variety of anthropogenic processes such as frequent disturbance, increased nutrients, landscape fragmentation, and facilitated dispersal<sup>11,12,48</sup>. Thus, the impact of urbanization on global species distribution has been characterized as Biotic Homogenization (BH)<sup>12,23,13</sup>. It has been suggested that BH is a major mechanism of global biodiversity loss<sup>11</sup>. There is conflict within the literature, however, as BH is often interpreted to mean that particular species are expected to appear across all urban areas and exclude endemic populations<sup>49</sup>. Although it is possible that urbanization leads to cosmopolitan species such as *Columba livia domestica* (pigeons) or *Festuca arundinacea* (tall fescue grass); it is also possible for two urban centers to contain different species, for example *F. arundinacea* and *Poa pratensis* (Kentucky blue grass), but converge in terms of functional traits<sup>50</sup>. Such convergence can nonetheless lead to biodiversity loss if it results in the exclusion of one guild in favor of another (e.g. opportunists over competitors)<sup>48</sup>.

Diversity metrics that are used by community ecologists capture positive occurrence patterns; because these metrics do not measure extinction directly,



biodiversity loss is determined based on inferences made from those metrics<sup>51</sup>. The techniques used to make these inferences depend on the species, tools available, and question being asked. In This case, I considered global biodiversity loss to be likely only if the microbial community exhibited a strong pattern of biogeography (representing the “average” degree of endemism, and thus vulnerability to extinction), exhibited a convergence in community composition similarity (implying a decreased prevalence of unique taxa), and a decrease in both community richness and abundance (demonstrating site specific decreases in biodiversity)<sup>51,52</sup>. Thus, I compared the communities from five cities on three continents to explore the relationship between urbanization, community convergence, and global biodiversity in soil microbes.

#### *The Global Urban Soil Ecology Education Network*

Soils were sampled from five cities: Baltimore, USA; Helsinki and Lahti, Finland; Budapest, Hungary; and Potchefstroom, South Africa (Figure 2.1). These cities are all part of the Global Urban Soil Ecology and Education Network<sup>47</sup>, a distributed network of urban ecologist using agreed upon protocols to investigate urban soils.

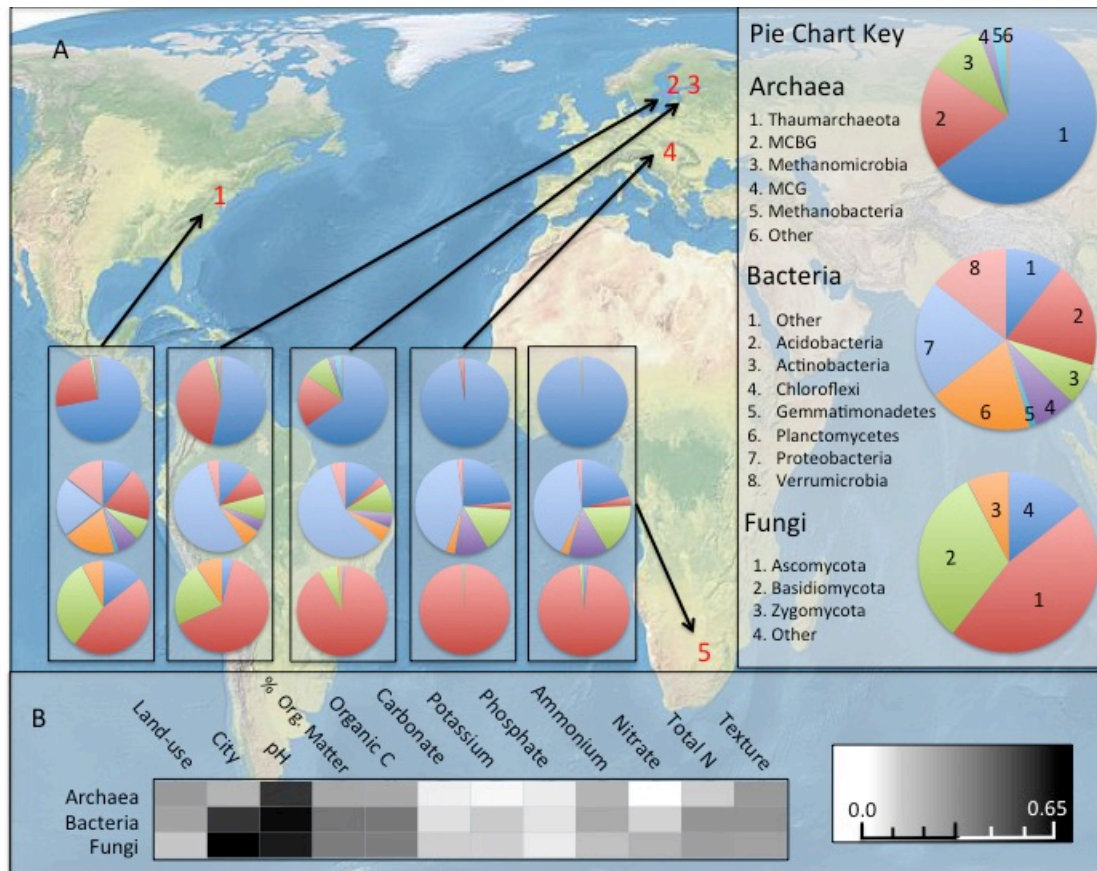


Figure 2.1: A) Geographic distribution of cities: 1) Baltimore, USA; 2) Helsinki, Finland; 3) Lahti, Finland; 4) Budapest, Hungary; 5) Potchefstroom, South Africa. Pie charts represent the average abundance of phyla across all samples for a given city (n=20). The top pie charts show the six most abundant archaeal class. The middle pie charts show the seven most abundant bacterial phyla. The bottom pie chart shows distribution of the most abundant fungal classes. B) The heat map represents the R-squared values of correlations between edaphic factors and community composition of each community.

The GLUSEEN experiment defined seven land-use categories that are designed to represent a range of disturbance and management impacts of the urban landscape; this portion of the pilot study employs four of these categories (Table 2.1). In our definition, disturbance is an event that substantially, physically disrupts the soil, which results in an undifferentiated soil profile (e.g. soils are massive). Foot traffic across a site, for example, would not be considered to be a disturbance event because the soil remains intact. Management is defined as an intentional alteration to the ecosystem state that persists through time. Our four land-uses were: reference, remnant, turf, and ruderal

(Table 2.1). Reference sites were unmanaged, undisturbed sites located outside the urban matrix that had similar soil characteristics to those found in the city<sup>47</sup>. Remnant sites are reflective of the historic ecosystem type, but are located within the city. Turf sites included park, residential, and municipal lawns maintained in a homeostatic state due to mowing and other management. Ruderal sites were chosen based on a recent history of substantial disturbance to the soil profile. Typically, ruderal sites were recent construction or demolition sites. Each land-use category was replicated five times within each city, totaling 100 sites in the study.

Table 1.1: A summary of the four site categories defined along a disturbance management spectrum from low disturbance and low management to high disturbance or high management.

Site	Within City	Disturbed soil	Unmanaged
Reference			✓
Remnant	✓		✓
Turf	✓	✓	
Ruderal	✓	✓	✓

### *Biogeography and Urbanization*

An important indicator of a community's vulnerability to biodiversity loss is the degree of endemism among its members<sup>53</sup>; cosmopolitan organisms are less vulnerable to extinction given a local habitat alteration. In the case of microbial communities, I interpret the strength of biogeographic patterns as indicative of the average degree of endemism among community taxa. I clustered microbes at 97% sequence similarity into Operational Taxonomic Units (OTU); analyses at other taxonomic resolution were

clustered according to putative taxonomy. The relative abundance of putatively defined groups across all the site categories were averaged, and separate abundance distributions were created for archaea, bacteria, and fungi (Figure 2.1A). The abundance of major taxonomic groups differed across cities for all three domains, but they were more pronounced in bacteria and fungi, compared to archaea.

In the bacterial community, the two Finnish cities were more similar and Baltimore, USA was the most distinct (Figure 2.1A). For example, Planctomycetes averaged 17% in Baltimore soils, but made up less than 7% of all sequences in the other four cities (Figure 2.1A). Proteobacteria were most abundant in the two Finnish cities and was the lowest in Baltimore. Thus, the bacteria exhibited a strong pattern of global biogeography ( $P < 0.001$ ), as cities generally separated from one another. Nested within each city, bacterial communities in the reference and remnant sites did tend to separate from turf and ruderal sites, but the turf and ruderal sites for the individual cities remained distinct (Figure 2.2D;  $P < 0.001$ ). This was especially true for Baltimore soils. Along with composition, bacterial gene quantity was determined by multiplying Q-PCR determined 16s gene copy numbers to the percent abundance of sequences matching bacterial OTUs. This multiplication was necessary, because I used the same primers for both Q-PCR and sequencing (Table 2.1), a primer set that simultaneously amplifies both archaea and bacteria (archaea discussed below). Bacterial gene copies numbers were lower in all three within city sites compared to the reference (Figure 2.2E:  $P = 0.011$ ) and did not differ in terms of species richness (Figure 2.2F).

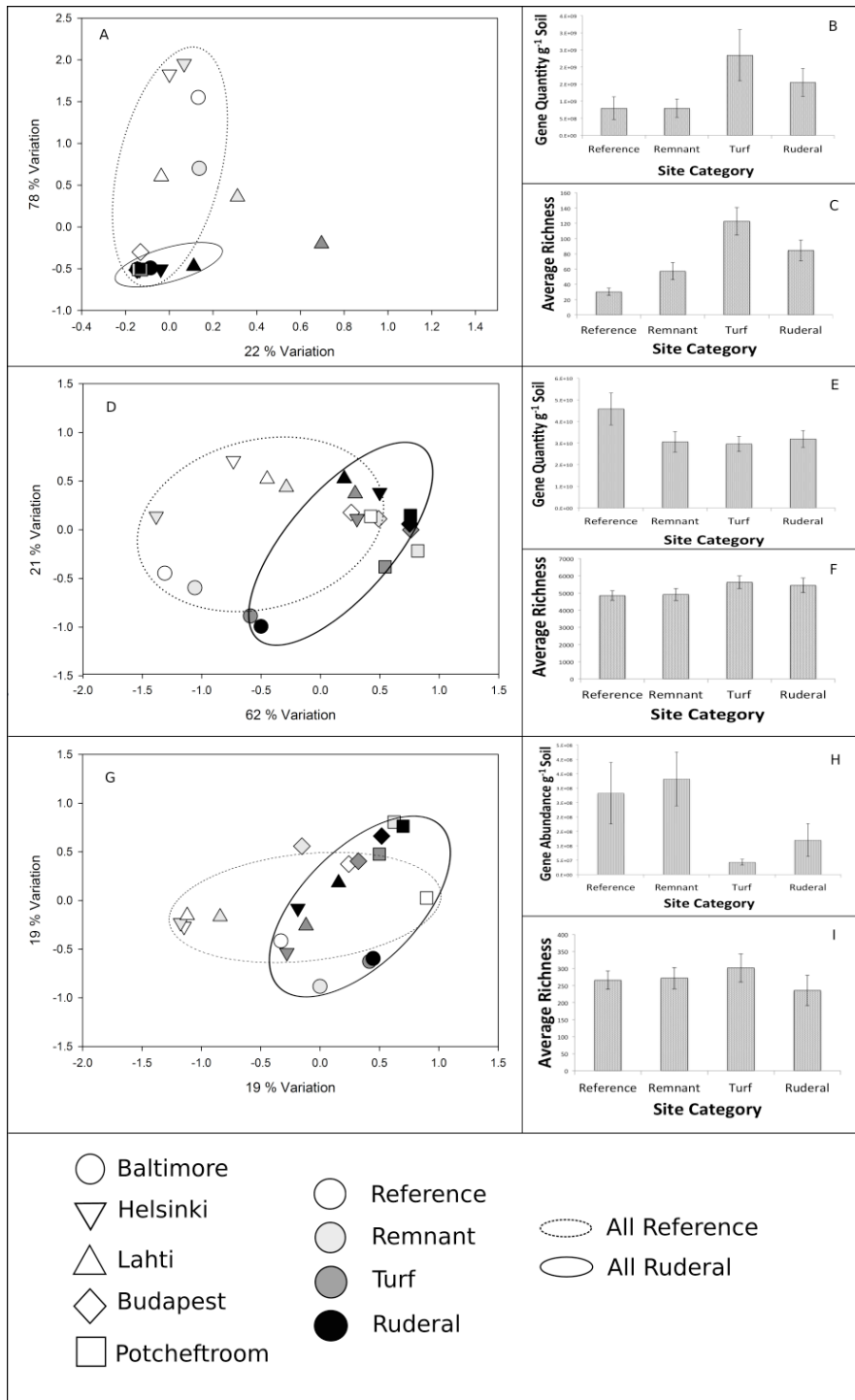


Figure 2.2: Non-metric multidimensional scaling ordinations of archaea (A), bacteria (D), and fungi (G). Gene quantities per gram of soil for archaea (B), bacteria (E), and fungi (H). Average species richness for archaea (C), bacteria (F), and fungi (I). In all cases, error bars represent standard error.

Table 2.1: Primer information for all analyses.

Target	Sequence	Gene info	Use
5.8S	CGC TGC GTT CTT CAT CG	Intergenic spacer region fungi	QPCR
ITS1F	CTTGGTCATTTAGAGGAAGTA	Intergenic spacer region fungi	QPCR
ITS1f+adapter	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG-CTTGGTCATTTAGAGGAAGTAA	Overhang adapter+ITS primer ITS1F	Sequencing
ITS2r+adapter	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG-GCTCGCTTCTCATCGATGC	Overhang adapter+ITS primer ITS2R	Sequencing
F515	GTGCCAGCMGCCGCGGTAA	16s rRNA	QPCR
R806	GGACTACVSGGGTATCTAAT	16s rRNA	QPCR
F515+adapter	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGGTGCCAGCMGCCGCGGTAA	Overhang adapter+16s primer ITS1F	Sequencing
R806+adapter	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGGGACTACVSGGGTATCTAAT	Overhang adapter+16s primer ITS2R	Sequencing

Similar to the bacteria, fungal communities, , showed a strong pattern of land-use (P<0.001) nested within a pattern of biogeography among fungal OTUs (P<0.001; Figure 2.2G), but unlike the bacteria, turf and ruderal sites contained significantly fewer fungal ITS gene copy numbers (Figure 2.2H; P=0.01). Species richness did not change between the four site categories (Figure 2.2I). The relative proportions of Ascomycota to Basidiomycota drove global differences in the fungal biogeography (Figure 2.1A). And similarly to bacteria, the fungal ordinations showed that in each site category, Baltimore sites separated from similar categories of the other four cities (Figures 2.2D and 2.2G).

In contrast, the archaeal communities had limited evidence for biogeographic distribution. Although there was some variation in the abundance of some phyla among cities, a PerMANOVA analysis shows no significant effect of city among archaeal genera (Figure 2.2A; P=0.17). More detailed abundance distribution is provided in the Supplementary Materials. Along with composition, archaeal gene quantity was determined by multiplying Q-PCR determined 16s gene copy numbers to the percent abundance of sequences matching archaeal OTUs. Surprisingly, turf sites contained the most archaeal 16S rRNA gene copy numbers (2.34E+9 gene copies g<sup>-1</sup> of soil; P=0.034), with fewer archaeal sequences detected in both the reference and remnant (Figure 2.2B). Likewise, archaeal species richness was also higher in the turf sites (Figure 2.2C);

$P < 0.001$ ). These data suggest that management practices such as increased nutrients may favor a larger, more diverse archaeal population (see Figure 2.5 for more detail).

Microbial community composition was correlated to a number of physiochemical parameters to further elucidate distribution patterns. Global bacterial and fungal community compositions were found to be highly correlated with soil pH, % organic matter, and organic C (Figure 2.1B). Physicochemical properties of these soils have been reported separately<sup>47</sup>. Soil pH strongly correlated with the bacterial community ( $R^2 = 0.61$ ; Figure 2.1B) and fungal community ( $R^2 = 0.57$ ; Figure 2.1B), a phenomena that has been reported in the literature previously<sup>54,55,56</sup>. The archaeal community composition also correlated with pH ( $R^2 = 0.51$ ; Figure 2.1B), but had lower correlation coefficients with % organic matter and organic C compared to bacteria and fungi (Figure 2.1B). These data suggest that archaeal distribution does not necessarily correspond to bacterial or fungal distribution, and in particular archaea appear to be less impacted by soil

### Urban Soil Convergence

Convergence of microbial communities represents a decrease in dominance of site-specific species, which may result in a loss of biodiversity. I measured convergence as a reduction of between-site variance through time, and regarded the reference sites as a proxy for the historic state of each city. I used Bray-Curtis dissimilarity index to compare site similarity. If microbial communities were converging, I expected to see dispersed reference sites for each city, but clustered (e.g. more similar) urban sites. The archaeal communities exhibited this relationship well; the reference and remnant sites were more variable especially from Baltimore, Helsinki, and Lahti, but the turf and ruderal sites were very similar (Figure 2.2A;  $P < 0.001$ ); thus a PerMANOVA analysis of archaeal

genera found no significant effect of city on community composition (e.g. no biogeography), but a significant effect of landuse ( $P < 0.001$ ).

I tested for convergence using the `betadisper` function in the `vegan`<sup>57</sup> statistical package in R (version 3.1.3), a multivariate analogue of the Levene's test of homogeneity of variances. I analyzed variance according to three site groupings (Figure 2.3); first I compared the four groups according to their land-use category, then I categorized sites into high or low impact groups (discussed below), and finally I categorized sites into groups based on whether they fall inside or outside of the urban matrix. These groupings were applied to the global dataset (Figure 2.3), and within each individual city (data not shown) in order to elucidate potential drivers of convergence. With the exception of archaeal ( $P = 0.037$ ) and fungal ( $P = 0.004$ ) communities in Baltimore, there were equal category-wise (land-use) variances of community composition in each individual city, indicating that generally convergence is not detectable at local scales.



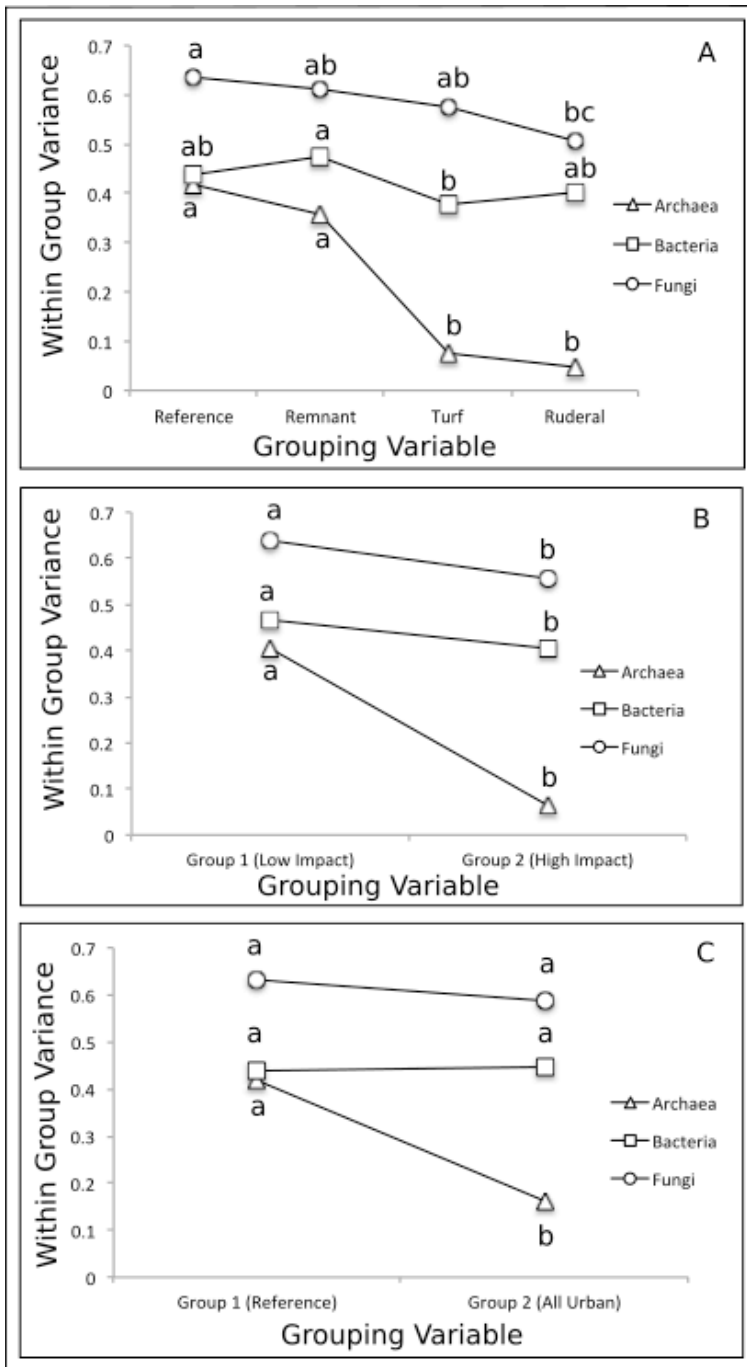


Figure 2.3: Euclidian distance from centroid, representing within-group variance. Points are the average of all cities. Groups were defined as A) Group 1 is reference, Group 2 is remnant, Group 3 is turf, Group 4 is ruderal B) Group 1 is reference and remnant sites, and Group 2 is turf and ruderal; C) Group 1 is reference sites and Group 2 is all other sites. Lowercase letters indicate significant difference between groups.

When I analyzed group variance according to site category, archaeal community converged ( $P < 0.001$ ); turf and ruderal site were less variable relative to reference and

remnant sites (Figure 2.3A). The fungal community also converged ( $P=0.047$ ); ruderal sites were less variable relative to reference sites. The bacterial community differed in variance between site categories ( $P=0.023$ ); however, a post hoc TukeyHSD indicated that this was driven by a slight increase in variance among remnant sites and slight decrease in variance among turf sites relative to reference. Thus, while the variance of remnant and turf sites differed, neither the variance of turf nor ruderal sites differed significantly from the reference (Figure 2.3A).

Using the same approach, I explored whether microbial communities converge simply because they exist within the urban matrix, or whether direct management and disturbance led to a greater degree of convergence. Thus, I conducted two tests to investigate the effect of management and disturbance on convergence. First, I grouped all reference and remnant sites together (Low impact), and compared them to a grouping of all turf and ruderal (High impact; Figure 2.3B). In this case, all three microbial communities converged (archaea  $P<0.001$ ; bacteria  $P=0.002$ ; and fungi  $P=0.014$ ). To test difference due to being within the city, reference sites were compared to a grouping of remnant, turf, and ruderal (Figure 2.3C). In this case, only the archaea converged ( $P<0.001$ ). These results suggest that human processes such as soil disturbance, and direct management of the flora, alter microbial communities more than other changes such as air temperature or atmospheric N deposition within an urban center. Thus, distinct human processes define each of the high impact sites, and yet when grouped together they have lower variation than the reference and remnant sites across all three domains, suggesting that there is either a common set of organisms that is able to exploit

these new environments, or as has been reported elsewhere in the BH literature, there is a consistent suppression of certain guilds<sup>48</sup>.

To answer whether urbanization suppresses organisms of certain metabolic strategies (or functions), I assigned functional guilds to our identified OTUs, using publicly available databases. Attempts to characterize bacterial and archaeal functional differences were hampered due to database limitations. I explore bacterial function using a metagenomics approach in Chapter 3. In the case of fungi, the fungal database program FUNGuilds was used to assign putative function to identified OTUs<sup>58</sup>. The majority of my fungal sequence data was binned into a functional category using this approach. The resulting data pointed to a loss of ectomycorrhizal fungi (ECM) as the main driver for convergence and differences between land-uses. When ECM sequences were examined at the family level, turf and ruderal sites significantly converged compared to reference and remnant ( $P=0.016$ ). At the OTU level, the turf and ruderal sites also converged (Figure 2.4A). There was also a significant decrease in sequence abundance ( $P=0.003$ , Figure 2.4B) and on OTU richness ( $P>0.001$ , Figure 2.4C) within the turf and ruderal sites. This supports previous research that also reported lower ECM colonization in disturbed urban soils<sup>59</sup>; to my knowledge ours is the first paper to report this trend across multiple cities.

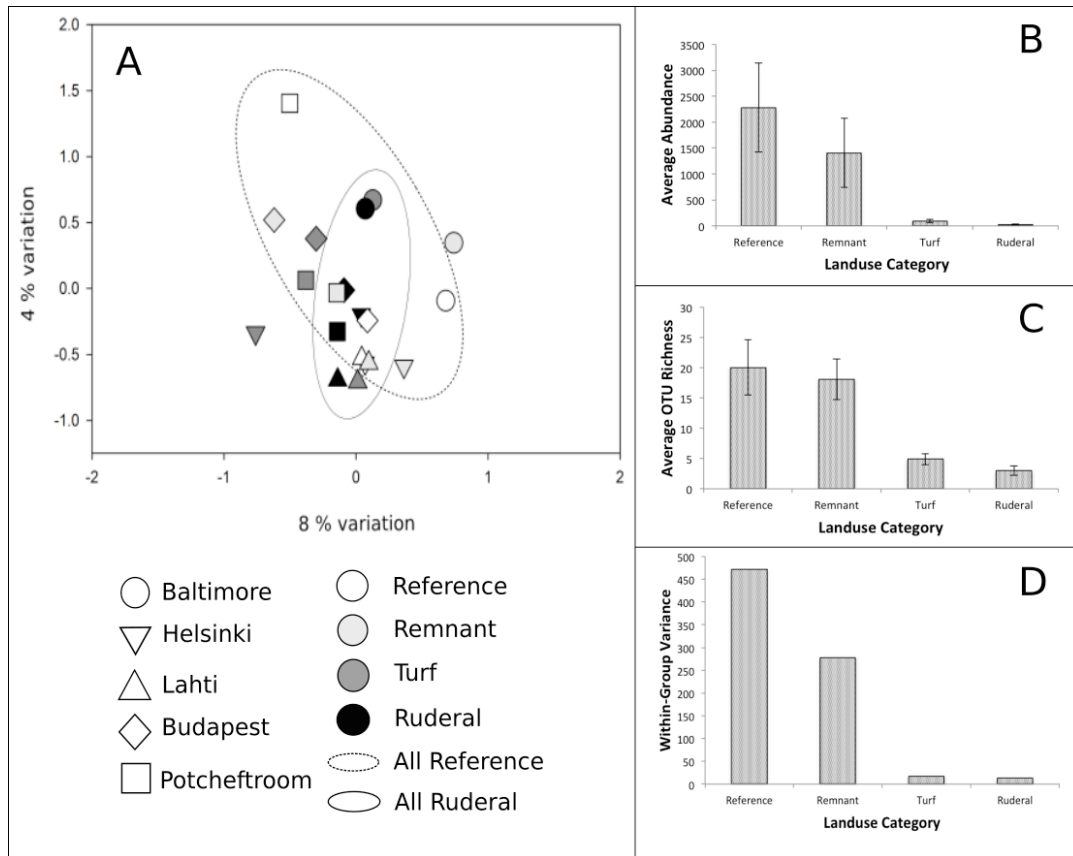


Figure 2.4: A) NMS ordination of the ectomycorrhizal community showing both land-use ( $P=0.004$ ) and city effects ( $P=0.001$ ). B) the average sequence abundance within each site category, measured after rarefying the fungal community to 20,000 OTU's. C) Average OTU richness of the ectomycorrhizal community, grouped according to land-use category. Error bars represent standard error. D) Within group variance, using groups defined by land-use categories, of the OTU richness.

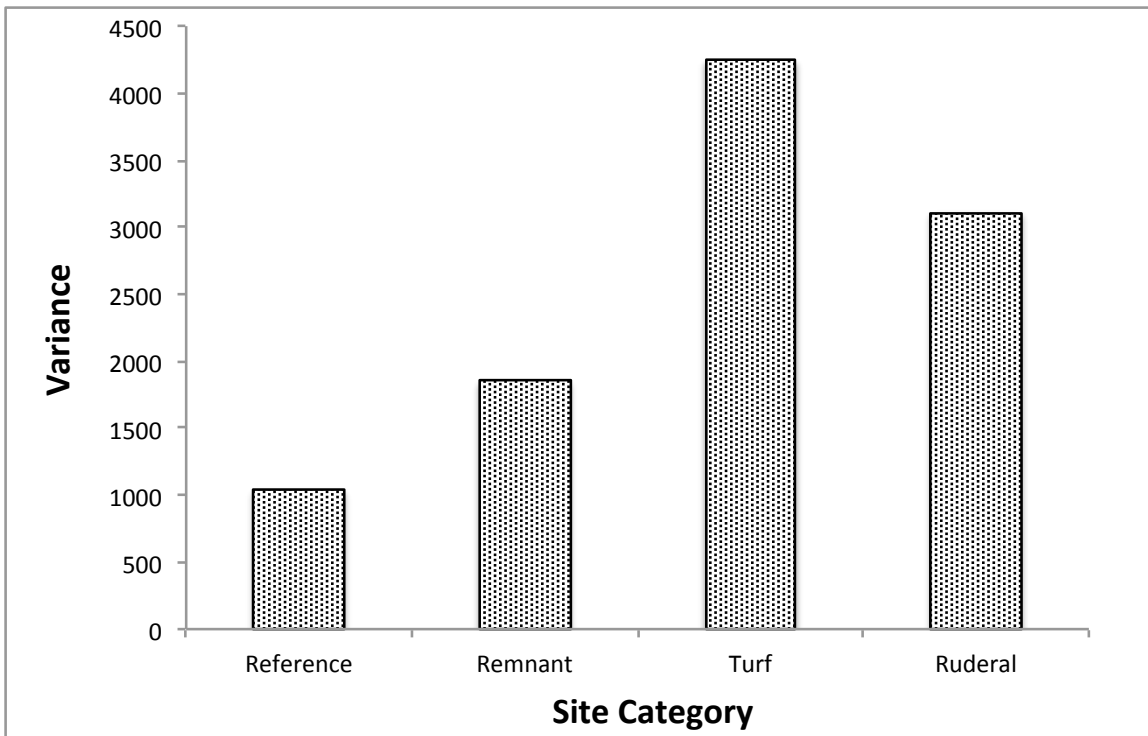


Figure 2.5: Shows a significant divergence of richness values ( $P > 0.001$ ) in the archaeal turf site. Convergence of Richness values has an implicit impact on biodiversity; The values shown above, along with a significant increase in gene quantity per gram of soil (shown in text, table 2.3), increase in richness value, and a convergence of bray-curtis dissimilarity indices among turf sites in particular, together points to an enrichment of archaeal community without exclusion (i.e. a few winners, and no losers).

### Evidence for Global Biodiversity Loss?

A major concern of urbanization is that BH will lead to global biodiversity loss. However, the pattern of convergence among urban biotic communities does not inherently imply a loss in biodiversity<sup>51</sup>. My fungal, archaeal, and bacterial datasets demonstrate how BH may be associated with biodiversity loss in some communities, but not others. My most compelling evidence for biodiversity loss was found in the fungal dataset, where I found evidence of endemism (biogeography) and an urban filter effect; the convergence of ruderal sites relative to reference shows that this filter is consistent

across cities globally. Likely many factors contribute to this observed change, including soil factors such as pH and nutrient concentration, but also the presence of particular host plants<sup>60</sup>. I found that the convergence of urban sites was associated with a precipitous loss of ectomycorrhizal fungi, which declined in abundance and richness. This data supports previous literature demonstrating that loss of diversity may be an important factor driving convergence<sup>48,51</sup>, and provides evidence supporting the connection between BH and global biodiversity loss<sup>11</sup>.

The convergence of the archaeal communities provides a contrasting scenario in which communities may converge without losing biodiversity. Archaeal communities converged in turf and ruderal sites, suggesting that urbanization is an environmental filter that increases community similarity. But there was little evidence of endemism, and my richness and abundance data suggests that urbanization enriches the archaeal community without necessarily excluding diversity. Thus I observe that if an introduced cosmopolitan species does not compete with endemic taxa, as is likely given archaeal metabolic strategy and the soil medium in which they grow<sup>21,61</sup>, then the community may experience convergence in composition without experiencing competitive exclusion and loss of biodiversity.

The bacterial community presents yet a third possibility. Even though the bacterial communities do exhibit strong pattern of biogeography and urbanization does appear to impact bacterial abundance, urbanization does not appear to impact bacterial diversity *per se*; there was little evidence for convergence and no change in richness. In such cases where decreased bacterial abundance and shifts in community composition do reflect an increased likelihood that some bacterial lineages are extirpated locally, the rapid rate of

bacterial evolution<sup>62,63</sup> and horizontal transfer of genetic materials<sup>64</sup> allow that bacterial diversity and function may nonetheless remain intact. This highlights the important interaction between time and evolutionary process, and raises intriguing questions about the impact of human management on global biodiversity at a geologic time-scale, should human management persist.

### Conclusions

My study represents one of the first comparisons of microbial community composition between different cities and across all three domains of microbial life. Although several previous studies have examined microbial communities within a single city, those studies could not infer global diversity loss, due to a lack of information concerning regional variation. I have shown that although urbanization leads to community composition changes across a wide range of microbes, that there were differences in specific interactions between land-use and community composition among the three microbial domains. I also found that ectomycorrhizal fungi may be the only group that experiences significant diversity loss due to urbanization. Because ECM are dependent on woody host plants that are often limited in cities compared to the surrounding environment, future city planning should include remnant sites that will help to support these microbes. Even if the host plant is present, large amounts of nutrients such as nitrogen<sup>65</sup> can further discourage ectomycorrhizal colonization. Nutrient management within urban centers and planting of host species will assist in remediating the loss of these valuable organisms.

## Methods:

### Soil Sampling

In each city, we took special care to ensure that soil type was consistent across all selected site categories (Table 1.1). In particular, we favored well-drained upland soils, avoiding riparian zones, and soils that are often saturated. Each sample consisted of five 2.5 x 10 cm deep soil cores, excluding the O horizon, that were homogenized in a sterile plastic bag using a sterile scoopula. A soil sample from each homogenized bag, weighing ~2 g, was preserved in 4 mL Lifeguard™ preservation solution (MoBio, Carlsbad, CA). This procedure was repeated at 5 locations per site category, resulting in 100 samples total (n=5).

Illumina amplicon sequencing returned 12.5 million archaeal and bacterial 16S rRNA sequences and 6.2 million fungal ITS sequences. By comparing 16S rRNA sequences to the Greengenes database at 97% similarity, 270,000 OTUs were identified across all samples. This included 3,700 archaeal OTUs; 255,481 bacterial OTUs; and 17,439 unassigned OTUs. Rarefaction analysis did not result in plateaued curves, suggesting additional sequencing would yield even more OTUs. On average, 62,132 sequences per sample were generated for the fungi and a total of 12,620 OTU's were identified by matching to the UNITE database<sup>66</sup>. Rarefaction curves of the fungal sequences indicated that this sequencing was sufficient to accurately sample fungal diversity. Rarefaction curves and sequences for each sample can be found in Figure 2.6, and Table 2.2 respectively. I document an OTU diversity in my global dataset that is approximately two times greater than the diversity documented in a recent study claiming



that soil biodiversity in New York's Central Park was equivalent to biodiversity found in global sampling<sup>67</sup>. In my analysis I used similar sequencing methods and clustering thresholds, but had one fifth the sampling depth (100 samples vs. 500 samples) and sequencing depth (12.5 million sequence reads vs. 50 million sequence reads).

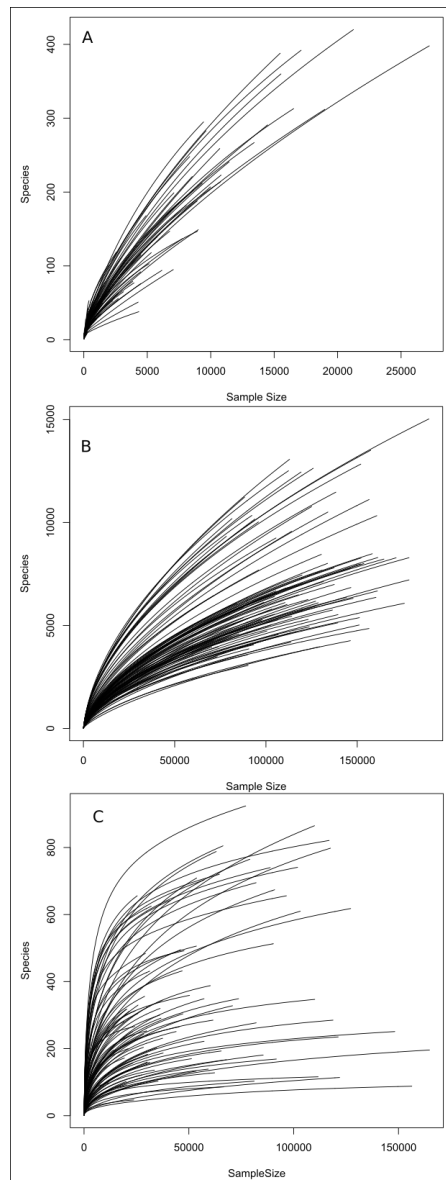


Figure 2.6: Rarefaction curve of A) archaea, B) bacterial and C) fungal sequence abundances.

Table 2.2: Raw and adjusted gene quantities, and sequence depths for each sample

Sample	City	Land-use	Total 16s Sequences	Bacterial Sequences	Archaeal Sequences	Total ITS Sequences	ECM Sequences	Total 16s QPCR	Bacterial 16s QPCR	Archaeal 16s QPCR	Fungal ITS QPCR
1	Baltimore	Rem	1.05E+05	9.82E+04	7.16E+03	8.94E+04	75	5.87E+10	5.27E+10	6.00E+09	9.57E+07
2	Baltimore	Rud	1.23E+05	1.18E+05	4.91E+03	1.08E+05	8	4.87E+10	4.53E+10	3.42E+09	3.71E+07
3	Baltimore	Rem	1.37E+05	1.36E+05	4.33E+02	1.11E+05	12241	5.86E+10	5.82E+10	3.63E+08	1.97E+08
4	Baltimore	Rud	9.21E+04	9.01E+04	1.99E+03	3.49E+04	32	3.95E+10	3.84E+10	1.12E+09	1.76E+07
5	Baltimore	Turf	9.83E+04	9.51E+04	3.21E+03	8.41E+04	226	4.04E+10	3.85E+10	1.85E+09	1.57E+07
6	Baltimore	Rem	1.30E+05	1.29E+05	1.05E+03	1.33E+05	4451	4.45E+10	4.39E+10	6.69E+08	5.71E+08
7	Baltimore	Turf	1.07E+05	1.00E+05	6.36E+03	7.55E+04	452	4.04E+10	3.67E+10	3.67E+09	4.53E+07
8	Baltimore	Rud	1.55E+05	1.42E+05	1.23E+04	1.22E+05	175	5.04E+10	4.16E+10	8.86E+09	1.97E+07
9	Baltimore	Rem	1.22E+05	1.21E+05	6.93E+02	7.77E+04	1428	1.03E+11	1.02E+11	1.02E+09	5.30E+07
10	Baltimore	Rud	1.74E+05	1.68E+05	6.27E+03	1.30E+05	77	2.40E+10	2.19E+10	2.15E+09	9.88E+06
11	Baltimore	Rem	1.07E+05	1.07E+05	2.70E+02	1.04E+05	12025	2.53E+10	2.52E+10	9.75E+07	2.06E+08
12	Baltimore	Turf	9.04E+04	8.15E+04	8.91E+03	4.87E+04	295	5.72E+10	4.99E+10	7.28E+09	4.75E+07
13	Baltimore	Turf	1.67E+05	1.61E+05	5.59E+03	6.72E+04	47	4.13E+10	3.80E+10	3.30E+09	1.26E+07
14	Baltimore	Ref	1.64E+05	1.64E+05	1.46E+02	1.21E+05	6630	4.98E+10	4.97E+10	1.04E+08	8.74E+07
15	Baltimore	Ref	1.41E+05	1.41E+05	3.05E+02	8.25E+04	702	2.63E+10	2.62E+10	1.15E+08	5.44E+07
16	Baltimore	Ref	1.93E+05	1.92E+05	5.27E+02	1.03E+05	195	4.18E+10	4.14E+10	3.14E+08	3.59E+07
17	Baltimore	Ref	1.17E+05	1.16E+05	6.89E+02	1.17E+05	14751	3.70E+10	3.67E+10	3.64E+08	7.46E+08
18	Baltimore	Ref	1.61E+05	1.60E+05	7.38E+02	1.49E+05	9695	2.28E+10	2.25E+10	2.40E+08	1.66E+08
19	Baltimore	Rud	1.22E+05	1.17E+05	4.77E+03	3.43E+04	75	3.96E+10	3.69E+10	2.70E+09	3.75E+06
20	Baltimore	Turf	9.31E+04	8.50E+04	8.06E+03	4.52E+04	23	4.95E+10	4.38E+10	5.71E+09	1.59E+07
21	Helsinki	Ref	9.16E+04	9.15E+04	9.60E+01	7.70E+04	1009	1.65E+10	1.65E+10	2.27E+07	1.84E+09
22	Helsinki	Ref	1.79E+05	1.79E+05	7.00E+01	1.29E+05	10761	7.94E+10	7.93E+10	7.94E+07	7.86E+07
23	Helsinki	Ref	1.73E+05	1.73E+05	8.30E+01	2.69E+04	3000	5.73E+10	5.72E+10	6.80E+07	2.03E+08
24	Helsinki	Ref	1.05E+05	1.04E+05	6.57E+02	2.04E+04	NA	7.51E+10	7.44E+10	7.05E+08	1.50E+08
25	Helsinki	Ref	1.78E+05	1.78E+05	8.60E+01	8.04E+04	1690	3.06E+10	3.06E+10	3.76E+07	1.21E+08
26	Helsinki	Rud	1.55E+05	1.54E+05	1.14E+03	2.26E+04	7	3.83E+10	3.77E+10	6.23E+08	3.10E+07
27	Helsinki	Rud	1.16E+05	1.13E+05	2.71E+03	3.08E+04	NA	9.26E+10	8.91E+10	3.58E+09	8.82E+07
28	Helsinki	Rud	1.38E+05	1.36E+05	1.62E+03	2.61E+04	30	5.18E+10	5.06E+10	1.20E+09	8.18E+06
29	Helsinki	Rud	1.30E+05	1.30E+05	1.42E+02	5.84E+02	NA	7.62E+10	7.61E+10	1.55E+08	6.53E+05
30	Helsinki	Rud	1.41E+05	1.40E+05	4.77E+02	5.45E+04	32	3.13E+10	3.11E+10	2.13E+08	1.59E+06
31	Helsinki	Turf	1.11E+05	1.11E+05	3.99E+02	3.55E+04	99	4.08E+10	4.05E+10	2.32E+08	9.20E+06
32	Helsinki	Turf	9.92E+04	9.72E+04	1.93E+03	9.50E+04	16	4.06E+10	3.95E+10	1.12E+09	5.07E+07
33	Helsinki	Turf	1.25E+05	1.24E+05	7.29E+02	2.70E+04	NA	4.24E+10	4.20E+10	4.42E+08	2.97E+07
34	Helsinki	Turf	1.57E+05	1.56E+05	9.93E+02	2.49E+04	394	1.46E+10	1.44E+10	2.08E+08	6.73E+05
35	Helsinki	Turf	1.35E+05	1.33E+05	2.07E+03	3.07E+04	1	4.10E+10	3.98E+10	1.21E+09	5.31E+06
36	Helsinki	Rem	1.61E+05	1.61E+05	2.46E+02	2.65E+04	331	1.29E+10	1.28E+10	4.53E+07	1.72E+09
37	Helsinki	Rem	8.57E+04	8.56E+04	1.13E+02	6.54E+04	482	2.89E+10	2.89E+10	4.67E+07	8.38E+08
38	Helsinki	Rem	7.64E+04	7.61E+04	2.92E+02	2.27E+04	829	1.12E+10	1.11E+10	4.67E+07	5.45E+08
39	Helsinki	Rem	7.60E+04	7.51E+04	8.61E+02	6.05E+04	675	7.06E+09	6.98E+09	8.69E+07	6.37E+08
40	Helsinki	Rem	1.25E+05	1.25E+05	4.80E+01	3.33E+04	1349	1.35E+10	1.35E+10	9.28E+06	2.20E+08
41	Lahti	Ref	9.49E+04	9.47E+04	1.68E+02	2.90E+04	323	4.54E+10	4.53E+10	1.09E+08	1.31E+09
42	Lahti	Ref	1.29E+05	1.29E+05	6.00E+01	4.58E+04	86	4.73E+10	4.73E+10	4.06E+07	5.83E+07
43	Lahti	Ref	9.00E+04	9.00E+04	4.00E+01	3.68E+04	88	3.26E+07	3.26E+07	1.86E+04	1.80E+04
44	Lahti	Ref	1.28E+05	1.28E+05	2.60E+01	3.67E+04	756	4.55E+10	4.55E+10	1.69E+07	4.55E+07
45	Lahti	Ref	7.07E+04	7.07E+04	3.30E+01	4.76E+04	2711	2.74E+10	2.74E+10	1.29E+07	9.79E+07
46	Lahti	Rud	1.07E+05	1.07E+05	2.80E+01	1.35E+05	16	4.19E+10	4.19E+10	1.68E+07	2.46E+08
47	Lahti	Rud	1.41E+05	1.40E+05	1.37E+03	5.85E+04	NA	2.59E+10	2.54E+10	5.08E+08	3.00E+08
48	Lahti	Rud	9.85E+04	9.84E+04	1.34E+02	1.36E+05	NA	2.42E+10	2.42E+10	4.64E+07	8.40E+07
49	Lahti	Rud	1.08E+05	1.07E+05	8.57E+02	2.94E+04	2	3.08E+10	3.05E+10	3.78E+08	2.33E+08
50	Lahti	Rud	1.28E+05	1.28E+05	4.53E+02	6.61E+04	0	2.39E+10	2.38E+10	1.55E+08	9.90E+07
51	Lahti	Turf	9.80E+04	9.79E+04	1.15E+02	1.48E+05	9	4.44E+10	4.43E+10	7.29E+07	1.40E+08
52	Lahti	Turf	1.66E+05	1.64E+05	1.63E+03	1.09E+05	19	2.50E+10	2.44E+10	5.82E+08	8.24E+07
53	Lahti	Turf	1.25E+05	1.25E+05	2.31E+02	3.12E+04	0	1.77E+10	1.76E+10	5.84E+07	2.48E+07
54	Lahti	Turf	1.40E+05	1.39E+05	1.03E+03	1.22E+05	0	1.28E+10	1.26E+10	1.88E+08	7.49E+07
55	Lahti	Turf	1.52E+05	1.52E+05	7.80E+01	2.59E+04	0	5.31E+06	5.30E+06	5.92E+03	1.38E+03
56	Lahti	Rem	1.04E+05	1.04E+05	6.50E+01	2.91E+04	127	3.53E+10	3.53E+10	3.28E+07	3.96E+08
57	Lahti	Rem	1.40E+05	1.40E+05	2.07E+02	2.33E+04	222	4.19E+10	4.18E+10	1.24E+08	7.59E+08
58	Lahti	Rem	1.57E+05	1.57E+05	1.00E+00	4.41E+04	35	1.74E+10	1.74E+10	2.48E+05	2.59E+08
59	Lahti	Rem	9.10E+04	9.10E+04	1.20E+01	5.74E+04	575	4.37E+10	4.37E+10	7.50E+06	2.72E+07
60	Lahti	Rem	1.61E+05	1.61E+05	2.50E+01	5.07E+04	268	2.25E+10	2.25E+10	8.03E+06	1.32E+07
61	Budapest	Ref	1.15E+05	1.14E+05	8.04E+02	5.49E+04	7	1.38E+10	1.37E+10	1.59E+08	1.63E+08
62	Budapest	Ref	9.49E+04	9.44E+04	4.96E+02	2.63E+04	1	5.94E+09	5.89E+09	4.21E+07	1.25E+09
63	Budapest	Ref	1.02E+05	9.88E+04	2.97E+03	3.87E+04	1	5.38E+09	5.15E+09	2.28E+08	4.11E+07
64	Budapest	Ref	1.19E+05	1.16E+05	2.50E+03	3.74E+03	NA	1.95E+10	1.88E+10	6.97E+08	2.59E+08
65	Budapest	Ref	1.13E+05	1.08E+05	4.39E+03	6.23E+04	3	1.26E+10	1.18E+10	7.91E+08	8.55E+07
66	Budapest	Rud	1.53E+05	1.52E+05	7.52E+02	6.87E+04	0	2.17E+10	2.14E+10	2.33E+08	4.89E+07
67	Budapest	Rud	8.79E+04	8.36E+04	4.24E+03	1.57E+05	0	1.15E+10	1.08E+10	6.97E+08	1.27E+09
68	Budapest	Rud	1.41E+05	1.36E+05	4.42E+03	1.65E+05	1	3.53E+10	3.30E+10	2.23E+09	9.16E+07
69	Budapest	Rud	1.03E+05	9.32E+04	9.79E+03	1.22E+05	0	3.48E+10	2.99E+10	4.86E+09	2.38E+08
70	Budapest	Rud	1.70E+05	1.67E+05	3.29E+03	5.94E+04	1	1.40E+10	1.34E+10	6.60E+08	2.92E+07
71	Budapest	Turf	1.52E+05	1.50E+05	2.24E+03	9.28E+04	1	2.88E+10	2.79E+10	9.21E+08	1.31E+08
72	Budapest	Turf	1.08E+05	1.03E+05	5.26E+03	1.11E+05	35	1.83E+10	1.70E+10	1.38E+09	1.49E+08
73	Budapest	Turf	1.53E+05	1.49E+05	3.91E+03	1.20E+05	13	1.24E+10	1.17E+10	6.90E+08	9.56E+07
74	Budapest	Turf	1.50E+05	1.49E+05	1.39E+03	1.36E+04	NA	2.85E+10	2.79E+10	5.65E+08	1.34E+07
75	Budapest	Turf	1.17E+05	1.11E+05	5.99E+03	3.05E+04	0	2.78E+10	2.54E+10	2.38E+09	6.28E+07
76	Budapest	Rem	1.71E+05	1.64E+05	7.68E+03	3.78E+04	8	3.06E+10	2.73E+10	3.36E+09	2.29E+08
77	Budapest	Rem	1.19E+05	1.18E+05	1.16E+03	1.12E+05	3	7.41E+09	7.29E+09	1.23E+08	1.21E+08
78	Budapest	Rem	1.18E+05	1.15E+05	3.46E+03	3.15E+04	22	3.22E+10	3.06E+10	1.59E+09	3.46E+07
79	Budapest	Rem	1.30E+05	1.25E+05	4.86E+03	5.51E+04	12	2.19E+10	2.03E+10	1.52E+09	1.84E+07
80	Budapest	Rem	9.29E+04	8.95E+04	3.39E+03	2.35E+04	4	8.13E+09	7.74E+09	3.94E+08	2.17E+07
81	Potchefstroom	Ref	1.29E+05	1.24E+05	4.90E+03	3.80E+04	12	7.04E+10	6.55E+10	4.93E+09	7.77E+07
82	Potchefstroom	Ref	1.46E+05	1.42E+05	3.76E+03	3.64E+04	27	1.29E+11	1.22E+11	6.91E+09	7.33E+07
83	Potchefstroom	Ref	1.35E+05	1.34E+05	1.37E+03	3.89E+04	6	1.46E+11	1.43E+11	2.87E+09	2.68E+07
84	Potchefstroom	Ref	1.81E+05	1.81E+05	2.67E+02	5.28E+04	7	1.12E+11	1.12E+11	4.28E+08	1.39E+07
85	Potchefstroom	Ref	1.16E+05	1.15E+05	8.63E+02	3.05E+04	0	4.79E+10	4.73E+10	5.90E+08	7.42E+07
86	Potchefstroom	Rem	1.25E+05	1.21E+05	3.45E+03	2.45E+04	10	2.98E+10	2.83E+10	1.47E+09	2.31E+08
87	Potchefstroom	Rem	9.10E+04	9.06E+04	4.02E+02	8.17E+04	0	8.38E+10	8.33E+10	4.81E+08	1.08E+09
88	Potchefstroom	Rem	7.92E+04	7.84E+04	8.28E+02	3.38E+04	4	1.47E+10	1.46E+10	1.74E+08	9.87E+05
89	Potchefstroom	Rem	1.56E+05	1.53E+05	3.18E+03	9.27E+04	0	1.44E+10	1.37E+10	6.51E+08	5.55E+06
90	Potchefstroom	Rem	1.19E+05	1.13E+05	6.37E+03	3.03E					

Each sample consisted of five-2.5 x 10 cm deep soil cores, excluding the O horizon, that were homogenized in a sterile plastic bag using a sterile scoopula. A soil sample from each homogenized bag, weighing ~2 g, was preserved in 4 mL Lifeguard™ preservation solution (MoBio, Carlsbad, CA). This procedure was repeated at 5 locations per site category, resulting in 100 samples total (n=5).

#### DNA Extraction and Preparation:

Soils were extracted following the MOBIO Laboratories inc. Powerlyzer™ Powersoils® DNA isolation kit protocol (MoBio, Carlsbad, CA, USA), with two exceptions. First, instead of adding 0.25 g of dry soil to the bead beating tubes in step two, 750 uL of soil suspended in Lifeguard™ solution was added to each tube. Second, a Fastprep®-24 set to 3.5 m/s for 45 s was used in cell lysis (MP Biomedical, Santa Ana, CA, USA). The exact weight of each soil sample used for extraction was calculated based on the measured weight of a 0.5 mL aliquot of soil slurry dried at 60° C for one week.

Extracted DNA was quantified using a QuBit® 2.0 Fluorometer (Invitrogen, Carlsbad, CA). DNA extracts were diluted to 1.25 ng/uL for sequencing amplification and QPCR.

DNA samples are stored at -20° C between each analysis.

#### Microbial Community Analysis:

Amplicon sequencing targeted a section of the 16S rRNA using primers 515F+adapter and 806R+adapter (Table 2.2) and the ITS1 region in fungi using primers ITS1F+adapter and ITS2+adapter (Table 2.2). Adapters were ligated to the forward and reverse strands, and the libraries were indexed using the Nextera XT 96 index kit

(Illumina, San Diego, CA, USA). Samples were sequenced using an Illumina MiSeq 2000 (Illumina, San Diego, CA, USA) at the Oregon State University Center for Genome Research and Biocomputing. The sequencing kit produced 250 bp, paired-end read, and was run with a 10% Phix.

Q-PCR for the combined bacterial and archaeal community was performed using the primers F515 and 806R (Table 2.2). Fungal community were quantified by targeting the ITS region using ITS1F and 5.8S (Table 2.2). The master mix for each QPCR reaction was as follows: 16 uL of Kicqstart Sybr Green Readymix With Rox (Sigma, St. Louis, MO), 1.0 uL of 10 uM forward and reverse primers, and 2 uL DNA diluted to 1.25 ng/mL. The standard curve was a 1:10 serial dilution of 2.5 ug/ml DNA, and concentrations are calculated based on the size of the plasmid containing an insert. An inhibition correction was applied using the following strategy: 2 uL of each sample was pooled and quantified using the flourometer; a soil standard curve consisted of 1:10 serial dilutions. I calculated a soil efficiency standard correction according to previously reported protocol<sup>68</sup>, but rejected this correction because it applied a more significant correction to samples with higher gene counts rather than lower gene counts.

#### Bioinformatics and Statistical Analyses:

The QIIME pipeline<sup>69</sup> was used in processing sequence libraries, with a few variations between the 16S and ITS sequence analysis. For ITS samples, the number of allowable homopolymer repeats in the split-libraries were increased from 6 to 8, because at the default of 6, 80% of the sequences were eliminated. The RDP<sup>70</sup> classifier was used to assign fungal taxonomy against the UNITE database<sup>66</sup>; and I used uclust to cluster the 16s OTUs against the Greengenes database<sup>71</sup>. Both datasets were clustered at 97%

similarity, followed by open-reference OTU-picking. The reverse primers were removed in the split-libraries step and the minimum overlap in joining the paired end reads (ea-utils<sup>72</sup>, fastq-join.py) was increased from 6 to 50 bases in both amplicon libraries. All other functions in the QIIME pipeline were run on default settings. Our QIIME pipeline workflow went as follows: fastq-join.py; convert\_fastqual\_Fastq.py; split-libraries.py; cat; pick-otus.py; pick\_rep\_set.py; assign\_taxonomy.py; make\_otu\_table.py; biom convert; and summarize\_taxa.py. I used outputs from the make\_otu\_table and summarize\_taxa functions in our downstream data analysis in R and PC-ORD. Full scripts can be found in the supplemental information.

Alpha diversity was calculated based on Shannon-Weiner diversity indices in each sample and Pielou's J was used to calculate evenness indices. Both R and PC-ORD platforms were used to construct Bray-Curtis distance matrices. PC-ORD<sup>73</sup> was used to run a nested Permanova to compare differences in community composition and the betadisper function (of the vegan package<sup>57</sup>) in R<sup>74</sup> (version 3.1.3) was used to test for difference in within group variance among site categories. The envfit (vegan package) function in R was used to generate correlations between microbial communities and environmental parameters. I also used the vegan stat package to run blocked one-way ANOVAs and Tukey post-hoc analyses for differences in gene abundances between site categories. All R scripts can be found in the supplemental material.

For all ECM analysis, the fungal communities were rarefied to 20,000 OTU's. R scripts for rarefaction and downstream statistics are available in the supplemental material. I used the R statistical packages vegan, stats<sup>74</sup> and lawstat<sup>75</sup> (scripts in supplemental; citations). 10 sites falling under 20,000 sequences were omitted from all

analyses, while an additional 13 sites were omitted from my community composition analysis because they lacked any ECM. Because I rarefied the community to equal sampling depth, I treat sequence abundance as accurately representing relative ECM abundance in the environment. Richness values were calculated as the total number of distinct OTU's at each site. Convergence was identified by comparing within-group variances among land-use categories, and tested using levene's test of equality of variances. The NMS ordination was constructed in PC-ORD73, with Beals smoothing applied to the distance matrix, accounting for the high incidence of zeroes within the ECM community.

## Chapter 3: A Metagenomics Approach to Inferring Microbial Function: Functions Differ Due to Geography and Land Use, but do not Converge due to Urbanization

### Introduction

Amplicon sequencing has been most commonly used to investigate soil microbial communities. Many studies have used 16S rRNA to reconstruct evolutionary history and infer phylogenetic relationships. However, a major shortcoming of amplicon sequencing is the absence of information on the metabolic characteristics of the organisms, and therefore also the inability to assess the potential function of the aggregate community. There have been a few attempts at addressing this issue; databases such as PICRUSt<sup>76</sup>, and FUNGuilds<sup>58</sup> attempt to predict metabolism or ecological function from 16S or other ribosomal RNA genes either using whole sequenced genomes of known organisms, or by applying literature reviews of representative taxa within a phylogeny. Because programs like PICRUSt leverage sequenced genomes to predict function, these models are limited in accuracy and breadth by organismal groups for which a representative genome has been sequenced. Another issue with using phylogenetic markers to infer function, is that difference in rRNA may not provide sufficient resolution to distinguish between strains. We know that in the case of *E. coli*, strains can have radically different ecological function<sup>77</sup> and up to one-third of the genome may differ within cells that are all considered the same species.

Shotgun metagenomic sequencing offers a complimentary approach to examine the functional capacity of the soil microbial community. Unlike, amplicon sequencing

that relies on targeting a specific gene of interest through PCR amplification, metagenomic sequencing does not use PCR, and random fragments of DNA are sequenced. Following the amplicon sequencing that was described in Chapter 2, I used metagenomic sequencing to further investigate the urban soil microbial communities, in this case focusing on differences in function.

The metagenomic sequence dataset can be analyzed by assigning metabolic pathways, or subsystems, to metagenomic datasets. Urban soil microbial data were analyzed using Metagenomic Rapid Assignment using Subsystems Technology (mg-RAST<sup>78</sup>) from Argon National Lab to make assignments based on the SEED database<sup>79</sup>. MG-RAST operates in three steps: 1) quality control filtering selected by the user; 2) assigning sequences to a likely protein via a database search (such as blast); and 3) annotating the sequences using the assigned subsystem. Subsystems are collections of genes that make up a metabolic pathway<sup>79</sup> and are curated manually, meaning they can change as more information about organismal metabolism becomes available. The shotgun sequencing approach primarily captures bacterial genes because of their dominant presence in bulk soils; therefore it should be considered a complimentary analysis to the bacterial 16S rRNA amplicon library from Chapter 2.

A number of edaphic factors are known to correlate to microbial community composition, including: soil texture, SOM concentration, C availability and pH. Previous research has shown that soil pH is perhaps the most significant variable correlated to landscape-level changes in community composition<sup>80</sup>. This is not surprising as soil pH controls nutrient availability and likely contributes to a host of microbial adaptations. Soil mineralogy has likewise been shown to strongly correlate to shifts in microbial



community composition<sup>81</sup>. One caveat of these studies, however, is that most are based on 16S rRNA. The 16S rRNA composition could appear different due to differences in environmental filtering caused by pH or mineralogy, but these factors may not necessarily lead to differences in functional pathways. Likewise, two soils may have similar communities based on 16S rRNA, but environmental variables may lead to the selection of strains that differ in functional composition<sup>82</sup>. My study is therefore one of the first to assess the role of these soil factors on soil metagenome composition.

For this study, the relationship between geographic distribution of edaphic properties such as mineralogy and pH is complicated by anthropogenic modification. Notably, carbon (C) concentration and pH of northern soils are significantly affected by urbanization. The increase in soil pH within cities has been attributed to the introduction of alkaline inputs in the form of concrete<sup>83</sup> (calcium carbonates and calcium). Management of the urban plant community<sup>23</sup> in combination with the physical disturbance of the soils, may result in C loss or accumulation<sup>84</sup>; my turf and ruderal site categories are consistent with land uses that may decrease soil carbon. The global convergence of soil pH and C content in urban soils has already been confirmed in these sites<sup>47</sup>. In this study, I used a metagenomics to assign potential function profiles and taxonomic community profiles to microbial communities sampled in five cities on three continents. I hypothesize that given predictable changes in urban soils such as increase pH and decreased C, the microbial communities within cities will converge in terms of functional characteristics, even if they do not converge in terms of community composition.

## Methods

### Site Description:

The Global Urban Soil Ecology Education Network (GLUSEEN) is a diffuse network of scientists using common methodology to answer basic questions about the ecology of urban ecosystems. Five cities distributed across three continents participated in this study: Baltimore, USA; Helsinki and Lahti, Finland; Budapest, Hungary; and Potchefstroom, South Africa. In each of these cities, four habitat types were defined: reference, remnant, turf, and ruderal<sup>47</sup>. These habitat types represent a range of disturbance and management inputs in soils. Reference sites are defined as undisturbed and unmanaged soils that are located outside of the urban matrix. Remnant sites are defined as undisturbed and unmanaged sites that are located within the urban matrix. Turf sites are defined as sites that are not recently disturbed (grading, soil transport etc. may have occurred at some point in the past), but are under management to maintain a turf ecosystem; typical examples include park lawns, municipal lawns, and residential lawns. Finally, ruderal sites are defined as having recently endured substantial disturbance to the soils structure, and include vacant lots and other abandoned properties. Soils for this study were sampled using a 2.5 cm diameter core to a depth of 10 cm; five separate cores were taken, and organic material was removed from the top leaving the mineral soil and in some cases an Oa horizon. These cores were homogenized in a sterile plastic bag, and a 2 g subsample was placed in a LifeGuard preservation solution.

The soils differ significantly in pH, organic matter concentration, and mineralogy among the various cities. For example, the soils of Lahti and Helsinki were spodosols<sup>47</sup>,

and have a higher organic fraction (our analysis included the Oa with the A mineral horizon) which represents a greater availability of organic C and N. Spodosols are acidic due to their high organic acid content, whereas the alphasols of Baltimore are buffered at around a pH of 5.2 by aluminum redox in the mineral matrix; arid soils such as those found around Potchefstroom are typically more alkaline, and Budapest sits on calcareous parent material, buffering soils to a more alkaline state<sup>47</sup>.

#### DNA Preparation:

DNA was extracted using the MoBIO (Carlsbad, California) Powersoils Powerlyzer DNA extraction kit. Q-PCR for the Ammonia oxidizing archaea and bacteria were quantified by targeting the ammonia monooxygenase gene, using previously published primer sets that were unique for each of the archaeal<sup>85</sup> and the bacterial<sup>86</sup> homologues. The master mix for each QPCR reaction was as follows: 16 uL of Kicqstart Sybr Green Readymix With Rox (Sigma, St. Louis, MO), 1.0 uL of 10 uM forward and reverse primers, and 2 uL DNA diluted to 1.25 ng/mL. The standard curve was a 1:10 serial dilution of 2.5 ug/ml DNA, and concentrations are calculated based on the size of the plasmid containing an insert. I only used QPCR data for which the run had 95-105% amplification efficiency, and an R-squared above 0.995.

The DNA library was prepared using the Nextera XT Tagmentation kit to prepare 96 samples, which were indexed using the Nextera XT 96 sample index. I followed Illumina recommended protocols (Part# 15044223 Rev. B). The resulting fragments ranged in size from 0.2 to 2.0 kb, determined by Bioanalyzer using the Agilent high sensitivity DNA assay at the University of Oregon Center of Genome Research and Biocomputing (Corvallis, OR). The initial objective of shotgun sequencing was to test a

new algorithm for detecting pathogens; our 16s rRNA library sequences indicated that I had *Yersinia* sp. (Figure 2.4) and I was looking to confirm the presence of *Y. pestis*. Therefore, I chose to omit four samples from Baltimore (samples 2 and 4 were ruderal sites, sample 1 was remnant, and sample 5 was turf) which had no *Yersinia* calls in the previous sequencing run. Paired-end sequencing, with 150bp reads, was conducted on an Illumina HiSeq 3000 platform, using the Nextera XT 96 sample indexes, at the University of Oregon Center of Genome Research and Biocomputing (Corvallis, OR).

#### Data Analysis:

Sequence annotation was done on the MG-RAST server (Argon National Lab, Chicago, IL); with no host filters applied, and no filter for PCR replication. I chose to use the SEED database<sup>79</sup> for function annotations in my analysis because this is the most robust database. Function and taxonomy data was downloaded using the matR package<sup>87</sup> in R (version 3.1.1), at two levels of ontology (level one and level three), and three levels of taxonomy (genus, family, and order; assigned based on the Greengenes database). Cutoff e-values were left at default (1e-5) for this analysis. The annotation data was then used to create a function matrix, which was the basis of my statistical analyses. I assessed the convergence of community function profiles using the betadisper function of the Vegan<sup>57</sup> package in R (version 3.1.1). Scripts can be found in the Appendix B. Beta diversity in the function profiles was assessed using a nested PerMANOVA analysis in PCORD<sup>73</sup>, and using the Vegan package in R.

## Results

At both ontology categorization levels, functional profiles were significantly affected by geography (e.g. City;  $P=0.007$ ,  $P<0.0001$ ) and land-use category ( $P=0.0004$ ,  $P<0.0001$ ). These effects are clearly visible on an NMS ordination (Figure 3.1). As in the 16s library, Baltimore sites clustered away from other cities and show a clear separation between site categories (Figure 2.2; Figure 3.1); Helsinki and Lahti also clustered together with distinctions among their constituent land-use categories, and Budapest and Potchefstroom cluster together. Although there were significantly different functional gene profiles present in each site category, there was no difference among the variances of each site category (i.e. there was no convergence;  $P=0.95$ ,  $P=0.99$ ).

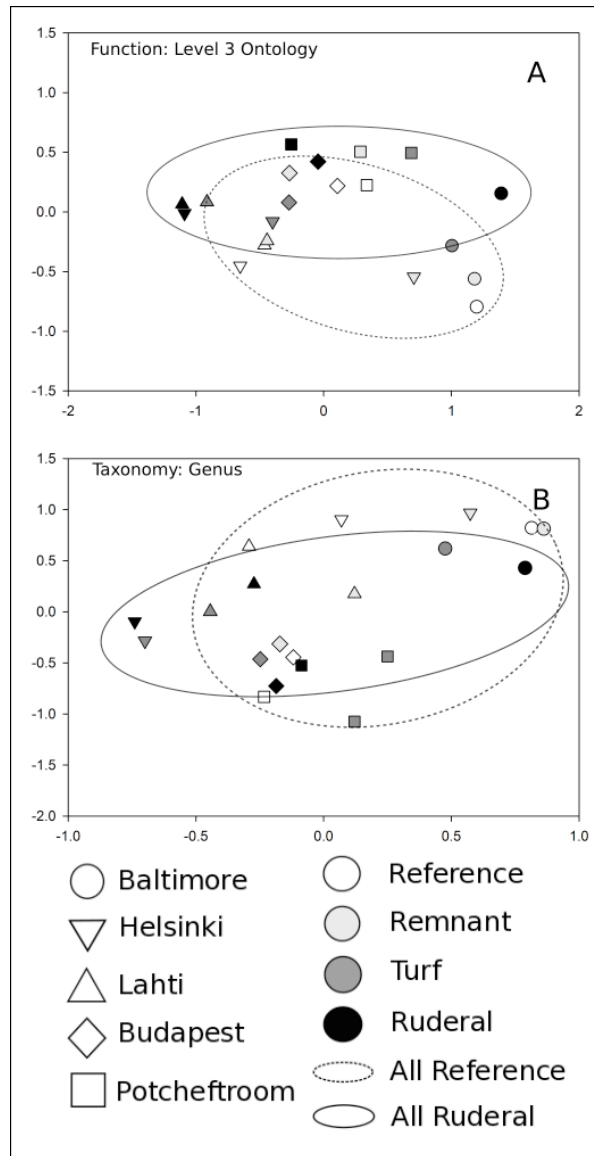


Figure 3.1: NMS ordinations representing the similarity of A) the functional composition of sites at level 3 ontology. Each symbol represents the centroid of all sites falling in the given land use category and city. B) taxonomy assignments of communities. Each symbol represents the centroid of all sites falling in the given land use category and city.

For the level one ontology group, the indicator categories in reference sites were:

“Virulence, disease and defense”; and “Potassium metabolism.” Level one ontology

groups with the highest abundance in remnant sites were: “sulfur metabolism”;

“Metabolism of Aromatic Compounds”; “Fatty Acids, Lipids, and Isoprenoids”;

“Secondary Metabolism”; and “Photosynthesis.” In ruderal sites the most abundant categories were: “Nitrogen Metabolism”; and “Iron Acquisition and Metabolism.” There were no indicators of turf at level one ontology. At level three ontology, which is higher resolution, the functions that strongly indicate each site category are genes that encode for maintenance, and metabolism (Table 3.1).

There were a total of 1155 level 3 subsystems identified across all 96 samples. Of those, 902 subsystems were significant indicators for at least one city ( $P < 0.05$ ). This is due to the strong geographic structure of this dataset. Across all cities, there were fewer indicators of site category. Ruderal sites had 69 significant indicator functions, turf sites had only one indicator function. Intermediate to these sites, 11 indicators were associated with remnant and 16 indicators were associated with reference. Though there were few statistically significant indicators of land use category, many of the functions had different expression patterns based on city and land use category.

Resistance to some metals, such as zinc, arsenic and cobalt-zinc-cadmium, had geographic structure. For example, zinc resistance was higher in Baltimore (Figure 3.2) than in any other city ( $P < 0.001$ ). In contrast, arsenic resistance was highest in Helsinki ruderal sites (Figure 3.3;  $P < 0.001$ ). Interestingly, some resistance pathways such as mercury, cadmium, and arsenic, exhibited as much variation within cities as among cities; in every city arsenic resistance was highest in turf or ruderal sites, as was mercury resistance (Figure 3.3, panel A;  $P < 0.001$ ). Meanwhile, Zinc-Cobalt-Cadmium resistance was lowest in turf and ruderal sites in all cities ( $P < 0.001$ ), and had the greatest abundance and differences among sites in northern cities ( $P < 0.001$ ). The abundance of zinc transporters was negatively correlated with zinc resistance ( $R = -0.559$ ). This may be

related to zinc availability and toxicity; zinc is a necessary micronutrient, but is also toxic at elevated levels. When it is scarce, I expect microbes to need genes that engage in active zinc transport into the cell, whereas when it is abundant microbes will need mechanisms for avoiding toxicity. Thus, genes for transport and resistance should be negatively correlated with one another.



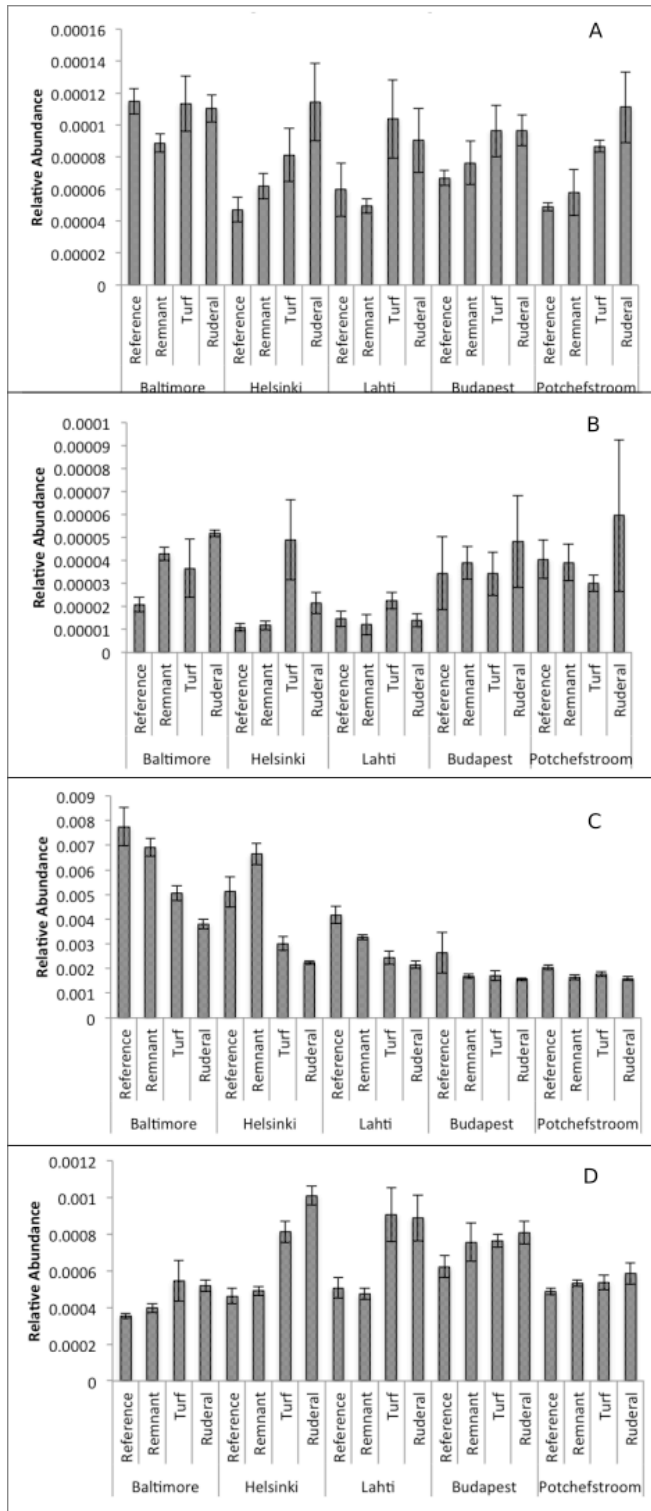


Figure 3.3: Metal Resistance genes for A) Mercury B) Cadmium C) Cobalt-Zinc-Cadmium D) Arsenic. Relative abundance determined using metagenomics annotation in MG-Rast

With the exception of Baltimore sites, Nickel and Cobalt transport was most abundant in turf and ruderal sites (figure 3.4). This is corroborated by the indicator analysis that found arsenic resistance was a strong indicator of ruderal sites ( $P=0.003$ ), as were copper homeostasis genes ( $p=0.001$ ), and Nickel and cobalt transport genes ( $P=0.004$ ). Manganese transport, on the other hand, was expressed least in turf and ruderal sites ( $P<0.001$ ). There was a significant land use effect on siderophore gene abundance (Figure 3.5), with gene abundance being on average highest in ruderal sites ( $P<0.001$ ). Iron metabolism exhibited a similar pattern, with Baltimore having the lowest abundance of these genes ( $P<0.001$ ), and ruderal sites having the highest average abundance. Iron metabolism was highly correlated with Iron transport (Figure 3.8;  $R=0.869$ ), and siderophore presence (Figure 3.8;  $R=0.899$ ).

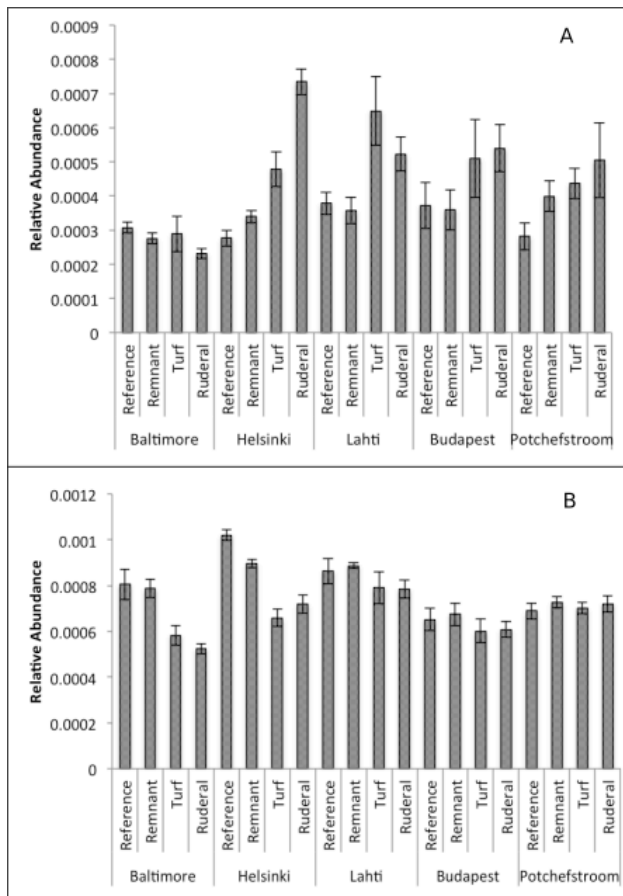


Figure 3.4: Relative abundance of metal transport genes for A) nickel and B) manganese and cobalt.

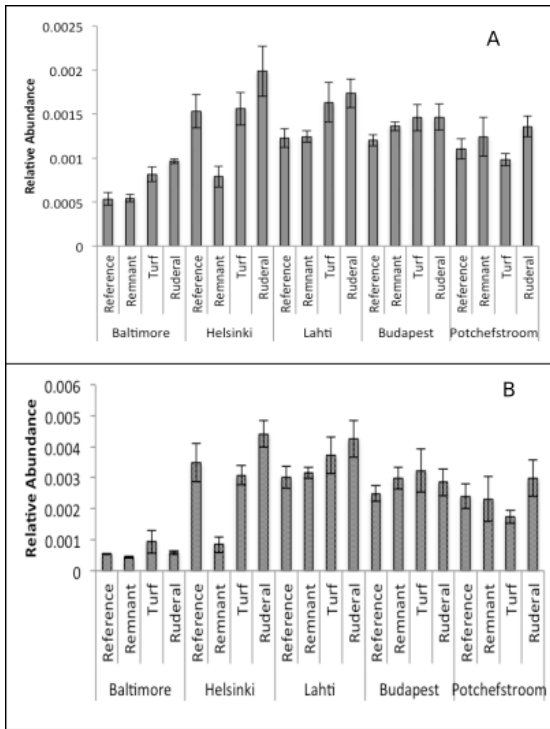


Figure 3.5: Relative abundance of A) iron transport genes, and B) combined siderophores.

Sulfur oxidation also exhibited a geographic pattern; Baltimore sites exhibited the highest abundance of Sulfur oxidation genes (Figure 3.6;  $P < 0.001$ ). Across land use, turf exhibited the highest number of annotations, and ruderal exhibited the lowest.

Methanogenesis exhibited a significant geographic expression pattern ( $P < 0.001$ ), but no relationship to land use (Figure 3.7;  $P = 0.116$ ). This pattern was mirrored in the particulate methane mono-oxygenase patterns ( $P = 0.007$ ;  $P = 0.305$ , geography and land use respectively). In both cases, abundance was highest in Baltimore. Carbon monoxide dehydrogenase, a gene involved in in methane oxidation as well as a diverse array of other carbon-oxidation metabolic pathways, was most abundant in Baltimore sites

( $P < 0.001$ ) and remnant sites (Figure 3.7;  $P < 0.001$ ). Nitrogen metabolism was a significant ontology level 1 indicator of ruderal sites. The level-three ontology indicated that the functions driving this annotation were nitrate and nitrite ammonification genes. Ammonia assimilation, an essential cell function for all life, exhibited no difference in expression profiles based on land use, but was significantly higher in Baltimore than all other cities (Figure 3.8;  $P < 0.001$ ). Expression of N fixation genes were again, highest in Baltimore ( $P < 0.001$ ), and exhibited no response to land use ( $P = 0.243$ ). N fixation was weakly and negatively correlated to nitrate and nitrite ammonification ( $R = -0.172$ ), and was most expressed in ruderal sites and in Helsinki and Lahti. Ammonia oxidation genes, a function which were not annotated to any sequences, were significantly more abundant in turf sites ( $P = 0.004$ ), and were positively correlated with the number of putatively identified ammonia oxidizing archaea in my the 16s amplicon dataset presented in Chapter 2 ( $R = 0.797$ , Figure 3.8).

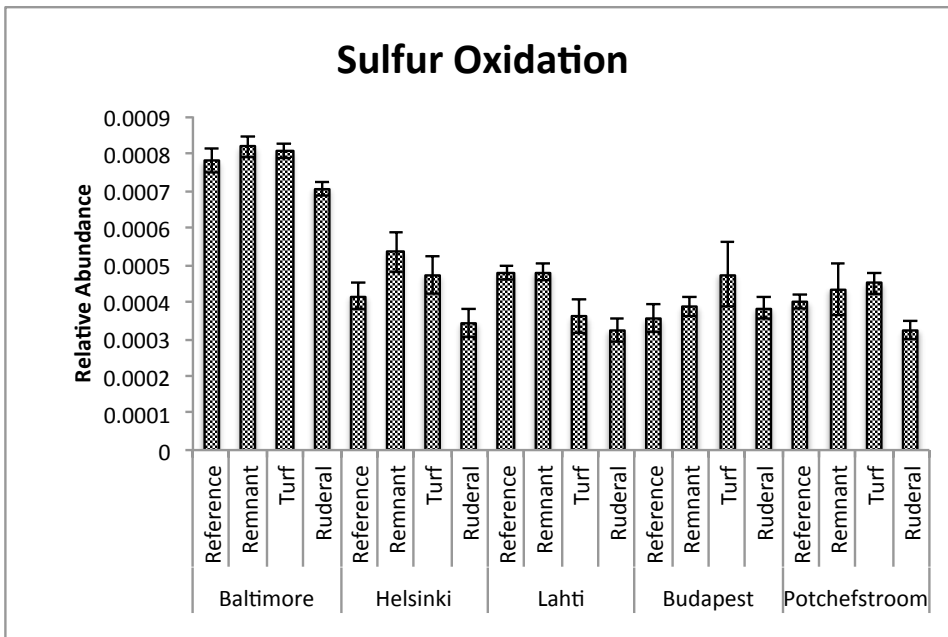


Figure 3.6: relative abundance of sulfur oxidation genes

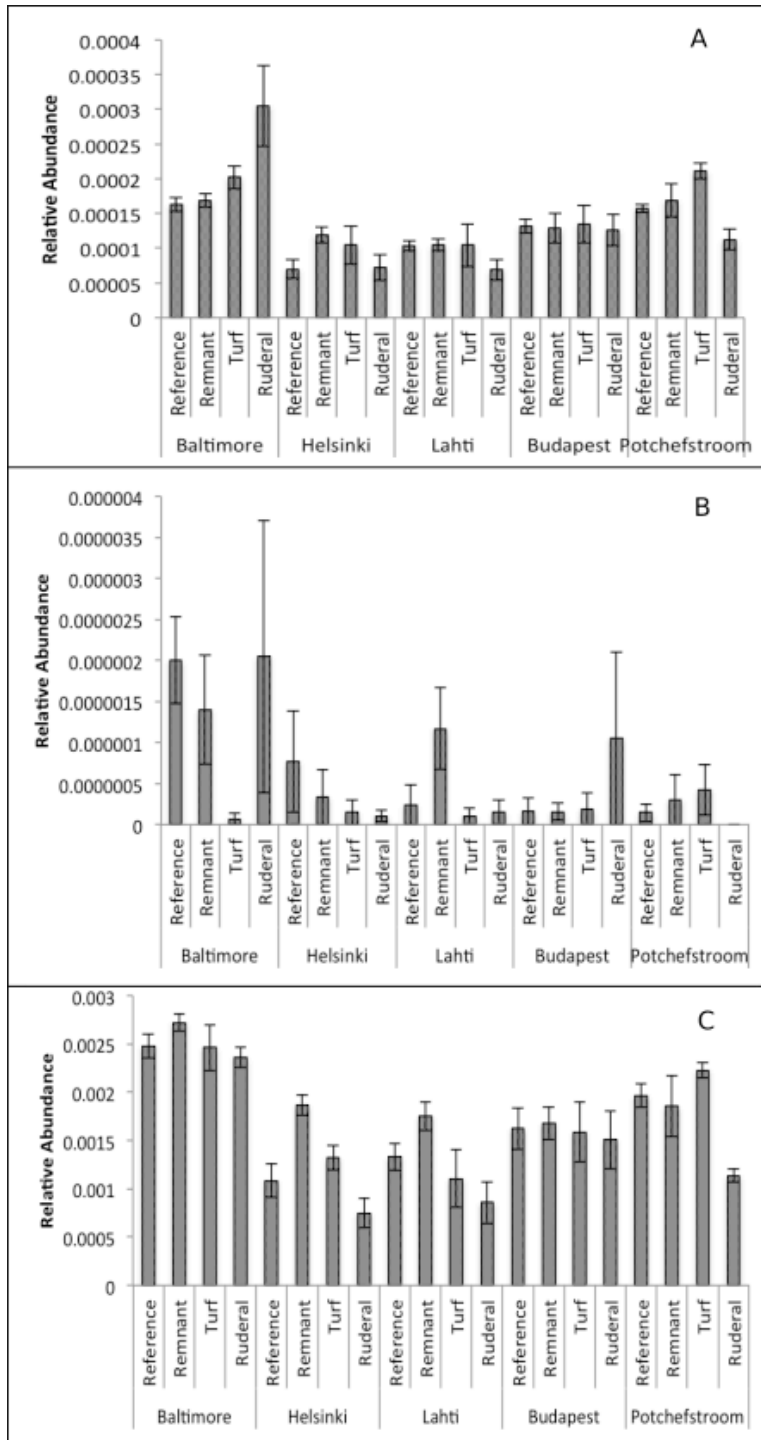


Figure 3.7: Relative abundance of A) methanogenesis genes, B) Particulate methane monooxygenase C) Carbon monoxide dehydrogenase.

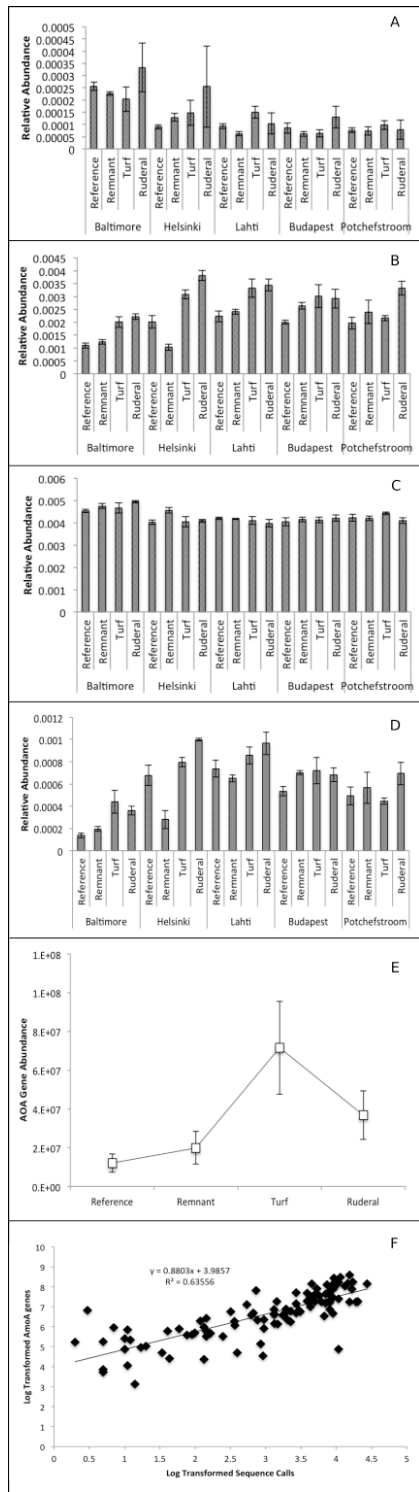


Figure 3.8: N metabolism Relative abundance of A) N fixation, B) nitrite/nitrate ammonification, C) ammonia assimilation, D) Nitrosative stress, E) actual abundance of ammonia oxidizing genes measured by QPCR, in each site category F) correlation between

ammonia oxidizing genes and taxa identified using 16s amplicon sequencing, which are putatively identified to engage in ammonia oxidation.

Nitrosative stress markers were found mostly in turf and ruderal sites (Figure 3.8,  $P < 0.001$ ), with Baltimore containing the fewest sequence matches. Other stress responses, such as osmotic stress (respectively, land use and city;  $P = 0.0147$ ;  $P < 0.001$ ), had similar patterns, while total stress response differed by geography (Figure 3.9;  $P < 0.001$ ), but not land use. Oxidative stress responses was not different across cities or landuse types. Periplasmic stress response was significantly different among cities ( $P < 0.001$ ) but not significantly different among land use ( $P = 0.06$ ), though it was generally highest in reference and remnant sites, with the exception of Baltimore and Potchefstroom. Genes associated with carbon starvation and biofilm adhesin production were prevalent in ruderal sites ( $P = 0.012$ , and  $P = 0.002$ , respectively).

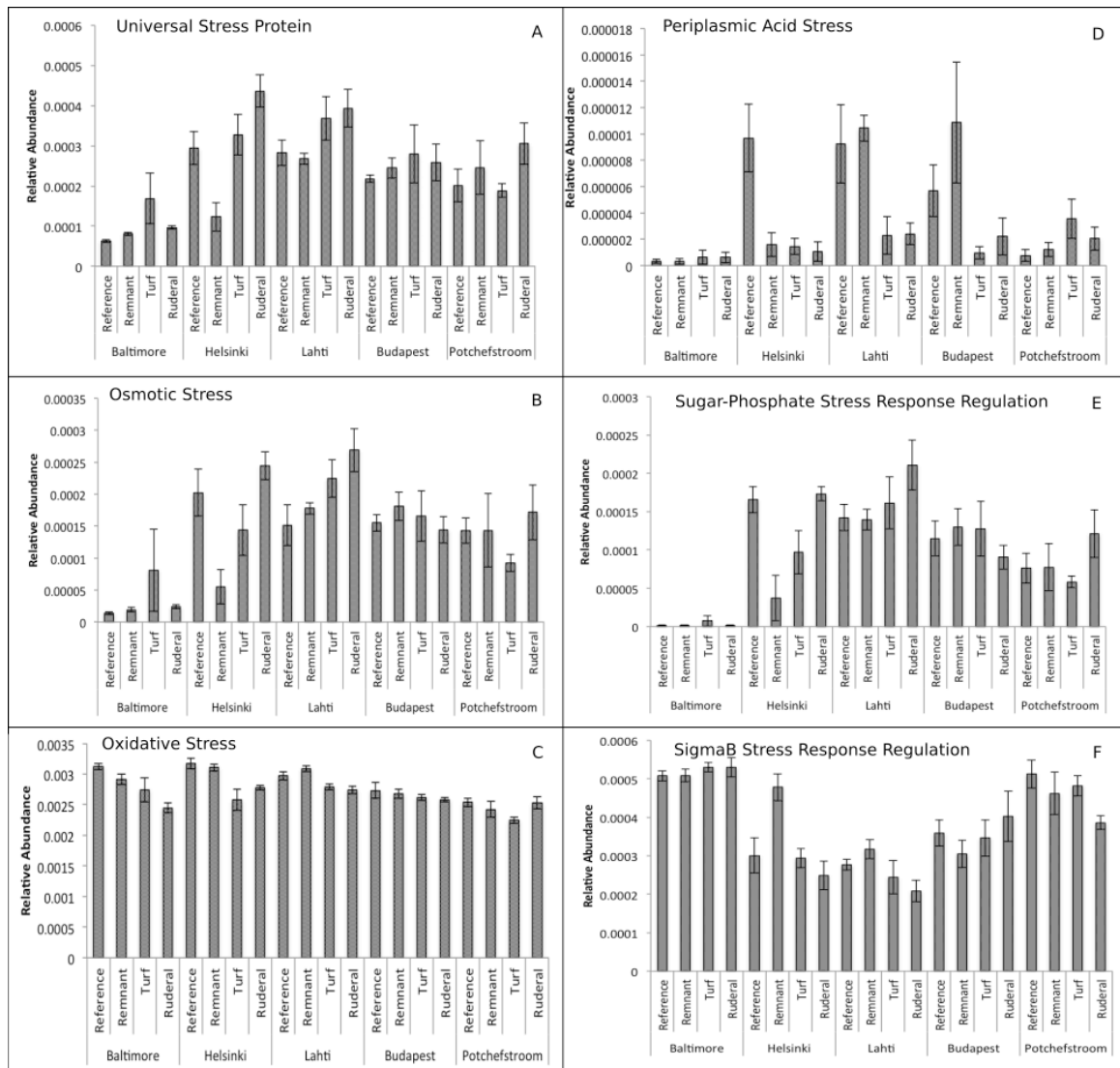


Figure 3.9: Relative abundance of stress response genes: A) universal stress protein, B) osmotic stress, C) oxidative stress, D) periplasmic acid stress response in enterobacteria, E) sugar-phosphate stress regulation, and F) SigmaB stress regulation.

Two diseases were annotated to indicate turf sites in particular cities: *Diphtheria* in Potchefstroom, and Staphylococcal pathogenicity islands in Baltimore. Pathogenicity hotspots for staphylococcus, vibrio and listeria each exhibited a different geographic expression pattern (Figure 3.10); Listeria was concentrated in Budapest ( $P < 0.001$ ), while vibrio pathogenicity island was in greater abundance in Helsinki, Lahti and Budapest ( $P < 0.001$ ). Neither pathogen exhibited a significant relationship to land use.



Staphylococcus was most abundant in Baltimore ruderal sites ( $P=0.008$ ); across all cities, it was most abundant in turf sites. Helsinki and Lahti had the lowest proportion of Staphylococcus pathogenicity hotspot SaPI ( $P<0.001$ ).

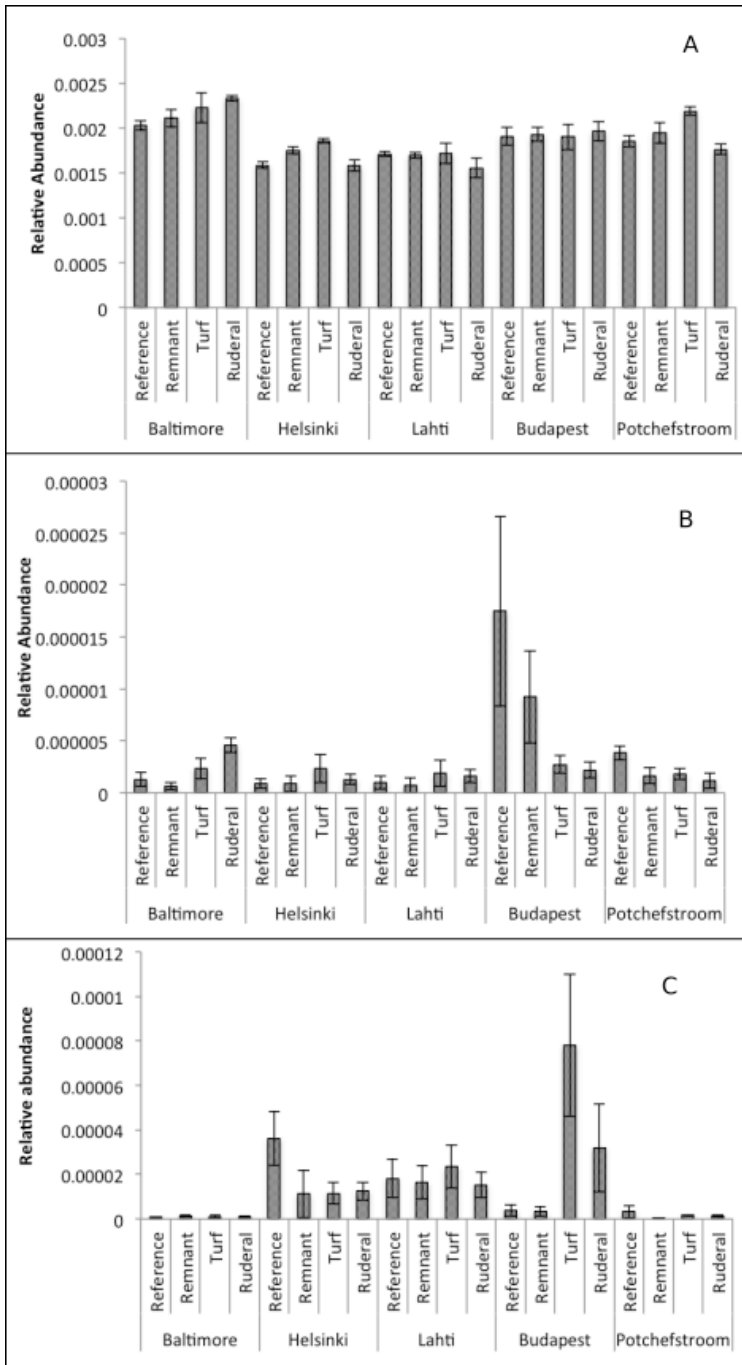


Figure 3.10: Pathogens Relative abundance of A) Staphylococcus pathogenicity island, B) Listeria pathogenicity island, C) Vibrio pathogenicity island.

### Discussion

The metagenomic data significantly differed based on geography and land-use, however, there was no significant convergence of function profiles according to land use. These results suggest that urbanization is not leading to functional convergence of the bacterial community. Previous research has shown that there may be a strong influence of local geology on the mineralogy of urban soils<sup>83</sup>. Many functions that were significantly different among cities were functions thought to be affected by mineralogy or climate. For example, Baltimore receives approximately twice the annual rainfall than any of the other cities, and its soils often have a seasonally high water table during the winter (I sampled the 14<sup>th</sup> and 15<sup>th</sup> of November). Thus these soils are more likely to periodically have lower redox potential. In addition, the moist climate may cause soluble nutrients such as nitrate to leach out. Functions such as methanogenesis, N fixation, and sulfur oxidation were highest in Baltimore. Conversely, functions related to the capture and transport of iron were least abundant. This may happen if iron is reduced, making it soluble and easily available. At the global scale, N fixation is negatively correlated with nitrate and nitrite ammonification. This suggests that in some areas, N recycling is more important to microbial communities in fixation.

Some of the differences in function may be driven by soil mineralogy. For example, resistance, uptake, and transport pathways for some metals, such as zinc, arsenic and cobalt-zinc-cadmium show distinct geographic distributions. Zinc resistance was higher in Baltimore than in any other city, while arsenic resistance was highest in

Helsinki ruderal sites (Figure 3.3). Genes responsible for cobalt, zinc and cadmium resistance were indicators of reference sites in Baltimore. This may be a result of the proximity of Baltimore reference sites to the “serpentine barrens,” a prairie ecosystem that is characterized by serpentine soils, which are ultramafic, with low Ca:Mg ratios, and are sometimes associated with higher concentrations of heavy metals<sup>88</sup>. The availability of these metals, may have been further modified by human intervention such as tillage and development that increases air and water deposition of these sediments.

Human alteration of pH and moisture content may mediate the availability of micronutrients. For example, oxidized iron is insoluble and not readily available to plants, whereas reduced iron is highly soluble and mobile in soils, and humans have a long history of manipulating local hydrology. Most metal micronutrients are most available at a pH between 6.2 and 6.8 (Brady and Weil, 2010); thus agricultural fields are limed to increase the availability of these micronutrients. However in the urban environment, in addition to parent material, heavy metals can be enriched in soils by industrial activity. Particularly, zinc enrichment occurs in close proximity to roadways<sup>89</sup>. The modification of soil pH may further enhance the bioavailability of zinc, and thus increase the abundance of genes. Although resistance and uptake pathways for metals varied according to city, they were also significantly impacted by urbanization. Arsenic resistance was a strong indicator of ruderal sites ( $P=0.003$ ), as were copper homeostasis genes ( $p=0.001$ ), as well as nickel and cobalt transport genes. These functions could be related to either a concentrating of heavy metal in ruderal soils, or an increased biological availability of heavy metal species.

Humans have other impacts as well. The widespread combustion of fossil fuels in industrialized nations is associated with higher concentrations of reactive nitrogen species in the atmosphere<sup>90</sup>. These reactive species may induce nitrosative stress. However, the nitrosative stress responses in the bacterial community is an indicator of greater oxidative stress that could be produced as a byproduct of denitrification, due to exposure to nitric oxide generated in the process. Bacterial DNA repair mechanisms were also more abundant in ruderal sites, which indirectly supports the observation that urban soil bacteria are responding to some sort of oxidative stress. While it is not possible to determine the driving mechanism exposing soil organisms to greater oxidative stress, this example illustrates that it is conceivable that soil microbial community respond to atmospheric pollutants even if there is no physical disruption of soil structure. This raises the interesting possibility that urban microbes may also be impacted by days with high ground-level ozone concentrations. Further research is needed to elucidate these interactions.

### Summary

I hypothesized that microbial function profiles would reflect differences that are adaptive to global patterns in the soil environment, such as parent material and climate. I hypothesized additionally that urban land use would modify those geographic patterns to reflect the new soil conditions imposed by land use. I also hypothesized that this interaction would cause highly modified environments, such as turf and ruderal sites, to converge relative to the historic state. In this dataset, I have shown that microbial functional gene profiles do reflect broad geographic and climactic patterns, while land

use effects were detectable nested within the geographic blocks (i.e. cities). However, I rejected my hypothesis that there would be a convergence of functional gene profiles. The differences I found in function profiles indicate the microbes are particularly responding to different mineral abundance of metals, nutrient status, and redox potential of the soil. In addition to the mineralogy of the parent material, moisture regime and pH are factors that interact to induce changes to redox potential and mineral solubility, and explain many of the global-scale and city-scale patterns I found in functional genes.

## Chapter 4: Conclusions

### Discussion

An important, but often unstated, assumption of the convergence and biotic homogenization hypotheses, is that closely related species are functionally similar. This is an assumption that is particularly important in interpreting amplicon sequencing, where there is no measure of function. In contrast, the metagenomics analysis assigns putative function to genes; putative function assignment is possible in amplicon libraries for organisms that are known to have high coherence of traits to phylogeny. To predict a convergence of community composition implies that similar functions among closely related organisms will cause them to become more abundant in the environment, driving convergence. I compared the detectability of a convergence pattern at each level of taxonomic assignment within each domain of life in this study (Figure 4.1) to determine how tightly coupled the traits under convergent selection are to phylogeny. What I found was that the ability to detect phylogenetic convergence patterns followed closely predictions we might make based on what we know about metabolic coherence of lineages within each domain, and what we know about our ability to accurately assign taxonomy. For example, certain metabolic traits, such as photosynthesis and methanogenesis, can be coherent to the phylum level<sup>91</sup>. In contrast, heterotrophic strategies (carbon oxidation) vary at finer taxonomy, sometimes to the order or class level<sup>34</sup>. Although I did not test functional traits of any of the organisms in this study, there were remarkable parallels between expected outcomes and coherence patterns. For

example, the archaeal community, which was dominated by methanogens and ammonium oxidizers, are generally known to have functional coherence to the class level. I observed that significant phylogenetic convergence also occurred at the class level. Similarly, the bacterial community, which has a greater proportion of members that use metabolic strategies with lower degrees of phylogenetic coherence, only exhibited a detectable convergence pattern at the family level or finer (Figure 4.1). Previous research has shown that bacterial niche parameters are randomly distributed at the class level<sup>82</sup>. My data agrees with these findings and suggest that community assembly should be studied at taxonomic resolution fine enough to distinguish metabolic strategies. Although there are still many unknowns, the archaeal and bacterial databases have considerably improved in recent years. The pattern of phylogenetic coherence in fungal community (Figure 4.1), however, suggests that unresolved taxonomy does not accurately represent true functional or phylogenetic relationships. I expect that once taxonomy can accurately be assigned, fungal convergence pattern will be similar to bacterial community patterns. Whether we expect community function, or community composition to converge, depends on a relationship between dispersal rate of organisms, rate of evolutionary change within organisms, and crucially, the distribution of functional traits across lineages.

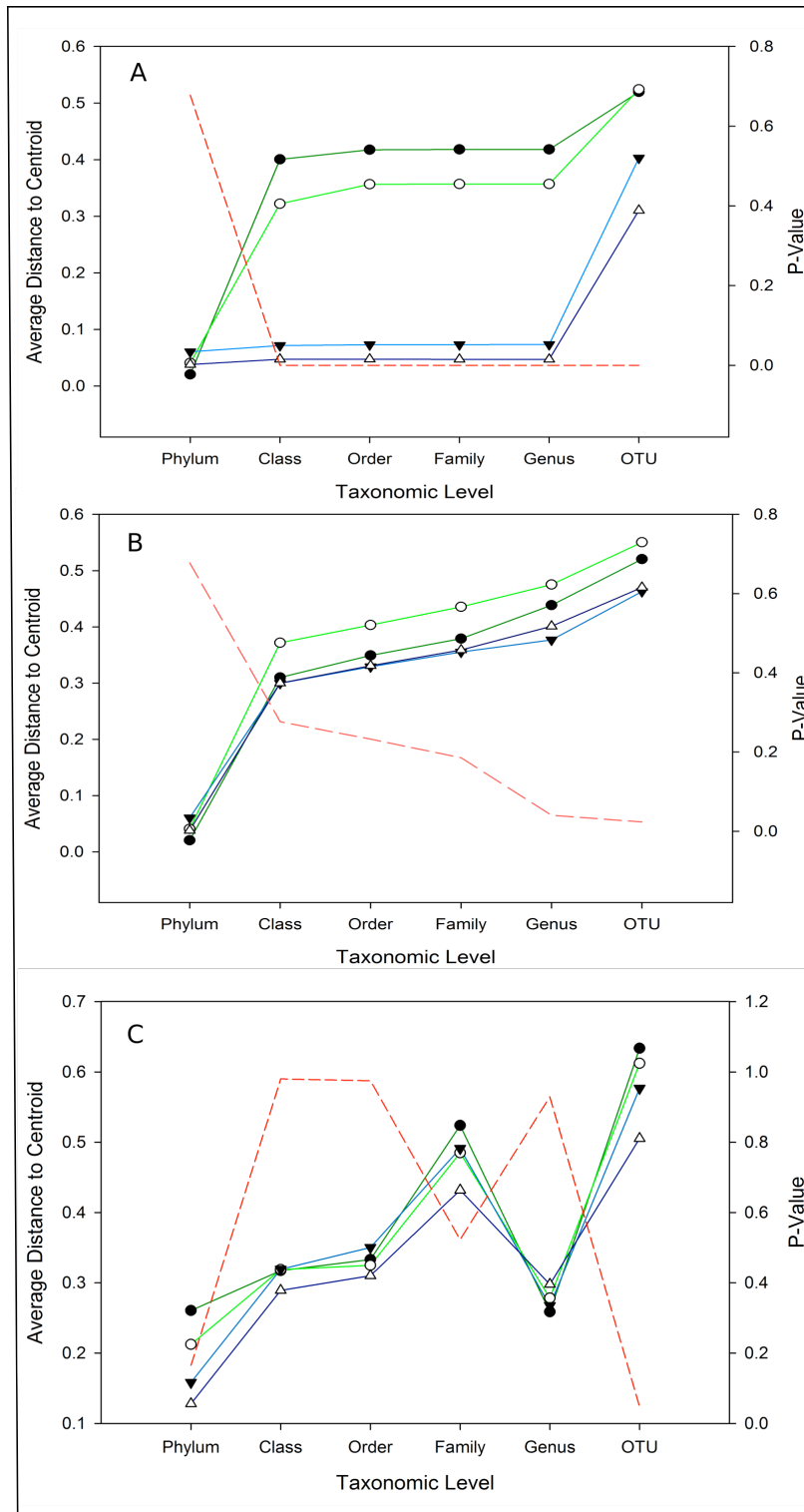


Figure 4.1: Taxonomic coherence of the global phylogenetic convergence pattern in A) archaea; B) bacteria; C) fungi. The dotted line represents the P-value at each taxonomic level. Solid circle is reference, open circle is remnant, open triangle is turf, closed circle is ruderal.



A key question driving the metagenomics approach was to ascertain whether there was a functional convergence within the bacterial community even when there was only weak evidence of convergence in community composition. My chapter three clearly demonstrates that there was not; there were significant effects of land-use and geography on function profiles, but these functional profiles did not converge. Furthermore, the metagenomics approach does also allow us to assign taxonomic profiles to the most abundant organisms. The patterns within the metagenome-derived community composition closely match those found in the functional profiles. Together, they also tell a similar story to the amplicon-derived community composition: that the bacterial community is structured by geography, and by landuse. But I find that the convergence of urban characteristics (pH, SOM etc.) is not associated with a convergence of bacterial community composition or potential function. This may be the result of unique interactions between different parent material and pH and SOM of soils.

There are a number of reasons why convergence might not be detectable, or may not have occurred, in the function dataset. There is a pervasive database bias in metagenomic annotations; annotations primarily derived of medical research, or agronomy (e.g. plant pathology). For example, *campylobacter* is a genus that the world health organization describes as causing intestinal infections (WHO Fact Sheet #255; published October, 2011), and according to my metagenomics annotations is responsible for pathways identified in iron metabolism, adhesion, and antibacterial drug resistance. And yet, there were no sequences identified as belonging to the campylobacter genus in my 16s community dataset. In fact, many of the annotated functions, including siderophore expression, biofilm formation, and antibacterial resistance pathways, are

derived from medical model organisms. For example, annotations of siderophores are associated with pathogens such as *Yersinia*, *Enterobactin*, and *campylobacter* (all engage in some pathogenicity); annotations of biofilms are nearly all related to pathogenesis pathways in *campylobacter* and *staphylococcus*; many drug resistance annotations are based on resistance to specific market antibiotics such as Erythromycin or Fosfomycin (though there are a few “broad-range” anti-bacterial resistance loci annotations), and the list goes on. In my preceding analysis, I have assumed that many of these annotations do represent accurately the functions, if perhaps not the specific organism from which they are derived.

It is possible, however, that some functions are simply not annotated yet. For example, I used the PICRUSSt tool to predict the functional potential of our 16s amplicon library to investigate the presence of ammonium oxidizing archaea and bacteria in turf site categories. Quite to my surprise, PICRUSSt predicted no ammonium oxidation. I collected QPCR data on Ammonium oxidizing archaeal genes and ammonium oxidizing bacterial genes using the ammonium monooxygenase subunit A. My data shows a significant correlation between AMOA genes and the identification of archaeal taxa that are generally known to engage in ammonium oxidation (Figure 3.8, Panel E and F). Therefore, I am confident that ammonium oxidizing archaea are a part of the community. In exploring the metagenomics data, which uses some of the same databases as PICRUSSt while employing a different annotation algorithm, I discovered that ammonium oxidation and methane oxidation are binned together because the enzymes are closely related. Thus, not only are some functions not annotated, the relationships between other annotations and environmental parameters may be obscured by the confounding effect of

inappropriate binning of enzymes. Potentially further obscuring these relationships, it is also possible that many enzymes that engage in homologous activity are not yet known. And, if pH is a master variable controlling microbial survival, then the proteins for some common cell functions are likely to have homologues that are optimized to operate under different pH environments; if these functions are binned together as homologues contributing to the same function, then the overall community function profile may not appear to converge even if the community becomes enriched with genes for resistant homologues of that enzyme. Thus, if the database is not curated in such a way as to be sensitive to the environmental performance of genes, we will not be able to detect a relationship between the expression of certain homologues and the environment from which they were sampled.

And finally, lack of convergence may be due to the nature of the similarity indices themselves. If the majority of genes identified are house-keeping genes that are expected to be present in some form in all samples, then the large body of genes that should not converge might mask the smaller fraction of those that should. Moreover, previous research has shown that the richness component of the index is highly correlated with convergences; that much of convergence is actually the richness of sites becoming more similar<sup>51</sup>. This is an irrelevant metric in metagenomics as the vast diversity of bacterial metabolic genes and housekeeping genes, means that it is highly unlikely that our sampling will detect a decrease in functional diversity even if a couple functional groups are indeed lost.

It is also possible that there was truly no convergence in function profiles. Changes in edaphic factors are certainly going to increase the expression of certain genes

relative to others. However, increased expression can occur without increasing the concentration of genes themselves. And reciprocally, cells will not express every gene they contain all the time. This is crucially important because in a given gram of soil, there will certainly exist many microsites for which the conditions differ dramatically from the average state, and the states of these microsites will vary through time. Thus, it is reasonable to assert that many genes coding for diverse functions may be maintained, and expressed when they are necessary. It is also important to consider that the scale at which we measure edaphic factors is much broader than the micro-site scale at which microbes face environmental selection, thus we certainly will not detect the existence of deviant microsite conditions; given our methodology, it is not possible to determine the variance in pH of the distribution of microsite pH conditions. Because we have no ability to quantify the variation of microsite conditions in situ, the relationship between annotated functions and the measured soil conditions may be obscured by the existence of divergent microsites. Widely divergent microsites that vary through time may maintain a broad functional diversity in the collective genome, in spite of an average state that converges on a global scale. Thus, while relatively broad scale measurements may be sufficient to predict aggregate functions across many grams of soil, they may not be sufficient to predict whether microsites will maintain selection on a diversity of facultative response genes.

The function of the urban ecosystem is a product of interacting physical, chemical and biotic alterations made by humans. Previous research indicates that mycorrhizal fungi can have a dramatic influence on seedling establishment<sup>37,92</sup>. The opposite effect is also true: the suppression of host species leads to a dramatic loss in ectomycorrhizal fungi.

This is a feedback that may make it harder for mycorrhizal plants to persist in urban areas. The suppression of ectomycorrhizal hosts also suppresses a mutualism that is particularly effective at capturing and cycling nutrient resources, and may contribute to the increased rate of nutrient loss in urban areas. In terms of the bacterial community, research indicates that landscape features are more useful in predicting denitrification activity than is community composition<sup>42</sup>, but this does not preclude disturbance from sufficiently altering the community composition to induce changes in function. It is also likely that within an urban center, disruption of past hydrology and community composition are not independent events. The “leakiness” of urban centers is likely due to a combination of altered landscape features, substantial N enrichment, and simultaneous disruption of nutrient cycling mechanisms. The loss of ectomycorrhizal fungi itself is a symptom of the larger problem: substantial nutrient enrichment and suppression of the native plant community. Remediating this situation necessarily means restructuring the landscape, selecting for the plant community, and substantially reducing nutrient inputs. But there are also opportunities; recent modeling work has shown that ectomycorrhizal fungi play an important role in carbon sequestration. In N-limited ecosystems with abundant ectomycorrhizal fungi, the fungi outcompete other decomposers for access to N, and carbon cycling in the soil is slowed leading to accumulating C in the soil<sup>93,94</sup>. By mitigating nutrient inputs, we may be able to engineer urban landscapes that are effective in capturing carbon. Since atmospheric N deposition represents a large portion of urban N flux<sup>95,96</sup>, converting to a fossil fuel-free transport infrastructure would have the synergistic effect of tilting the soil community towards one that more efficiently captures carbon, and reducing urban emissions. In order to create an environment where urban

areas are in equilibrium with the surrounding landscape, we must understand how microbial communities respond to our management, and be able to predict the likely biogeochemical outcomes of those responses. This thesis represents one small step towards generating the knowledge necessary to complete such a project.

### Summary

In each data chapter, I used a different molecular technique to address the microbial community response to urbanization. This provided me the opportunity to directly compare shotgun sequencing to amplicon sequencing techniques in terms of the patterns they are capable of detecting, and the information they provide. In Chapter 2, I argue that the archaeal community has the strongest convergence effect but that this effect is driven by the dominance of just a few common lineages without evidence for biodiversity loss; the fungal communities are strongly structured by biogeography, and converge due to a loss in ectomycorrhizal fungi. The bacterial community provides strong evidence of a geographic distribution, but weak evidence of convergence. In fact, the global patterns discovered in Chapter 3 correspond well to bacterial distribution patterns found in Chapter 2; both analyses found significant differences among cities in community composition or function profiles, and significant differences among land-use categories within each city. And, in neither analysis was there a significant convergence of ruderal or turf sites relative to the reference. While Chapter 3 focused on the assigned functional traits, metagenomics can also be used to assign taxonomy; I compared amplicon and shotgun generated taxonomic profiles directly. The taxonomy assignments generated from mg-RAST show the exact same patterns as the functional assignments; geographic structure, land-use filter, and no convergence. Amplicon sequencing gives better

resolution (more taxa identified per sample); the remarkable congruence between these two approaches gives me confidence that the patterns are real. Patterns among mineral transport and resistance genes suggest that urban impacted sites are affected by the local mineralogy. The lack of convergence of functional profiles suggests one of three things: that our test is not sensitive to changes in function; or it indicates that real variation is driven either by a persistence of native edaphic factors in spite of urbanization, or that certain human impacts increase the variability of soil conditions. assembly.

## Appendices

### *Appendix A: Anova Tables*

#### Amplicon

##### **Convergence Bacteria**

###### Phylum

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Groups	3	0.04233	0.014111	1.0224	0.3863
Residuals	96	1.32496	0.013802		

###### Class

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Groups	3	0.08956	0.029852	1.3066	0.2768
Residuals	96	2.19327	0.022847		

###### Order

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Groups	3	0.09002	0.030006	1.46	0.2303
Residuals	96	1.97295	0.020552		

##### a. Family

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Groups	3	0.10318	0.034394	1.6391	0.1854
Residuals	96	2.01446	0.020984		

###### Genus

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Groups	3	0.13964	0.046545	2.8755	0.04014
Residuals	96	1.55392	0.016187		

###### OTU



	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Groups	3	0.13091	0.043636	3.3148	0.02321
Residuals	96	1.26373	0.013164		

### Convergence Archaea

#### Phylum

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Groups	3	0.02006	0.0066855	0.5078	0.6779
Residuals	96	1.264	0.0131667		

#### Class

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Groups	3	2.3638	0.78792	18.231	1.93E-09
Residuals	96	4.149	0.04322		

#### Order

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Groups	3	2.7261	0.90871	19.421	6.38E-10
Residuals	96	4.4919	0.04679		

#### Family

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Groups	3	2.7356	0.91187	19.363	6.73E-10
Residuals	96	4.521	0.04709		

#### Genus

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Groups	3	2.7342	0.9114	19.31	7.06E-10
Residuals	96	4.5311	0.0472		

#### OTU

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Groups	3	0.78984	0.26328	19.078	8.75E-10
Residuals	96	1.3248	0.0138		

## Convergence Fungi

### Phylum

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Groups	3	0.258	0.085992	1.7333	0.1653
Residuals	96	4.7628	0.049612		

### Class

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Groups	3	0.0162	0.005396	0.0607	0.9803
Residuals	96	8.5314	0.088869		

### Order

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Groups	3	0.021	0.006992	0.0716	0.975
Residuals	96	9.3795	0.097703		

### Family

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Groups	3	0.1086	0.036187	0.7532	0.5232
Residuals	96	4.6125	0.048047		

### Genus

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Groups	3	0.0212	0.00708	0.1504	0.9292
Residuals	96	4.5181	0.047064		

### OTU

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Groups	3	0.2369	0.078967	2.7335	0.04792
Residuals	96	2.7733	0.028888		

## Convergence of Function

### Convergence Function Level1

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Groups	3	0.00005	1.59E-05	0.11	0.95
Residuals	92	0.01296	1.41E-04		

### Convergence Function Level 3

	Df	Sum Sq	Mean Sq F	value	Pr(>F)
Groups	3	0.0001	0.000035	0.04	0.99
Residuals	92	0.0795	0.000864		

## Biogeography

### Permanova Bacteria

Source	d.f.	SS	MS	F	p
CITY	4	8.5963	2.1491	4.7234	0.0006
TRT	15	6.8247	0.45498	5.1392	0.0002
Residual	80	7.0825	8.85E-02		
Total	99	22.503			

### Permanova Archaea

Source	d.f.	SS	MS	F	p
CITY	4	3.2304	0.80761	1.6994	0.1722
TRT	15	7.1287	0.47524	9.7136	0.0002
Residual	80	3.9141	4.89E-02		
Total	99	14.273			

### Permanova Fungi

Source	d.f.	SS	MS	F	p
CITY	4	10.817	2.7042	3.6415	0.0002
TRT	15	11.139	0.74262	3.4314	0.0002
Residual	80	17.313	0.21642		
Total	99	39.27			

#### Permanova Function 1

Source	d.f.	SS	MS	F	p
CITY	3	1.31E-02	4.36E-03	3.9404	0.007
TRT	12	1.33E-02	1.11E-03	2.6903	0.0004
Residual	64	2.63E-02	4.12E-04		
Total	79	5.27E-02			

#### Permanova Function 3

Source	d.f.	SS	MS	F	p
CITY	3	7.79E-02	2.60E-02	2.4933	0.0242
TRT	12	0.12495	1.04E-02	3.2651	0.0002
Residual	64	0.2041	3.19E-03		
Total	79	0.40693			

### Metagenome Functions

#### Fatty Acid Regulation

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
TRT	3	2.17E-05	7.22E-06	14.185	1.98E-07
CITY	4	3.31E-05	8.28E-06	16.273	1.14E-09
TRT:CITY	12	2.21E-05	1.84E-06	3.618	0.00027
Residuals	76	3.87E-05	5.09E-07		

#### Pyruvate Metabolism/acetyl CoA acetogenesis from pyruvate

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
TRT	3	2.26E-06	7.53E-07	8.75	4.68E-05
CITY	4	5.15E-06	1.29E-06	14.962	4.61E-09
TRT:CITY	12	1.55E-06	1.29E-07	1.496	0.144
Residuals	76	6.54E-06	8.61E-08		

#### Universal GTPases

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
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TRT	3	3.46E-07	1.15E-07	0.641	0.59085
CITY	4	8.43E-06	2.11E-06	11.714	1.84E-07
TRT:CITY	12	6.37E-06	5.31E-07	2.95	0.00205
Residuals	76	1.37E-05	1.80E-07		

Methionine  
Biosynthesis

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
TRT	3	2.28E-07	7.61E-08	1.207	0.31291
CITY	4	1.11E-06	2.78E-07	4.408	0.00294
TRT:CITY	12	1.27E-06	1.06E-07	1.674	0.0895
Residuals	76	4.79E-06	6.31E-08		

Glutamine/Glutamate and Aspartamine/Aspartamate biosynthesis

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
TRT	3	5.57E-07	1.86E-07	2.259	0.08839
CITY	4	4.66E-06	1.17E-06	14.163	1.11E-08
TRT:CITY	12	2.44E-06	2.03E-07	2.469	0.00882
Residuals	76	6.25E-06	8.22E-08		

Peptidoglycan Biosynthesis

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
TRT	3	1.50E-06	4.99E-07	6.972	0.000331
CITY	4	7.80E-06	1.95E-06	27.228	4.96E-14
TRT:CITY	12	1.69E-06	1.41E-07	1.968	0.039074
Residuals	76	5.44E-06	7.16E-08		

TCA Cycle

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
TRT	3	1.24E-06	4.12E-07	2.156	0.1002
CITY	4	2.18E-05	5.44E-06	28.516	1.78E-14
TRT:CITY	12	4.31E-06	3.59E-07	1.879	0.0503
Residuals	76	1.45E-05	1.91E-07		

PROSC

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
TRT	3	2.24E-07	7.48E-08	2.304	0.0836
CITY	4	9.23E-07	2.31E-07	7.113	6.42E-05
TRT:CITY	12	8.04E-07	6.70E-08	2.065	0.0294

Residuals	76	2.47E-06	3.25E-08
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#### tRNA modification in Bacteria

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
TRT	3	7.90E-06	2.63E-06	13.936	2.50E-07
CITY	4	9.96E-06	2.49E-06	13.185	3.32E-08
TRT:CITY	12	5.61E-06	4.67E-07	2.473	0.00874
Residuals	76	1.44E-05	1.89E-07		

#### DNA Replication

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
TRT	3	3.90E-06	1.30E-06	16.045	3.57E-08
CITY	4	1.60E-05	4.00E-06	49.375	< 2e-16
TRT:CITY	12	1.02E-06	8.50E-08	1.045	0.418
Residuals	76	6.16E-06	8.10E-08		

#### Sugar Utilization in thermotogales

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
TRT	3	2.41E-06	8.04E-07	1.75	0.16389
CITY	4	2.13E-05	5.33E-06	11.617	2.06E-07
TRT:CITY	12	1.40E-05	1.16E-06	2.536	0.00722
Residuals	76	3.49E-05	4.59E-07		

#### YgfZ

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
TRT	3	8.41E-07	2.80E-07	0.902	0.4444
CITY	4	2.81E-05	7.01E-06	22.549	2.64E-12
TRT:CITY	12	7.75E-06	6.46E-07	2.078	0.0283
Residuals	76	2.36E-05	3.11E-07		

#### Serine glyoxelate cycle

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
TRT	3	1.58E-05	5.25E-06	7.719	0.000144
CITY	4	7.95E-05	1.99E-05	29.196	1.04E-14
TRT:CITY	12	2.57E-05	2.14E-06	3.146	0.00113
Residuals	76	5.17E-05	6.80E-07		

#### Photosystem 1

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
TRT	3	1.42E-11	4.74E-12	1.871	0.14166

CITY	4	8.67E-11	2.17E-11	8.558	9.34E-06
TRT:CITY	12	8.08E-11	6.74E-12	2.661	0.00494
Residuals	76	1.92E-10	2.53E-12		

photosystem II

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
TRT	3	1.06E-11	3.52E-12	4.02	0.0104
CITY	4	2.28E-12	5.69E-13	0.651	0.628
TRT:CITY	12	2.36E-11	1.96E-12	2.244	0.0174
Residuals	76	6.65E-11	8.75E-13		

Photosystem II type photosynthetic reaction center

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
TRT	3	1.26E-10	4.19E-11	3.138	0.0302
CITY	4	3.09E-11	7.72E-12	0.578	0.6798
TRT:CITY	12	1.45E-10	1.21E-11	0.904	0.5467
Residuals	76	1.02E-09	1.34E-11		

Bacteria light harvesting proteins

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
TRT	3	3.90E-12	1.30E-12	1.786	0.157
CITY	4	2.78E-12	6.95E-13	0.954	0.438
TRT:CITY	12	1.21E-11	1.01E-12	1.38	0.194
Residuals	76	5.54E-11	7.28E-13		

Particulate methane monooxygenase

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
TRT	3	3.50E-12	1.17E-12	1.228	0.30525
CITY	4	1.46E-11	3.65E-12	3.848	0.00669
TRT:CITY	12	1.54E-11	1.29E-12	1.355	0.20659
Residuals	76	7.21E-11	9.49E-13		

CO dehydrogenase

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
TRT	3	6.21E-06	2.07E-06	11.201	3.63E-06
CITY	4	1.78E-05	4.46E-06	24.115	6.67E-13
TRT:CITY	12	3.61E-06	3.01E-07	1.628	0.101
Residuals	76	1.41E-05	1.85E-07		

Methanogenesis

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
TRT	3	1.09E-08	3.62E-09	2.038	0.115576
CITY	4	1.54E-07	3.85E-08	21.724	5.57E-12
TRT:CITY	12	7.11E-08	5.92E-09	3.339	0.000628
Residuals	76	1.35E-07	1.77E-09		

#### Methanogenesis from methylated compounds

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
TRT	3	1.06E-09	3.54E-10	6.337	0.000681
CITY	4	1.07E-09	2.68E-10	4.795	0.001673
TRT:CITY	12	5.43E-10	4.52E-11	0.81	0.63927
Residuals	76	4.24E-09	5.58E-11		

#### Siderophore Enterobactin

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
TRT	3	4.04E-06	1.35E-06	9.061	3.35E-05
CITY	4	9.85E-06	2.46E-06	16.578	8.31E-10
TRT:CITY	12	4.56E-06	3.80E-07	2.562	0.00667
Residuals	76	1.13E-05	1.49E-07		

#### Siderophore Desferroxamine

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
TRT	3	2.02E-10	6.75E-11	0.992	0.40121
CITY	4	1.37E-09	3.43E-10	5.044	0.00117
TRT:CITY	12	5.20E-10	4.33E-11	0.637	0.80449
Residuals	76	5.17E-09	6.80E-11		

#### Iron Chelation (all siderophores)

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
TRT	3	1.84E-05	6.12E-06	6.467	0.000588
CITY	4	8.25E-05	2.06E-05	21.787	5.26E-12
TRT:CITY	12	2.97E-05	2.47E-06	2.614	0.005703
Residuals	76	7.19E-05	9.46E-07		

#### Sulfur Oxidation

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
TRT	3	2.27E-07	7.58E-08	9.325	2.53E-05
CITY	4	1.78E-06	4.45E-07	54.742	< 2e-16
TRT:CITY	12	1.32E-07	1.10E-08	1.355	0.207



Residuals	76	6.18E-07	8.10E-09
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#### Campylobacter Iron metabolism

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
TRT	3	7.00E-07	2.33E-07	9.363	2.43E-05
CITY	4	1.85E-06	4.63E-07	18.579	1.10E-10
TRT:CITY	12	7.51E-07	6.26E-08	2.512	0.00776
Residuals	76	1.89E-06	2.49E-08		

#### Pyruvateferredoxinoxidoreductase

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
TRT	3	3.86E-07	1.29E-07	10.52	7.26E-06
CITY	4	5.17E-07	1.29E-07	10.572	7.30E-07
TRT:CITY	12	5.79E-07	4.83E-08	3.944	0.000102
Residuals	76	9.30E-07	1.22E-08		

#### Nitrate and Nitrite ammonification

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
TRT	3	3.00E-05	1.00E-05	34.091	4.57E-14
CITY	4	1.41E-05	3.52E-06	11.98	1.34E-07
TRT:CITY	12	1.24E-05	1.04E-06	3.524	0.00036
Residuals	76	2.23E-05	2.94E-07		

#### N fixation

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
TRT	3	4.81E-08	1.60E-08	1.421	0.243
CITY	4	3.59E-07	8.97E-08	7.956	2.06E-05
TRT:CITY	12	8.26E-08	6.88E-09	0.611	0.827
Residuals	76	8.57E-07	1.13E-08		

#### Dissimilatory nitrite reductase

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
TRT	3	2.25E-08	7.49E-09	3.411	0.02165
CITY	4	1.30E-07	3.25E-08	14.799	5.50E-09
TRT:CITY	12	6.90E-08	5.75E-09	2.618	0.00563
Residuals	76	1.67E-07	2.20E-09		

#### Nitriliase

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
TRT	3	4.44E-11	1.48E-11	0.485	0.694

CITY	4	2.21E-10	5.53E-11	1.812	0.135
TRT:CITY	12	5.01E-10	4.18E-11	1.368	0.2
Residuals	76	2.32E-09	3.05E-11		

#### Ammonia assimilation

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
TRT	3	3.50E-07	1.17E-07	1.364	0.26
CITY	4	3.98E-06	9.95E-07	11.644	2.00E-07
TRT:CITY	12	1.49E-06	1.24E-07	1.452	0.162
Residuals	76	6.50E-06	8.55E-08		

#### Urea decomposition

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
TRT	3	1.08E-06	3.61E-07	6.493	0.00057
CITY	4	4.01E-06	1.00E-06	18.052	1.85E-10
TRT:CITY	12	1.17E-06	9.75E-08	1.755	0.07152
Residuals	76	4.22E-06	5.56E-08		

#### NiFe hydrogenase maturation

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
TRT	3	2.83E-08	9.44E-09	0.736	0.53355
CITY	4	3.16E-07	7.90E-08	6.158	0.00024
TRT:CITY	12	1.20E-07	1.00E-08	0.78	0.66864
Residuals	76	9.75E-07	1.28E-08		

#### Putative hemin transporter

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
TRT	3	8.69E-08	2.90E-08	1.63	0.18943
CITY	4	1.54E-06	3.85E-07	21.66	5.93E-12
TRT:CITY	12	5.91E-07	4.92E-08	2.77	0.00354
Residuals	76	1.35E-06	1.78E-08		

#### Transport of Iron

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
TRT	3	3.46E-06	1.15E-06	10.942	4.72E-06
CITY	4	6.86E-06	1.72E-06	16.254	1.16E-09
TRT:CITY	12	2.87E-06	2.39E-07	2.265	0.0163
Residuals	76	8.02E-06	1.06E-07		

#### Transport of Manganese

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
TRT	3	3.69E-07	1.23E-07	14.341	1.71E-07
CITY	4	6.00E-07	1.50E-07	17.514	3.18E-10
TRT:CITY	12	3.25E-07	2.71E-08	3.158	0.00109
Residuals	76	6.51E-07	8.57E-09		

#### Transport of Molybdenum

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
TRT	3	1.15E-08	3.85E-09	1.392	0.2517
CITY	4	1.15E-07	2.88E-08	10.411	8.90E-07
TRT:CITY	12	6.93E-08	5.77E-09	2.09	0.0274
Residuals	76	2.10E-07	2.76E-09		

#### Transport of Nickel and Cobalt

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
TRT	3	7.21E-07	2.40E-07	13.928	2.52E-07
CITY	4	3.67E-07	9.17E-08	5.311	0.000796
TRT:CITY	12	5.00E-07	4.17E-08	2.416	0.010369
Residuals	76	1.31E-06	1.73E-08		

#### Transport of Zinc

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
TRT	3	1.00E-07	3.33E-08	2.941	0.03838
CITY	4	9.09E-07	2.27E-07	20.038	2.68E-11
TRT:CITY	12	3.56E-07	2.97E-08	2.619	0.00561
Residuals	76	8.61E-07	1.13E-08		

#### Potassium homeostasis

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
TRT	3	1.20E-06	4.00E-07	2.33	0.081
CITY	4	3.43E-05	8.56E-06	49.866	<2e-16
TRT:CITY	12	5.26E-06	4.38E-07	2.551	0.0069
Residuals	76	1.31E-05	1.72E-07		

#### Zinc Resistance

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
TRT	3	1.42E-08	4.70E-09	0.756	0.522
CITY	4	2.96E-06	7.41E-07	118.005	<2e-16
TRT:CITY	12	9.46E-08	7.90E-09	1.256	0.263

Residuals	76	4.77E-07	6.30E-09
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#### Arsenic Resistance

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
TRT	3	1.46E-06	4.88E-07	22.05	2.26E-10
CITY	4	1.03E-06	2.56E-07	11.58	2.15E-07
TRT:CITY	12	7.43E-07	6.19E-08	2.8	0.00323
Residuals	76	1.68E-06	2.21E-08		

#### Cadmium Resistance

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
TRT	3	2.69E-09	8.96E-10	1.351	0.26425
CITY	4	1.02E-08	2.55E-09	3.841	0.00676
TRT:CITY	12	7.29E-09	6.08E-10	0.917	0.53476
Residuals	76	5.04E-08	6.63E-10		

#### Cobalt-zinc-cadmium resistance

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
TRT	3	7.60E-05	2.53E-05	39.77	1.47E-15
CITY	4	2.23E-04	5.57E-05	87.435	< 2e-16
TRT:CITY	12	4.91E-05	4.09E-06	6.427	9.78E-08
Residuals	76	4.84E-05	6.40E-07		

#### Mercury resistance operon

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
TRT	3	2.71E-08	9.02E-09	8.923	3.89E-05
CITY	4	1.42E-08	3.54E-09	3.501	0.0112
TRT:CITY	12	1.08E-08	8.96E-10	0.887	0.5635
Residuals	76	7.68E-08	1.01E-09		

#### Resistance to chromium compounds

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
TRT	3	2.09E-08	6.97E-09	2.641	0.0554
CITY	4	1.94E-07	4.85E-08	18.375	1.35E-10
TRT:CITY	12	3.70E-08	3.09E-09	1.17	0.3195
Residuals	76	2.00E-07	2.64E-09		

#### Acid resistance mechanisms

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
TRT	3	1.15E-07	3.84E-08	2.278	0.0863

CITY	4	1.60E-06	4.00E-07	23.68	9.73E-13
TRT:CITY	12	4.44E-07	3.70E-08	2.192	0.0202
Residuals	76	1.28E-06	1.69E-08		

#### Mercuric reductase

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
TRT	3	2.04E-07	6.79E-08	13.036	5.91E-07
CITY	4	5.52E-07	1.38E-07	26.507	8.91E-14
TRT:CITY	12	2.13E-07	1.78E-08	3.411	0.000505
Residuals	76	3.96E-07	5.21E-09		

#### Multidrug resistance efflux pump

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
TRT	3	5.90E-06	1.97E-06	8.47	6.33E-05
CITY	4	2.93E-05	7.32E-06	31.505	1.81E-15
TRT:CITY	12	4.30E-06	3.59E-07	1.544	0.127
Residuals	76	1.77E-05	2.32E-07		

#### Themdt ABCD mutidrug resittance cluster

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
TRT	3	4.50E-07	1.50E-07	3.137	0.0302
CITY	4	3.71E-06	9.26E-07	19.367	5.10E-11
TRT:CITY	12	1.11E-06	9.28E-08	1.94	0.0423
Residuals	76	3.64E-06	4.78E-08		

#### lipid AAra4N pathway polymyxine resistance

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
TRT	3	1.44E-07	4.81E-08	3.366	0.0229
CITY	4	1.37E-06	3.43E-07	23.99	7.43E-13
TRT:CITY	12	9.92E-08	8.30E-09	0.578	0.8532
Residuals	76	1.09E-06	1.43E-08		

#### Nitrosative stress

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
TRT	3	1.24E-06	4.14E-07	14.988	9.35E-08
CITY	4	2.69E-06	6.71E-07	24.316	5.61E-13
TRT:CITY	12	1.12E-06	9.29E-08	3.365	0.000581
Residuals	76	2.10E-06	2.76E-08		

#### SigmaB stress response regulation

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
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TRT	3	6.28E-08	2.10E-08	3.346	0.02344
CITY	4	7.64E-07	1.91E-07	30.519	3.79E-15
TRT:CITY	12	2.05E-07	1.71E-08	2.727	0.00404
Residuals	76	4.76E-07	6.26E-09		

Periplasmic Stress response

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
TRT	3	2.70E-07	8.99E-08	2.47	0.0682
CITY	4	2.16E-06	5.40E-07	14.832	5.31E-09
TRT:CITY	12	8.29E-07	6.90E-08	1.897	0.0478
Residuals	76	2.77E-06	3.64E-08		

Osmotic cluster regulation

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
TRT	3	5.32E-08	1.77E-08	3.729	0.0147
CITY	4	2.73E-07	6.82E-08	14.345	9.05E-09
TRT:CITY	12	1.30E-07	1.08E-08	2.28	0.0156
Residuals	76	3.61E-07	4.75E-09		

total stress (all stress indicators combined)

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
TRT	3	2.08E-07	6.92E-08	0.332	0.802118
CITY	4	4.96E-06	1.24E-06	5.954	0.000319
TRT:CITY	12	2.78E-06	2.32E-07	1.112	0.362931
Residuals	76	1.58E-05	2.08E-07		

Listeria Pathogenicity island LIPI1

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
TRT	3	1.32E-10	4.38E-11	1.475	0.228154
CITY	4	6.30E-10	1.57E-10	5.297	0.000812
TRT:CITY	12	6.91E-10	5.76E-11	1.937	0.042709
Residuals	76	2.26E-09	2.97E-11		

Staphylococcal pathogenicity island SaPI

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
TRT	3	4.35E-07	1.45E-07	4.204	0.00832
CITY	4	2.87E-06	7.18E-07	20.799	1.31E-11
TRT:CITY	12	6.66E-07	5.55E-08	1.608	0.10717
Residuals	76	2.62E-06	3.45E-08		

Vibrio pathogenicity Island

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
TRT	3	3.77E-09	1.26E-09	2.414	0.073141
CITY	4	1.11E-08	2.78E-09	5.335	0.000769
TRT:CITY	12	1.71E-08	1.43E-09	2.744	0.003841
Residuals	76	3.96E-08	5.21E-10		

### Appendix B: R Scripts

#### **Shannon Weiner Index**

```
library(vegan)
Bacteria=read.csv('Desktop/Thesis/AlphaDiversity/Bacteria_OTU.csv')
attach(Bacteria)
diversity(Bacteria, index="Shannon", MARGIN=1, base=exp(1))
```

```
Archaea=read.csv('Desktop/Thesis/AlphaDiversity/ArchaeaOTUtable.csv')
attach(Archaea)
diversity(Archaea, index="Shannon", MARGIN=1, base=exp(1))
```

```
Fungi=read.csv('Desktop/Thesis/AlphaDiversity/Fungal_OTU.csv')
attach(Fungal)
diversity(Fungal, index="Shannon", MARGIN=1, base=exp(1))
```

#### **Pielou's J**

```
library(asbio)
```

```
Bacteria=read.csv('Desktop/Thesis/AlphaDiversity/Bacteria_OTU.csv')
Archaea=read.csv('Desktop/Thesis/AlphaDiversity/ArchaeaOTUtable.csv')
Fungi=read.csv('Desktop/Thesis/AlphaDiversity/Fungal_OTU.csv')
```

```
evenness(Bacteria)
evenness(Archaea)
evenness(Fungi)
```

#### **Convergence Archaea:**

```
ArchaealCommunity_Phylum=read.csv('Desktop/Thesis/R/BetaDiversityAnalysis_073115/PCOA/Archaea/Archaea_CommunityData_Phylum_101915.csv')
```

```
ArchaeaPhylum_DistanceMatrix=vegdist(ArchaealCommunity_Phylum, method="bray", binary=FALSE, diag=FALSE, upper=FALSE, na.rm=FALSE)
```

```
Sup_Data=read.csv('Desktop/Thesis/R/BetaDiversityAnalysis_073115/PCOA/SecondMatrix.csv')
```

```
attach(Sup_Data)
GroupA=BLK2
GroupB=BLK
GroupC=TRT
```

```
ArchaeaPhylum_modA=betadisper(ArchaeaPhylum_DistanceMatrix, GroupA,
type=c("median"))
ArchaeaPhylum_modB=betadisper(ArchaeaPhylum_DistanceMatrix, GroupB,
type=c("median"))
ArchaeaPhylum_modC=betadisper(ArchaeaPhylum_DistanceMatrix, GroupC,
type=c("median"))
```

```
anova(ArchaeaPhylum_modA)
anova(ArchaeaPhylum_modB)
anova(ArchaeaPhylum_modC)
```

```
permutest(ArchaeaPhylum_modA)
permutest(ArchaeaPhylum_modB)
permutest(ArchaeaPhylum_modC)
```

```
ArchaeaPhylum_modA
ArchaeaPhylum_modB
ArchaeaPhylum_modC
```

```
#####CLASS#####
```

```
ArchaeaCommunity_Class=read.csv('Desktop/Thesis/R/BetaDiversityAnalysis_073115/P
COA/Archaea/Archaea_CommunityData_Class_101915.csv')
```

```
ArchaeaClass_DistanceMatrix=vegdist(ArchaeaCommunity_Class, method="bray",
binary=FALSE, diag=FALSE, upper=FALSE, na.rm=FALSE)
```

```
ArchaeaClass_modA=betadisper(ArchaeaClass_DistanceMatrix, GroupA,
type=c("median"))
ArchaeaClass_modB=betadisper(ArchaeaClass_DistanceMatrix, GroupB,
type=c("median"))
ArchaeaClass_modC=betadisper(ArchaeaClass_DistanceMatrix, GroupC,
type=c("median"))
```

```
anova(ArchaeaClass_modA)
anova(ArchaeaClass_modB)
anova(ArchaeaClass_modC)
```

```
permutest(ArchaeaClass_modA)
permutest(ArchaeaClass_modB)
```



```
permutest(ArchaeaClass_modC)
```

```
ArchaeaClass_modA
```

```
ArchaeaClass_modB
```

```
ArchaeaClass_modC
```

```
#####ORDER#####
```

```
ArchaeaCommunity_Order=read.csv('Desktop/Thesis/R/BetaDiversityAnalysis_073115/  
PCOA/Archaea/Archaea_CommunityData_Order_101915.csv')
```

```
ArchaeaOrder_DistanceMatrix=vegdist(ArchaeaCommunity_Order, method="bray",  
binary=FALSE, diag=FALSE, upper=FALSE, na.rm=FALSE)
```

```
ArchaeaOrder_modA=betadisper(ArchaeaOrder_DistanceMatrix, GroupA,  
type=c("median"))
```

```
ArchaeaOrder_modB=betadisper(ArchaeaOrder_DistanceMatrix, GroupB,  
type=c("median"))
```

```
ArchaeaOrder_modC=betadisper(ArchaeaOrder_DistanceMatrix, GroupC,  
type=c("median"))
```

```
anova(ArchaeaOrder_modA)
```

```
anova(ArchaeaOrder_modB)
```

```
anova(ArchaeaOrder_modC)
```

```
permutest(ArchaeaOrder_modA)
```

```
permutest(ArchaeaOrder_modB)
```

```
permutest(ArchaeaOrder_modC)
```

```
ArchaeaOrder_modA
```

```
ArchaeaOrder_modB
```

```
ArchaeaOrder_modC
```

```
#####FAMILY#####
```

```
ArchaeaCommunity_Family=read.csv('Desktop/Thesis/R/BetaDiversityAnalysis_073115/  
/PCOA/Archaea/Archaea_CommunityData_Family_101915.csv')
```

```
ArchaeaFamily_DistanceMatrix=vegdist(ArchaeaCommunity_Family, method="bray",  
binary=FALSE, diag=FALSE, upper=FALSE, na.rm=FALSE)
```

```
ArchaeaFamily_modA=betadisper(ArchaeaFamily_DistanceMatrix, GroupA,  
type=c("median"))
```

```
ArchaeaFamily_modB=betadisper(ArchaeaFamily_DistanceMatrix, GroupB,  
type=c("median"))
```

```
ArchaeaFamily_modC=betadisper(ArchaeaFamily_DistanceMatrix, GroupC,  
type=c("median"))
```

```
anova(ArchaeaFamily_modA)
```

```
anova(ArchaeaFamily_modB)
```

```
anova(ArchaeaFamily_modC)
```

```
permutest(ArchaeaFamily_modA)
```

```
permutest(ArchaeaFamily_modB)
```

```
permutest(ArchaeaFamily_modC)
```

```
ArchaeaFamily_modA
```

```
ArchaeaFamily_modB
```

```
ArchaeaFamily_modC
```

```
#####GENUS#####
```

```
ArchaeaCommunity_Genus=read.csv('Desktop/Thesis/R/BetaDiversityAnalysis_073115/  
PCOA/Archaea/Archaea_CommunityData_Genus_073115.csv')
```

```
ArchaeaGenus_DistanceMatrix=vegdist(ArchaeaCommunity_Genus, method="bray",  
binary=FALSE, diag=FALSE, upper=FALSE, na.rm=FALSE)
```

```
ArchaeaGenus_modA=betadisper(ArchaeaGenus_DistanceMatrix, GroupA,  
type=c("median"))
```

```
ArchaeaGenus_modB=betadisper(ArchaeaGenus_DistanceMatrix, GroupB,  
type=c("median"))
```

```
ArchaeaGenus_modC=betadisper(ArchaeaGenus_DistanceMatrix, GroupC,  
type=c("median"))
```

```
anova(ArchaeaGenus_modA)
```

```
anova(ArchaeaGenus_modB)
```

```
anova(ArchaeaGenus_modC)
```

```
permutest(ArchaeaGenus_modA)
```

```
permutest(ArchaeaGenus_modB)
```

```
permutest(ArchaeaGenus_modC)
```

```
ArchaeaGenus_modA  
ArchaeaGenus_modB  
ArchaeaGenus_modC
```

```
#####Archaea OTU#####
```

```
ArchaeaOTU=read.csv('Desktop/Thesis/R/AlphaDiversity/ArchaeaOTUtable.csv')
```

```
ArchaeaOTU_DistanceMatrix=vegdist(ArchaeaOTU, method="bray", binary=FALSE,  
diag=FALSE, upper=FALSE, na.rm=FALSE)
```

```
ArchaeaOTU_modA=betadisper(ArchaeaOTU_DistanceMatrix, GroupA,  
type=c("median"))  
ArchaeaOTU_modB=betadisper(ArchaeaOTU_DistanceMatrix, GroupB,  
type=c("median"))  
ArchaeaOTU_modC=betadisper(ArchaeaOTU_DistanceMatrix, GroupC,  
type=c("median"))
```

```
anova(ArchaeaOTU_modA)  
anova(ArchaeaOTU_modB)  
anova(ArchaeaOTU_modC)
```

```
permutest(ArchaeaOTU_modA)  
permutest(ArchaeaOTU_modB)  
permutest(ArchaeaOTU_modC)
```

```
ArchaeaOTU_modA  
ArchaeaOTU_modB  
ArchaeaOTU_modC
```

```
#####
```

```
Treatment by City
```

```
#####
```

```
Baltimore
```

```
Archaea_Baltimore=read.csv('Desktop/Thesis/R/BetaDiversityAnalysis_073115/PCOA/  
Archaea/Archaea_PCOA_Genus_Baltimore.csv')
```

```
ArchaeaBaltimore_DistanceMatrix=vegdist(Archaea_Baltimore, method="bray",  
binary=FALSE, diag=FALSE, upper=FALSE, na.rm=FALSE)
```

```
Sup_DataBaltimore=read.csv('Desktop/Thesis/R/BetaDiversityAnalysis_073115/PCOA/  
Second_Baltimore.csv')
```

```
GroupC=TRT
```

```
ArchaeaBaltimore_modC=betadisper(ArchaeaBaltimore_DistanceMatrix, GroupC,  
type=c("median"))
```

```
anova(ArchaeaBaltimore_modC)
```

```
permutest(ArchaeaBaltimore_modC)
```

```
ArchaeaBaltimore_modC
```

```
#####BY CITY#####  
#####Baltimore#####
```

```
Archaea_Baltimore=read.csv('Desktop/Thesis/R/BetaDiversityAnalysis_073115/PCOA/  
Archaea/Archaea_PCOA_Genus_Baltimore.csv')
```

```
ArchaeaBaltimore_DistanceMatrix=vegdist(Archaea_Baltimore, method="bray",  
binary=FALSE, diag=FALSE, upper=FALSE, na.rm=FALSE)
```

```
Sup_DataBaltimore=read.csv('Desktop/Thesis/R/BetaDiversityAnalysis_073115/PCOA/  
Second_Baltimore.csv')  
attach(Sup_DataBaltimore)
```

```
GroupC=TRT
```

```
ArchaeaBaltimore_modC=betadisper(ArchaeaBaltimore_DistanceMatrix, GroupC,  
type=c("median"))
```

```
anova(ArchaeaBaltimore_modC)
```

```
permutest(ArchaeaBaltimore_modC)
```

```
ArchaeaBaltimore_modC
```

```
#####helsinki#####
```

```
Archaea_Helsinki=read.csv('Desktop/Thesis/R/BetaDiversityAnalysis_073115/PCOA/Ar  
chaea/Archaea_PCOA_Genus_Helsinki.csv')
```

```

ArchaeaHelsinki_DistanceMatrix=vegdist(Archaea_Helsinki, method="bray",
binary=FALSE, diag=FALSE, upper=FALSE, na.rm=FALSE)

Sup_DataHelsinki=read.csv('Desktop/Thesis/R/BetaDiversityAnalysis_073115/PCOA/Se
cond_Helsinki.csv')
attach(Sup_DataHelsinki)

GroupC=TRT

ArchaeaHelsinki_modC=betadisper(ArchaeaHelsinki_DistanceMatrix, GroupC,
type=c("median"))

anova(ArchaeaHelsinki_modC)

permutest(ArchaeaHelsinki_modC)

ArchaeaHelsinki_modC

#####Lahti#####
Archaea_Lahti=read.csv('Desktop/Thesis/R/BetaDiversityAnalysis_073115/PCOA/Archae
a/Archaea_PCOA_Genus_Lahti.csv')

ArchaeaLahti_DistanceMatrix=vegdist(Archaea_Lahti, method="bray", binary=FALSE,
diag=FALSE, upper=FALSE, na.rm=FALSE)

Sup_DataLahti=read.csv('Desktop/Thesis/R/BetaDiversityAnalysis_073115/PCOA/Seco
nd_Lahti.csv')
attach(Sup_DataLahti)

GroupC=TRT

ArchaeaLahti_modC=betadisper(ArchaeaLahti_DistanceMatrix, GroupC,
type=c("median"))

anova(ArchaeaLahti_modC)

permutest(ArchaeaLahti_modC)

ArchaeaLahti_modC

#####Budapest#####
Archaea_Budapest=read.csv('Desktop/Thesis/R/BetaDiversityAnalysis_073115/PCOA/A
rchaea/Archaea_PCOA_Genus_Budapest.csv')

```

```
ArchaeaBudapest_DistanceMatrix=vegdist(Archaea_Budapest, method="bray",  
binary=FALSE, diag=FALSE, upper=FALSE, na.rm=FALSE)
```

```
Sup_DataBudapest=read.csv('Desktop/Thesis/R/BetaDiversityAnalysis_073115/PCOA/S  
econd_Budapest.csv')  
attach(Sup_DataBudapest)
```

```
GroupC=TRT
```

```
ArchaeaBudapest_modC=betadisper(ArchaeaBudapest_DistanceMatrix, GroupC,  
type=c("median"))
```

```
anova(ArchaeaBudapest_modC)
```

```
permutest(ArchaeaBudapest_modC)
```

```
ArchaeaBudapest_modC
```

```
#####Potchefstroom#####
```

```
Archaea_Potchefstroom=read.csv('Desktop/Thesis/R/BetaDiversityAnalysis_073115/PC  
OA/Archaea/Archaea_PCOA_Genus_Potchefstroom.csv')
```

```
ArchaeaPotchefstroom_DistanceMatrix=vegdist(Archaea_Potchefstroom,  
method="bray", binary=FALSE, diag=FALSE, upper=FALSE, na.rm=FALSE)
```

```
Sup_DataPotchefstroom=read.csv('Desktop/Thesis/R/BetaDiversityAnalysis_073115/PC  
OA/Second_Potchefstroom.csv')  
attach(Sup_DataPotchefstroom)
```

```
GroupC=TRT
```

```
ArchaeaPotchefstroom_modC=betadisper(ArchaeaPotchefstroom_DistanceMatrix,  
GroupC, type=c("median"))
```

```
anova(ArchaeaPotchefstroom_modC)
```

```
permutest(ArchaeaPotchefstroom_modC)
```

```
ArchaeaPotchefstroom_modC
```

### **Convergence Bacteria**

```
BacterialCommunity_Phylum=read.csv('/Volumes/Seagate/R/BetaDiversityAnalysis_073  
115/PCOA/Bacteria_CommunityData_Phylum.csv')
```

```
BacteriaPhylum_DistanceMatrix=vegdist(BacterialCommunity_Phylum, method="bray",  
binary=FALSE, diag=FALSE, upper=FALSE, na.rm=FALSE)
```

```
Sup_Data=read.csv('Desktop/Thesis/R/BetaDiversityAnalysis_073115/PCOA/SecondMa  
trix.csv')
```

```
attach(Sup_Data)  
GroupA=BLK2  
GroupB=BLK  
GroupC=TRT
```

```
BacteriaPhylum_modA=betadisper(BacteriaPhylum_DistanceMatrix, GroupA,  
type=c("median"))  
BacteriaPhylum_modB=betadisper(BacteriaPhylum_DistanceMatrix, GroupB,  
type=c("median"))  
BacteriaPhylum_modC=betadisper(BacteriaPhylum_DistanceMatrix, GroupC,  
type=c("median"))
```

```
anova(BacteriaPhylum_modA)  
anova(BacteriaPhylum_modB)  
anova(BacteriaPhylum_modC)
```

```
permutest(BacteriaPhylum_modA)  
permutest(BacteriaPhylum_modB)  
permutest(BacteriaPhylum_modC)
```

```
BacteriaPhylum_modA  
BacteriaPhylum_modB  
BacteriaPhylum_modC
```

```
#####CLASS#####
```

```
BacterialCommunity_Class=read.csv('/Volumes/Seagate/R/BetaDiversityAnalysis_07311  
5/PCOA/Bacteria_CommunityData_Class.csv')
```

```
BacteriaClass_DistanceMatrix=vegdist(BacterialCommunity_Class, method="bray",  
binary=FALSE, diag=FALSE, upper=FALSE, na.rm=FALSE)
```

```
BacteriaClass_modA=betadisper(BacteriaClass_DistanceMatrix, GroupA,  
type=c("median"))  
BacteriaClass_modB=betadisper(BacteriaClass_DistanceMatrix, GroupB,  
type=c("median"))  
BacteriaClass_modC=betadisper(BacteriaClass_DistanceMatrix, GroupC,  
type=c("median"))
```

```
anova(BacteriaClass_modA)
```

```
anova(BacteriaClass_modB)
anova(BacteriaClass_modC)
```

```
permutest(BacteriaClass_modA)
permutest(BacteriaClass_modB)
permutest(BacteriaClass_modC)
```

```
BacteriaClass_modA
BacteriaClass_modB
BacteriaClass_modC
```

```
#####ORDER#####
```

```
BacterialCommunity_Order=read.csv('/Volumes/Seagate/R/BetaDiversityAnalysis_0731
15/PCOA/Bacteria_CommunityData_Order.csv')
```

```
BacteriaOrder_DistanceMatrix=vegdist(BacterialCommunity_Order, method="bray",
binary=FALSE, diag=FALSE, upper=FALSE, na.rm=FALSE)
```

```
BacteriaOrder_modA=betadisper(BacteriaOrder_DistanceMatrix, GroupA,
type=c("median"))
BacteriaOrder_modB=betadisper(BacteriaOrder_DistanceMatrix, GroupB,
type=c("median"))
BacteriaOrder_modC=betadisper(BacteriaOrder_DistanceMatrix, GroupC,
type=c("median"))
```

```
anova(BacteriaOrder_modA)
anova(BacteriaOrder_modB)
anova(BacteriaOrder_modC)
```

```
permutest(BacteriaOrder_modA)
permutest(BacteriaOrder_modB)
permutest(BacteriaOrder_modC)
```

```
BacteriaOrder_modA
BacteriaOrder_modB
BacteriaOrder_modC
```

```
#####FAMILY#####
```



```
BacterialCommunity_Family=read.csv('/Volumes/Seagate/R/BetaDiversityAnalysis_073115/PCOA/Bacteria_CommunityData_Family.csv')
```

```
BacteriaFamily_DistanceMatrix=vegdist(BacterialCommunity_Family, method="bray", binary=FALSE, diag=FALSE, upper=FALSE, na.rm=FALSE)
```

```
BacteriaFamily_modA=betadisper(BacteriaFamily_DistanceMatrix, GroupA, type=c("median"))
```

```
BacteriaFamily_modB=betadisper(BacteriaFamily_DistanceMatrix, GroupB, type=c("median"))
```

```
BacteriaFamily_modC=betadisper(BacteriaFamily_DistanceMatrix, GroupC, type=c("median"))
```

```
anova(BacteriaFamily_modA)
```

```
anova(BacteriaFamily_modB)
```

```
anova(BacteriaFamily_modC)
```

```
permutest(BacteriaFamily_modA)
```

```
permutest(BacteriaFamily_modB)
```

```
permutest(BacteriaFamily_modC)
```

```
BacteriaFamily_modA
```

```
BacteriaFamily_modB
```

```
BacteriaFamily_modC
```

```
#####GENUS#####
```

```
BacterialCommunity_Genus=read.csv('/Volumes/Seagate/R/BetaDiversityAnalysis_073115/PCOA/Bacteria_CommunityData_Genus_073115.csv')
```

```
BacteriaGenus_DistanceMatrix=vegdist(BacterialCommunity_Genus, method="bray", binary=FALSE, diag=FALSE, upper=FALSE, na.rm=FALSE)
```

```
BacteriaGenus_modA=betadisper(BacteriaGenus_DistanceMatrix, GroupA, type=c("median"))
```

```
BacteriaGenus_modB=betadisper(BacteriaGenus_DistanceMatrix, GroupB, type=c("median"))
```

```
BacteriaGenus_modC=betadisper(BacteriaGenus_DistanceMatrix, GroupC, type=c("median"))
```

```
anova(BacteriaGenus_modA)
```

```
anova(BacteriaGenus_modB)
```

```
anova(BacteriaGenus_modC)
```

```
permutest(BacteriaGenus_modA)
```

```
permutest(BacteriaGenus_modB)
```

```
permutest(BacteriaGenus_modC)
```

```
BacteriaGenus_modA
```

```
BacteriaGenus_modB
```

```
BacteriaGenus_modC
```

```
#####Bacteria OTU#####
```

```
read.tcsv = function(file, header=TRUE, sep=",", ...) {
```

```
  n = max(count.fields(file, sep=","), na.rm=TRUE)
```

```
  x = readLines(file)
```

```
  .splitvar = function(x, sep, n) {
```

```
    var = unlist(strsplit(x, split=sep))
```

```
    length(var) = n
```

```
    return(var)
```

```
  }
```

```
  x = do.call(cbind, lapply(x, .splitvar, sep=sep, n=n))
```

```
  x = apply(x, 1, paste, collapse=sep)
```

```
  out = read.csv(text=x, sep=sep, header=header, ...)
```

```
  return(out)
```

```
}
```

```
bacteria_OTU=read.tcsv('Desktop/Thesis/R/AlphaDiversity/Bacteria_OTU.csv')
```

```
BacteriaOTU_DistanceMatrix=vegdist(bacteria_OTU, method="bray", binary=FALSE,  
diag=FALSE, upper=FALSE, na.rm=FALSE)
```

```
BacteriaOTU_modA=betadisper(BacteriaOTU_DistanceMatrix, GroupA,  
type=c("median"))
```

```
BacteriaOTU_modB=betadisper(BacteriaOTU_DistanceMatrix, GroupB,  
type=c("median"))
```

```
BacteriaOTU_modC=betadisper(BacteriaOTU_DistanceMatrix, GroupC,  
type=c("median"))
```

```
anova(BacteriaOTU_modA)
```

```
anova(BacteriaOTU_modB)
anova(BacteriaOTU_modC)
```

```
permutest(BacteriaOTU_modA)
permutest(BacteriaOTU_modB)
permutest(BacteriaOTU_modC)
```

```
BacteriaOTU_modA
BacteriaOTU_modB
BacteriaOTU_modC
```

```
#####BY CITY#####
#####Baltimore#####
```

```
Bacteria_Baltimore=read.csv('Desktop/Thesis/R/BetaDiversityAnalysis_073115/PCOA/
Bacteria/Bacteria_PCOA_Genus_Baltimore.csv')
```

```
BacteriaBaltimore_DistanceMatrix=vegdist(Bacteria_Baltimore, method="bray",
binary=FALSE, diag=FALSE, upper=FALSE, na.rm=FALSE)
```

```
Sup_DataBaltimore=read.csv('Desktop/Thesis/R/BetaDiversityAnalysis_073115/PCOA/
Second_Baltimore.csv')
attach(Sup_DataBaltimore)
```

```
GroupC=TRT
```

```
BacteriaBaltimore_modC=betadisper(BacteriaBaltimore_DistanceMatrix, GroupC,
type=c("median"))
```

```
anova(BacteriaBaltimore_modC)
```

```
permutest(BacteriaBaltimore_modC)
```

```
BacteriaBaltimore_modC
```

```
#####helsinki#####
```

```
Bacteria_Helsinki=read.csv('Desktop/Thesis/R/BetaDiversityAnalysis_073115/PCOA/Ba
cteria/Bacteria_PCOA_Genus_Helsinki.csv')
```

```
BacteriaHelsinki_DistanceMatrix=vegdist(Bacteria_Helsinki, method="bray",
binary=FALSE, diag=FALSE, upper=FALSE, na.rm=FALSE)
```

```
Sup_DataHelsinki=read.csv('Desktop/Thesis/R/BetaDiversityAnalysis_073115/PCOA/Se
cond_Helsinki.csv')
attach(Sup_DataHelsinki)
```

```

GroupC=TRT

BacteriaHelsinki_modC=betadisper(BacteriaHelsinki_DistanceMatrix, GroupC,
type=c("median"))

anova(BacteriaHelsinki_modC)

permutest(BacteriaHelsinki_modC)

BacteriaHelsinki_modC

#####Lahti#####
Bacteria_Lahti=read.csv('Desktop/Thesis/R/BetaDiversityAnalysis_073115/PCOA/Bacteria/Bacteria_PCOA_Genus_Lahti.csv')

BacteriaLahti_DistanceMatrix=vegdist(Bacteria_Lahti, method="bray", binary=FALSE,
diag=FALSE, upper=FALSE, na.rm=FALSE)

Sup_DataLahti=read.csv('Desktop/Thesis/R/BetaDiversityAnalysis_073115/PCOA/Second_Lahti.csv')
attach(Sup_DataLahti)

GroupC=TRT

BacteriaLahti_modC=betadisper(BacteriaLahti_DistanceMatrix, GroupC,
type=c("median"))

anova(BacteriaLahti_modC)

permutest(BacteriaLahti_modC)

BacteriaLahti_modC

#####Budapest#####
Bacteria_Budapest=read.csv('Desktop/Thesis/R/BetaDiversityAnalysis_073115/PCOA/Bacteria/Bacteria_PCOA_Genus_Budapest.csv')

BacteriaBudapest_DistanceMatrix=vegdist(Bacteria_Budapest, method="bray",
binary=FALSE, diag=FALSE, upper=FALSE, na.rm=FALSE)

Sup_DataBudapest=read.csv('Desktop/Thesis/R/BetaDiversityAnalysis_073115/PCOA/Second_Budapest.csv')
attach(Sup_DataBudapest)

GroupC=TRT

```

```
BacteriaBudapest_modC=betadisper(BacteriaBudapest_DistanceMatrix, GroupC,  
type=c("median"))
```

```
anova(BacteriaBudapest_modC)
```

```
permutest(BacteriaBudapest_modC)
```

```
BacteriaBudapest_modC
```

```
#####Potchefstroom#####
```

```
Bacteria_Potchefstroom=read.csv('Desktop/Thesis/R/BetaDiversityAnalysis_073115/PC  
OA/Bacteria/Bacteria_PCOA_Genus_Potchefstroom.csv')
```

```
BacteriaPotchefstroom_DistanceMatrix=vegdist(Bacteria_Potchefstroom,  
method="bray", binary=FALSE, diag=FALSE, upper=FALSE, na.rm=FALSE)
```

```
Sup_DataPotchefstroom=read.csv('Desktop/Thesis/R/BetaDiversityAnalysis_073115/PC  
OA/Second_Potchefstroom.csv')  
attach(Sup_DataPotchefstroom)
```

```
GroupC=TRT
```

```
BacteriaPotchefstroom_modC=betadisper(BacteriaPotchefstroom_DistanceMatrix,  
GroupC, type=c("median"))
```

```
anova(BacteriaPotchefstroom_modC)
```

```
permutest(BacteriaPotchefstroom_modC)
```

```
BacteriaPotchefstroom_modC
```

### **Convergence Fungi**

```
FungilCommunity_Phylum=read.csv('Desktop/Thesis/R/BetaDiversityAnalysis_073115/  
PCOA/Fungi/Fungal_CommunityData_Phylum_101915.csv')
```

```
FungiPhylum_DistanceMatrix=vegdist(FungilCommunity_Phylum, method="bray",  
binary=FALSE, diag=FALSE, upper=FALSE, na.rm=FALSE)
```

```
Sup_Data=read.csv('Volumes/Seagate/R/BetaDiversityAnalysis_073115/PCOA/Second  
Matrix.csv')
```

```
attach(Sup_Data)
```

```
GroupA=BLK2
GroupB=BLK
GroupC=TRT
```

```
FungiPhylum_modA=betadisper(FungiPhylum_DistanceMatrix, GroupA,
type=c("median"))
FungiPhylum_modB=betadisper(FungiPhylum_DistanceMatrix, GroupB,
type=c("median"))
FungiPhylum_modC=betadisper(FungiPhylum_DistanceMatrix, GroupC,
type=c("median"))
```

```
anova(FungiPhylum_modA)
anova(FungiPhylum_modB)
anova(FungiPhylum_modC)
```

```
permutest(FungiPhylum_modA)
permutest(FungiPhylum_modB)
permutest(FungiPhylum_modC)
```

```
FungiPhylum_modA
FungiPhylum_modB
FungiPhylum_modC
```

```
#####CLASS#####
```

```
FungiCommunity_Class=read.csv('Desktop/Thesis/R/BetaDiversityAnalysis_073115/PC
OA/Fungi/Fungal_CommunityData_Class_101915.csv')
```

```
FungiClass_DistanceMatrix=vegdist(FungiCommunity_Class, method="bray",
binary=FALSE, diag=FALSE, upper=FALSE, na.rm=FALSE)
```

```
FungiClass_modA=betadisper(FungiClass_DistanceMatrix, GroupA, type=c("median"))
FungiClass_modB=betadisper(FungiClass_DistanceMatrix, GroupB, type=c("median"))
FungiClass_modC=betadisper(FungiClass_DistanceMatrix, GroupC, type=c("median"))
```

```
anova(FungiClass_modA)
anova(FungiClass_modB)
anova(FungiClass_modC)
```

```
permutest(FungiClass_modA)
permutest(FungiClass_modB)
permutest(FungiClass_modC)
```

```
FungiClass_modA
FungiClass_modB
FungiClass_modC
```

```
#####ORDER#####
```

```
FungiCommunity_Order=read.csv('Desktop/Thesis/R/BetaDiversityAnalysis_073115/PC  
OA/Fungi/Fungal_CommunityData_Order_101915.csv')
```

```
FungiOrder_DistanceMatrix=vegdist(FungiCommunity_Order, method="bray",  
binary=FALSE, diag=FALSE, upper=FALSE, na.rm=FALSE)
```

```
FungiOrder_modA=betadisper(FungiOrder_DistanceMatrix, GroupA, type=c("median"))  
FungiOrder_modB=betadisper(FungiOrder_DistanceMatrix, GroupB, type=c("median"))  
FungiOrder_modC=betadisper(FungiOrder_DistanceMatrix, GroupC, type=c("median"))
```

```
anova(FungiOrder_modA)  
anova(FungiOrder_modB)  
anova(FungiOrder_modC)
```

```
permutest(FungiOrder_modA)  
permutest(FungiOrder_modB)  
permutest(FungiOrder_modC)
```

```
FungiOrder_modA  
FungiOrder_modB  
FungiOrder_modC
```

```
#####FAMILY#####
```

```
FungiCommunity_Family=read.csv('Desktop/Thesis/R/BetaDiversityAnalysis_073115/P  
COA/Fungi/Fungal_CommunityData_Family_101915.csv')
```

```
FungiFamily_DistanceMatrix=vegdist(FungiCommunity_Family, method="bray",  
binary=FALSE, diag=FALSE, upper=FALSE, na.rm=FALSE)
```

```
FungiFamily_modA=betadisper(FungiFamily_DistanceMatrix, GroupA,  
type=c("median"))  
FungiFamily_modB=betadisper(FungiFamily_DistanceMatrix, GroupB,  
type=c("median"))  
FungiFamily_modC=betadisper(FungiFamily_DistanceMatrix, GroupC,  
type=c("median"))
```

```
anova(FungiFamily_modA)
anova(FungiFamily_modB)
anova(FungiFamily_modC)
```

```
permutest(FungiFamily_modA)
permutest(FungiFamily_modB)
permutest(FungiFamily_modC)
```

```
FungiFamily_modA
FungiFamily_modB
FungiFamily_modC
```

```
#####GENUS#####
```

```
FungiCommunity_Genus=read.csv('Desktop/Thesis/R/BetaDiversityAnalysis_073115/P
COA/Fungi/Fungal_CommunitData_Genus_090215.csv')
```

```
FungiGenus_DistanceMatrix=vegdist(FungiCommunity_Genus, method="bray",
binary=FALSE, diag=FALSE, upper=FALSE, na.rm=FALSE)
```

```
FungiGenus_modA=betadisper(FungiGenus_DistanceMatrix, GroupA,
type=c("median"))
FungiGenus_modB=betadisper(FungiGenus_DistanceMatrix, GroupB,
type=c("median"))
FungiGenus_modC=betadisper(FungiGenus_DistanceMatrix, GroupC,
type=c("median"))
```

```
anova(FungiGenus_modA)
anova(FungiGenus_modB)
anova(FungiGenus_modC)
```

```
permutest(FungiGenus_modA)
permutest(FungiGenus_modB)
permutest(FungiGenus_modC)
```

```
FungiGenus_modA
FungiGenus_modB
FungiGenus_modC
```

```
#####Fungi OTU#####
```



```
Fungi_OTU=read.csv('Desktop/Thesis/R/BetaDiversityAnalysis_073115/PCOA/Fungi/Fungal_CommunitData_OTU_073115.csv')
```

```
FungiOTU_DistanceMatrix=vegdist(Fungi_OTU, method="bray", binary=FALSE, diag=FALSE, upper=FALSE, na.rm=FALSE)
```

```
FungiOTU_modA=betadisper(FungiOTU_DistanceMatrix, GroupA, type=c("median"))  
FungiOTU_modB=betadisper(FungiOTU_DistanceMatrix, GroupB, type=c("median"))  
FungiOTU_modC=betadisper(FungiOTU_DistanceMatrix, GroupC, type=c("median"))
```

```
anova(FungiOTU_modA)  
anova(FungiOTU_modB)  
anova(FungiOTU_modC)
```

```
permutest(FungiOTU_modA)  
permutest(FungiOTU_modB)  
permutest(FungiOTU_modC)
```

```
FungiOTU_modA  
FungiOTU_modB  
FungiOTU_modC
```

```
#####BY CITY#####  
#####Baltimore#####
```

```
Fungi_Baltimore=read.csv('Desktop/Thesis/R/BetaDiversityAnalysis_073115/PCOA/Fungi/Fungi_PCOA_OTU_Baltimore.csv')
```

```
FungiBaltimore_DistanceMatrix=vegdist(Fungi_Baltimore, method="bray", binary=FALSE, diag=FALSE, upper=FALSE, na.rm=FALSE)
```

```
Sup_DataBaltimore=read.csv('Desktop/Thesis/R/BetaDiversityAnalysis_073115/PCOA/Second_Baltimore.csv')  
attach(Sup_DataBaltimore)
```

```
GroupC=TRT
```

```
FungiBaltimore_modC=betadisper(FungiBaltimore_DistanceMatrix, GroupC, type=c("median"))
```

```
anova(FungiBaltimore_modC)
```

```
permutest(FungiBaltimore_modC)
```

```
FungiBaltimore_modC
```

```
#####helsinki#####
```

```
Fungi_Helsinki=read.csv('Desktop/Thesis/R/BetaDiversityAnalysis_073115/PCOA/Fungi/Fungi_PCOA_OTU_Helsinki.csv')
```

```
FungiHelsinki_DistanceMatrix=vegdist(Fungi_Helsinki, method="bray",  
binary=FALSE, diag=FALSE, upper=FALSE, na.rm=FALSE)
```

```
Sup_DataHelsinki=read.csv('Desktop/Thesis/R/BetaDiversityAnalysis_073115/PCOA/Second_Helsinki.csv')  
attach(Sup_DataHelsinki)
```

```
GroupC=TRT
```

```
FungiHelsinki_modC=betadisper(FungiHelsinki_DistanceMatrix, GroupC,  
type=c("median"))
```

```
anova(FungiHelsinki_modC)
```

```
permutest(FungiHelsinki_modC)
```

```
FungiHelsinki_modC
```

```
#####Lahti#####
```

```
Fungi_Lahti=read.csv('Desktop/Thesis/R/BetaDiversityAnalysis_073115/PCOA/Fungi/Fungi_PCOA_OTU_Lahti.csv')
```

```
FungiLahti_DistanceMatrix=vegdist(Fungi_Lahti, method="bray", binary=FALSE,  
diag=FALSE, upper=FALSE, na.rm=FALSE)
```

```
Sup_DataLahti=read.csv('Desktop/Thesis/R/BetaDiversityAnalysis_073115/PCOA/Second_Lahti.csv')  
attach(Sup_DataLahti)
```

```
GroupC=TRT
```

```
FungiLahti_modC=betadisper(FungiLahti_DistanceMatrix, GroupC, type=c("median"))
```

```
anova(FungiLahti_modC)
```

```
permutest(FungiLahti_modC)
```

FungiLahti\_modC

#####Budapest#####

```
Fungi_Budapest=read.csv('Desktop/Thesis/R/BetaDiversityAnalysis_073115/PCOA/Fungi/Fungi_PCOA_OTU_Budapest.csv')
```

```
FungiBudapest_DistanceMatrix=vegdist(Fungi_Budapest, method="bray", binary=FALSE, diag=FALSE, upper=FALSE, na.rm=FALSE)
```

```
Sup_DataBudapest=read.csv('Desktop/Thesis/R/BetaDiversityAnalysis_073115/PCOA/Seond_Budapest.csv')  
attach(Sup_DataBudapest)
```

GroupC=TRT

```
FungiBudapest_modC=betadisper(FungiBudapest_DistanceMatrix, GroupC, type=c("median"))
```

```
anova(FungiBudapest_modC)
```

```
permutest(FungiBudapest_modC)
```

FungiBudapest\_modC

#####Potchefstroom#####

```
Fungi_Potchefstroom=read.csv('Desktop/Thesis/R/BetaDiversityAnalysis_073115/PCOA/Fungi/Fungi_PCOA_OTU_Potchefstroom.csv')
```

```
FungiPotchefstroom_DistanceMatrix=vegdist(Fungi_Potchefstroom, method="bray", binary=FALSE, diag=FALSE, upper=FALSE, na.rm=FALSE)
```

```
Sup_DataPotchefstroom=read.csv('Desktop/Thesis/R/BetaDiversityAnalysis_073115/PCOA/Second_Potchefstroom.csv')  
attach(Sup_DataPotchefstroom)
```

GroupC=TRT

```
FungiPotchefstroom_modC=betadisper(FungiPotchefstroom_DistanceMatrix, GroupC, type=c("median"))
```

```
anova(FungiPotchefstroom_modC)
```

```
permutest(FungiPotchefstroom_modC)
```

```
FungiPotchefstroom_modC
```

## Anova

```
library(vegan)
```

```
qpcr.data=read.csv('Desktop/Thesis/R/QPCR/QPCR_Data_100615.csv')
```

```
attach(qpcr.data)
```

```
ITS.TRT=aov(its ~ TRT + CITY, data=qpcr.data)
```

```
summary(ITS.TRT)
```

```
X16s.TRT=aov(X16s ~ TRT+ CITY, data=qpcr.data)
```

```
summary(X16s.TRT)
```

```
AOA.TRT=aov(AOA~ TRT+ CITY, data=qpcr.data)
```

```
summary(AOA.TRT)
```

```
logaoa.TRT=aov(logaoa ~ TRT+ CITY, data=qpcr.data)
```

```
summary(logaoa.TRT)
```

```
logaob.TRT=aov(logaob ~ TRT+ CITY, data=qpcr.data)
```

```
summary(logaob.TRT)
```

```
AOB.TRT=aov(AOB ~ TRT+ CITY, data=qpcr.data)
```

```
summary(AOB.TRT)
```

```
PICRUStAmoa.TRT=aov(PICRUStAmoa ~ TRT+ CITY, data=qpcr.data)
```

```
summary(PICRUStAmoa.TRT)
```

```
F_Pathotroph.TRT=aov(F_Pathotroph ~ TRT+ CITY, data=qpcr.data)
```

```
summary(F_Pathotroph.TRT)
```

```
F_Symbiotroph.TRT=aov(F_Symbiotroph ~ TRT+ CITY, data=qpcr.data)
```

```
summary(F_Symbiotroph.TRT)
```

```
F_Saprotroph.TRT=aov(F_Saprotroph ~ TRT+ CITY, data=qpcr.data)
```

```
summary(F_Saprotroph.TRT)
```

```
Archseq_Thaum=aov(Arch_Amoa~TRT + CITY, data=qpcr.data)
```

```
summary(Archseq_Thaum)
```

```
Archseq_Therm=aov(Arch_acid~TRT+ CITY, data=qpcr.data)
```

```
summary(Archseq_Therm)
```

```
Archseq_Meth=aov(Arch_Meth~TRT+ CITY, data=qpcr.data)
```

```
summary(Archseq_Meth)
```

## Post-hoc Tukey HSD

```
TukeyHSD(ITS.TRT, ordered=FALSE, conf.level=0.95)
```

```
TukeyHSD(AOA.TRT, ordered=FALSE, conf.level=0.95)
```

```
TukeyHSD(Logaoa.TRT, ordered=FALSE, conf.level=0.95)
```

```
TukeyHSD(PICRUStAmoa.TRT, ordered=FALSE, conf.level=0.95)
```

```
TukeyHSD(F_Symbiotroph.TRT, ordered=FALSE, conf.level=0.95)
```

```
TukeyHSD(Archseq_Thaum, ordered=FALSE, conf.level=0.95)
```

## *Environmental correlation*

```
archaeacmatrix1=read.csv('Desktop/Thesis/R/MainMatrix/Archaea_Genus_Main.csv')  
ArchOrd1=metaMDS(archaeacmatrix1)
```

```
Bacteriamatrix1=read.csv('Desktop/Thesis/R/MainMatrix/Bacteria_Genus_Main.csv')  
BacOrd1=metaMDS(Bacteriamatrix1)
```

```
Fungimatrix1=read.csv('Desktop/Thesis/R/MainMatrix/Fungi_OTU_Main.csv')  
FunOrd1=metaMDS(Fungimatrix1)
```

```
Data.env=read.csv('Desktop/Thesis/R/MainMatrix/Second.csv')  
ArchFit=envfit(ArchOrd1, Data.env)  
ArchFit
```

```
BacFit=envfit(BacOrd1, Data.env)  
BacFit
```

```
FunFit=envfit(FunOrd1, Data.env)  
FunFit
```

## Appendix C: Qiime Scripts

### **16s Library:**

```
fastq-join -m 50 -r /media/Seagate/QIIME/16s/Joinreport/DES_1_Joinreport  
Illumina/Data/MiSeq_67_Sequences/files.cgrb.oregonstate.edu/Illumina/140331_M0149  
8_0067_000000000-A8U60/BaseCalls/lane1-s008-indexN708-S501-CAGAGAGG-  
TAGATCGC-8_S8_L001_R1_001.fastq.gz  
Illumina/Data/MiSeq_67_Sequences/files.cgrb.oregonstate.edu/Illumina/140331_M0149  
8_0067_000000000-A8U60/BaseCalls/lane1-s008-indexN708-S501-CAGAGAGG-  
TAGATCGC-8_S8_L001_R2_001.fastq.gz -o  
/media/Seagate/QIIME/16s/DES_1_Unpaired_R1 -o  
/media/Seagate/QIIME/16s/DES_1_Unpaired_R2 -o  
/media/Seagate/QIIME/16s/DES_1_Joined;fastq-join -m 50 -r  
/media/Seagate/QIIME/16s/Joinreport/DES_2_Joinreport  
Illumina/Data/MiSeq_67_Sequences/files.cgrb.oregonstate.edu/Illumina/140331_M0149  
8_0067_000000000-A8U60/BaseCalls/lane1-s020-indexN708-S502-CAGAGAGG-  
CTCTCTAT-20_S20_L001_R1_001.fastq.gz  
Illumina/Data/MiSeq_67_Sequences/files.cgrb.oregonstate.edu/Illumina/140331_M0149  
8_0067_000000000-A8U60/BaseCalls/lane1-s020-indexN708-S502-CAGAGAGG-  
CTCTCTAT-20_S20_L001_R2_001.fastq.gz -o  
/media/Seagate/QIIME/16s/DES_2_Unpaired_R1 -o
```

```

/media/Seagate/QIIME/16s/DES_2_Unpaired_R2 -o
/media/Seagate/QIIME/16s/DES_2_Joined;fastq-join -m 50 -r
/media/Seagate/QIIME/16s/Joinreport/DES_3_Joinreport
Illumina/Data/MiSeq_67_Sequences/files.cgrb.oregonstate.edu/Illumina/140331_M0149
8_0067_000000000-A8U60/BaseCalls/lane1-s032-indexN708-S503-CAGAGAGG-
TATCCTCT-32_S32_L001_R1_001.fastq.gz
Illumina/Data/MiSeq_67_Sequences/files.cgrb.oregonstate.edu/Illumina/140331_M0149
8_0067_000000000-A8U60/BaseCalls/lane1-s032-indexN708-S503-CAGAGAGG-
TATCCTCT-32_S32_L001_R2_001.fastq.gz -o
/media/Seagate/QIIME/16s/DES_3_Unpaired_R1 -o
/media/Seagate/QIIME/16s/DES_3_Unpaired_R2 -o
/media/Seagate/QIIME/16s/DES_3_Joined;fastq-join -m 50 -r
/media/Seagate/QIIME/16s/Joinreport/DES_4_Joinreport
Illumina/Data/MiSeq_67_Sequences/files.cgrb.oregonstate.edu/Illumina/140331_M0149
8_0067_000000000-A8U60/BaseCalls/lane1-s044-indexN708-S504-CAGAGAGG-
AGAGTAGA-44_S44_L001_R1_001.fastq.gz
Illumina/Data/MiSeq_67_Sequences/files.cgrb.oregonstate.edu/Illumina/140331_M0149
8_0067_000000000-A8U60/BaseCalls/lane1-s044-indexN708-S504-CAGAGAGG-
AGAGTAGA-44_S44_L001_R2_001.fastq.gz -o
/media/Seagate/QIIME/16s/DES_4_Unpaired_R1 -o
/media/Seagate/QIIME/16s/DES_4_Unpaired_R2 -o
/media/Seagate/QIIME/16s/DES_4_Joined;fastq-join -m 50 -r
/media/Seagate/QIIME/16s/Joinreport/DES_5_Joinreport
Illumina/Data/MiSeq_67_Sequences/files.cgrb.oregonstate.edu/Illumina/140331_M0149
8_0067_000000000-A8U60/BaseCalls/lane1-s056-indexN708-S505-CAGAGAGG-
GTAAGGAG-56_S56_L001_R1_001.fastq.gz
Illumina/Data/MiSeq_67_Sequences/files.cgrb.oregonstate.edu/Illumina/140331_M0149
8_0067_000000000-A8U60/BaseCalls/lane1-s056-indexN708-S505-CAGAGAGG-
GTAAGGAG-56_S56_L001_R2_001.fastq.gz -o
/media/Seagate/QIIME/16s/DES_5_Unpaired_R1 -o
/media/Seagate/QIIME/16s/DES_5_Unpaired_R2 -o
/media/Seagate/QIIME/16s/DES_5_Joined;fastq-join -m 50 -r
/media/Seagate/QIIME/16s/Joinreport/DES_6_Joinreport
Illumina/Data/MiSeq_67_Sequences/files.cgrb.oregonstate.edu/Illumina/140331_M0149
8_0067_000000000-A8U60/BaseCalls/lane1-s068-indexN708-S506-CAGAGAGG-
ACTGCATA-68_S68_L001_R1_001.fastq.gz
Illumina/Data/MiSeq_67_Sequences/files.cgrb.oregonstate.edu/Illumina/140331_M0149
8_0067_000000000-A8U60/BaseCalls/lane1-s068-indexN708-S506-CAGAGAGG-
ACTGCATA-68_S68_L001_R2_001.fastq.gz -o
/media/Seagate/QIIME/16s/DES_6_Unpaired_R1 -o
/media/Seagate/QIIME/16s/DES_6_Unpaired_R2 -o
/media/Seagate/QIIME/16s/DES_6_Joined;fastq-join -m 50 -r
/media/Seagate/QIIME/16s/Joinreport/DES_7_Joinreport
Illumina/Data/MiSeq_67_Sequences/files.cgrb.oregonstate.edu/Illumina/140331_M0149
8_0067_000000000-A8U60/BaseCalls/lane1-s080-indexN708-S507-CAGAGAGG-
AAGGAGTA-80_S80_L001_R1_001.fastq.gz

```

Illumina/Data/MiSeq\_67\_Sequences/files.cgrb.oregonstate.edu/Illumina/140331\_M0149  
 8\_0067\_000000000-A8U60/BaseCalls/lane1-s080-indexN708-S507-CAGAGAGG-  
 AAGGAGTA-80\_S80\_L001\_R2\_001.fastq.gz -o  
 /media/Seagate/QIIME/16s/DES\_7\_Unpaired\_R1 -o  
 /media/Seagate/QIIME/16s/DES\_7\_Unpaired\_R2 -o  
 /media/Seagate/QIIME/16s/DES\_7\_Joined;fastq-join -m 50 -r  
 /media/Seagate/QIIME/16s/Joinreport/DES\_8\_Joinreport  
 Illumina/Data/MiSeq\_67\_Sequences/files.cgrb.oregonstate.edu/Illumina/140331\_M0149  
 8\_0067\_000000000-A8U60/BaseCalls/lane1-s092-indexN708-S508-CAGAGAGG-  
 CTAAGCCT-92\_S92\_L001\_R1\_001.fastq.gz  
 Illumina/Data/MiSeq\_67\_Sequences/files.cgrb.oregonstate.edu/Illumina/140331\_M0149  
 8\_0067\_000000000-A8U60/BaseCalls/lane1-s092-indexN708-S508-CAGAGAGG-  
 CTAAGCCT-92\_S92\_L001\_R2\_001.fastq.gz -o  
 /media/Seagate/QIIME/16s/DES\_8\_Unpaired\_R1 -o  
 /media/Seagate/QIIME/16s/DES\_8\_Unpaired\_R2 -o  
 /media/Seagate/QIIME/16s/DES\_8\_Joined;fastq-join -m 50 -r  
 /media/Seagate/QIIME/16s/Joinreport/DES\_9\_Joinreport  
 Illumina/Data/MiSeq\_67\_Sequences/files.cgrb.oregonstate.edu/Illumina/140331\_M0149  
 8\_0067\_000000000-A8U60/BaseCalls/lane1-s009-indexN709-S501-GCTACGCT-  
 TAGATCGC-9\_S9\_L001\_R1\_001.fastq.gz  
 Illumina/Data/MiSeq\_67\_Sequences/files.cgrb.oregonstate.edu/Illumina/140331\_M0149  
 8\_0067\_000000000-A8U60/BaseCalls/lane1-s009-indexN709-S501-GCTACGCT-  
 TAGATCGC-9\_S9\_L001\_R2\_001.fastq.gz -o  
 /media/Seagate/QIIME/16s/DES\_9\_Unpaired\_R1 -o  
 /media/Seagate/QIIME/16s/DES\_9\_Unpaired\_R2 -o  
 /media/Seagate/QIIME/16s/DES\_9\_Joined;fastq-join -m 50 -r  
 /media/Seagate/QIIME/16s/Joinreport/DES\_10\_Joinreport  
 Illumina/Data/MiSeq\_67\_Sequences/files.cgrb.oregonstate.edu/Illumina/140331\_M0149  
 8\_0067\_000000000-A8U60/BaseCalls/lane1-s021-indexN709-S502-GCTACGCT-  
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```











```

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```

```

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truncate_remove -o
/media/Seagate/QIIME/16s/split_libraries_output/split_library_1;split_libraries.py -m
/media/Seagate/QIIME/16s/Mapping_Files/MappingFile_DES_2.txt -f
/media/Seagate/QIIME/16s/Fastaqual_output/DES_2_Joined.fna -q
/media/Seagate/QIIME/16s/Fastaqual_output/DES_2_Joined.qual -b 0 -z
truncate_remove -o

```

```
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truncate_remove -o
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truncate_remove -o
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truncate_remove -o
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/media/Seagate/QIIME/16s/Fastaqual_output/DES_6_Joined.fna -q
/media/Seagate/QIIME/16s/Fastaqual_output/DES_6_Joined.qual -b 0 -z
truncate_remove -o
/media/Seagate/QIIME/16s/split_libraries_output/split_library_6;split_libraries.py -m
/media/Seagate/QIIME/16s/Mapping_Files/MappingFile_DES_7.txt -f
/media/Seagate/QIIME/16s/Fastaqual_output/DES_7_Joined.fna -q
/media/Seagate/QIIME/16s/Fastaqual_output/DES_7_Joined.qual -b 0 -z
truncate_remove -o
/media/Seagate/QIIME/16s/split_libraries_output/split_library_7;split_libraries.py -m
/media/Seagate/QIIME/16s/Mapping_Files/MappingFile_DES_8.txt -f
/media/Seagate/QIIME/16s/Fastaqual_output/DES_8_Joined.fna -q
/media/Seagate/QIIME/16s/Fastaqual_output/DES_8_Joined.qual -b 0 -z
truncate_remove -o
/media/Seagate/QIIME/16s/split_libraries_output/split_library_8;split_libraries.py -m
/media/Seagate/QIIME/16s/Mapping_Files/MappingFile_DES_9.txt -f
/media/Seagate/QIIME/16s/Fastaqual_output/DES_9_Joined.fna -q
/media/Seagate/QIIME/16s/Fastaqual_output/DES_9_Joined.qual -b 0 -z
truncate_remove -o
/media/Seagate/QIIME/16s/split_libraries_output/split_library_9;split_libraries.py -m
/media/Seagate/QIIME/16s/Mapping_Files/MappingFile_DES_10.txt -f
/media/Seagate/QIIME/16s/Fastaqual_output/DES_10_Joined.fna -q
/media/Seagate/QIIME/16s/Fastaqual_output/DES_10_Joined.qual -b 0 -z
truncate_remove -o
/media/Seagate/QIIME/16s/split_libraries_output/split_library_10;split_libraries.py -m
/media/Seagate/QIIME/16s/Mapping_Files/MappingFile_DES_11.txt -f
/media/Seagate/QIIME/16s/Fastaqual_output/DES_11_Joined.fna -q
/media/Seagate/QIIME/16s/Fastaqual_output/DES_11_Joined.qual -b 0 -z
truncate_remove -o
/media/Seagate/QIIME/16s/split_libraries_output/split_library_11;split_libraries.py -m
```

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/media/Seagate/QIIME/16s/Mapping_Files/MappingFile_DES_12.txt -f
/media/Seagate/QIIME/16s/Fastaqual_output/DES_12_Joined.fna -q
/media/Seagate/QIIME/16s/Fastaqual_output/DES_12_Joined.qual -b 0 -z
truncate_remove -o
/media/Seagate/QIIME/16s/split_libraries_output/split_library_12;split_libraries.py -m
/media/Seagate/QIIME/16s/Mapping_Files/MappingFile_DES_13.txt -f
/media/Seagate/QIIME/16s/Fastaqual_output/DES_13_Joined.fna -q
/media/Seagate/QIIME/16s/Fastaqual_output/DES_13_Joined.qual -b 0 -z
truncate_remove -o
/media/Seagate/QIIME/16s/split_libraries_output/split_library_13;split_libraries.py -m
/media/Seagate/QIIME/16s/Mapping_Files/MappingFile_DES_14.txt -f
/media/Seagate/QIIME/16s/Fastaqual_output/DES_14_Joined.fna -q
/media/Seagate/QIIME/16s/Fastaqual_output/DES_14_Joined.qual -b 0 -z
truncate_remove -o
/media/Seagate/QIIME/16s/split_libraries_output/split_library_14;split_libraries.py -m
/media/Seagate/QIIME/16s/Mapping_Files/MappingFile_DES_15.txt -f
/media/Seagate/QIIME/16s/Fastaqual_output/DES_15_Joined.fna -q
/media/Seagate/QIIME/16s/Fastaqual_output/DES_15_Joined.qual -b 0 -z
truncate_remove -o
/media/Seagate/QIIME/16s/split_libraries_output/split_library_15;split_libraries.py -m
/media/Seagate/QIIME/16s/Mapping_Files/MappingFile_DES_16.txt -f
/media/Seagate/QIIME/16s/Fastaqual_output/DES_16_Joined.fna -q
/media/Seagate/QIIME/16s/Fastaqual_output/DES_16_Joined.qual -b 0 -z
truncate_remove -o
/media/Seagate/QIIME/16s/split_libraries_output/split_library_16;split_libraries.py -m
/media/Seagate/QIIME/16s/Mapping_Files/MappingFile_DES_17.txt -f
/media/Seagate/QIIME/16s/Fastaqual_output/DES_17_Joined.fna -q
/media/Seagate/QIIME/16s/Fastaqual_output/DES_17_Joined.qual -b 0 -z
truncate_remove -o
/media/Seagate/QIIME/16s/split_libraries_output/split_library_17;split_libraries.py -m
/media/Seagate/QIIME/16s/Mapping_Files/MappingFile_DES_18.txt -f
/media/Seagate/QIIME/16s/Fastaqual_output/DES_18_Joined.fna -q
/media/Seagate/QIIME/16s/Fastaqual_output/DES_18_Joined.qual -b 0 -z
truncate_remove -o
/media/Seagate/QIIME/16s/split_libraries_output/split_library_18;split_libraries.py -m
/media/Seagate/QIIME/16s/Mapping_Files/MappingFile_DES_19.txt -f
/media/Seagate/QIIME/16s/Fastaqual_output/DES_19_Joined.fna -q
/media/Seagate/QIIME/16s/Fastaqual_output/DES_19_Joined.qual -b 0 -z
truncate_remove -o
/media/Seagate/QIIME/16s/split_libraries_output/split_library_19;split_libraries.py -m
/media/Seagate/QIIME/16s/Mapping_Files/MappingFile_DES_20.txt -f
/media/Seagate/QIIME/16s/Fastaqual_output/DES_20_Joined.fna -q
/media/Seagate/QIIME/16s/Fastaqual_output/DES_20_Joined.qual -b 0 -z
truncate_remove -o
/media/Seagate/QIIME/16s/split_libraries_output/split_library_20;split_libraries.py -m
/media/Seagate/QIIME/16s/Mapping_Files/MappingFile_DES_21.txt -f

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/media/Seagate/QIIME/16s/Fastaqual_output/DES_21_Joined.fna -q
/media/Seagate/QIIME/16s/Fastaqual_output/DES_21_Joined.qual -b 0 -z
truncate_remove -o
/media/Seagate/QIIME/16s/split_libraries_output/split_library_21;split_libraries.py -m
/media/Seagate/QIIME/16s/Mapping_Files/MappingFile_DES_22.txt -f
/media/Seagate/QIIME/16s/Fastaqual_output/DES_22_Joined.fna -q
/media/Seagate/QIIME/16s/Fastaqual_output/DES_22_Joined.qual -b 0 -z
truncate_remove -o
/media/Seagate/QIIME/16s/split_libraries_output/split_library_22;split_libraries.py -m
/media/Seagate/QIIME/16s/Mapping_Files/MappingFile_DES_23.txt -f
/media/Seagate/QIIME/16s/Fastaqual_output/DES_23_Joined.fna -q
/media/Seagate/QIIME/16s/Fastaqual_output/DES_23_Joined.qual -b 0 -z
truncate_remove -o
/media/Seagate/QIIME/16s/split_libraries_output/split_library_23;split_libraries.py -m
/media/Seagate/QIIME/16s/Mapping_Files/MappingFile_DES_24.txt -f
/media/Seagate/QIIME/16s/Fastaqual_output/DES_24_Joined.fna -q
/media/Seagate/QIIME/16s/Fastaqual_output/DES_24_Joined.qual -b 0 -z
truncate_remove -o
/media/Seagate/QIIME/16s/split_libraries_output/split_library_24;split_libraries.py -m
/media/Seagate/QIIME/16s/Mapping_Files/MappingFile_DES_25.txt -f
/media/Seagate/QIIME/16s/Fastaqual_output/DES_25_Joined.fna -q
/media/Seagate/QIIME/16s/Fastaqual_output/DES_25_Joined.qual -b 0 -z
truncate_remove -o
/media/Seagate/QIIME/16s/split_libraries_output/split_library_25;split_libraries.py -m
/media/Seagate/QIIME/16s/Mapping_Files/MappingFile_DES_26.txt -f
/media/Seagate/QIIME/16s/Fastaqual_output/DES_26_Joined.fna -q
/media/Seagate/QIIME/16s/Fastaqual_output/DES_26_Joined.qual -b 0 -z
truncate_remove -o
/media/Seagate/QIIME/16s/split_libraries_output/split_library_26;split_libraries.py -m
/media/Seagate/QIIME/16s/Mapping_Files/MappingFile_DES_27.txt -f
/media/Seagate/QIIME/16s/Fastaqual_output/DES_27_Joined.fna -q
/media/Seagate/QIIME/16s/Fastaqual_output/DES_27_Joined.qual -b 0 -z
truncate_remove -o
/media/Seagate/QIIME/16s/split_libraries_output/split_library_27;split_libraries.py -m
/media/Seagate/QIIME/16s/Mapping_Files/MappingFile_DES_28.txt -f
/media/Seagate/QIIME/16s/Fastaqual_output/DES_28_Joined.fna -q
/media/Seagate/QIIME/16s/Fastaqual_output/DES_28_Joined.qual -b 0 -z
truncate_remove -o
/media/Seagate/QIIME/16s/split_libraries_output/split_library_28;split_libraries.py -m
/media/Seagate/QIIME/16s/Mapping_Files/MappingFile_DES_29.txt -f
/media/Seagate/QIIME/16s/Fastaqual_output/DES_29_Joined.fna -q
/media/Seagate/QIIME/16s/Fastaqual_output/DES_29_Joined.qual -b 0 -z
truncate_remove -o
/media/Seagate/QIIME/16s/split_libraries_output/split_library_29;split_libraries.py -m
/media/Seagate/QIIME/16s/Mapping_Files/MappingFile_DES_30.txt -f
/media/Seagate/QIIME/16s/Fastaqual_output/DES_30_Joined.fna -q

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/media/Seagate/QIIME/16s/Fastaqual_output/DES_30_Joined.qual -b 0 -z
truncate_remove -o
/media/Seagate/QIIME/16s/split_libraries_output/split_library_30;split_libraries.py -m
/media/Seagate/QIIME/16s/Mapping_Files/MappingFile_DES_31.txt -f
/media/Seagate/QIIME/16s/Fastaqual_output/DES_31_Joined.fna -q
/media/Seagate/QIIME/16s/Fastaqual_output/DES_31_Joined.qual -b 0 -z
truncate_remove -o
/media/Seagate/QIIME/16s/split_libraries_output/split_library_31;split_libraries.py -m
/media/Seagate/QIIME/16s/Mapping_Files/MappingFile_DES_32.txt -f
/media/Seagate/QIIME/16s/Fastaqual_output/DES_32_Joined.fna -q
/media/Seagate/QIIME/16s/Fastaqual_output/DES_32_Joined.qual -b 0 -z
truncate_remove -o
/media/Seagate/QIIME/16s/split_libraries_output/split_library_32;split_libraries.py -m
/media/Seagate/QIIME/16s/Mapping_Files/MappingFile_DES_33.txt -f
/media/Seagate/QIIME/16s/Fastaqual_output/DES_33_Joined.fna -q
/media/Seagate/QIIME/16s/Fastaqual_output/DES_33_Joined.qual -b 0 -z
truncate_remove -o
/media/Seagate/QIIME/16s/split_libraries_output/split_library_33;split_libraries.py -m
/media/Seagate/QIIME/16s/Mapping_Files/MappingFile_DES_34.txt -f
/media/Seagate/QIIME/16s/Fastaqual_output/DES_34_Joined.fna -q
/media/Seagate/QIIME/16s/Fastaqual_output/DES_34_Joined.qual -b 0 -z
truncate_remove -o
/media/Seagate/QIIME/16s/split_libraries_output/split_library_34;split_libraries.py -m
/media/Seagate/QIIME/16s/Mapping_Files/MappingFile_DES_35.txt -f
/media/Seagate/QIIME/16s/Fastaqual_output/DES_35_Joined.fna -q
/media/Seagate/QIIME/16s/Fastaqual_output/DES_35_Joined.qual -b 0 -z
truncate_remove -o
/media/Seagate/QIIME/16s/split_libraries_output/split_library_35;split_libraries.py -m
/media/Seagate/QIIME/16s/Mapping_Files/MappingFile_DES_36.txt -f
/media/Seagate/QIIME/16s/Fastaqual_output/DES_36_Joined.fna -q
/media/Seagate/QIIME/16s/Fastaqual_output/DES_36_Joined.qual -b 0 -z
truncate_remove -o
/media/Seagate/QIIME/16s/split_libraries_output/split_library_36;split_libraries.py -m
/media/Seagate/QIIME/16s/Mapping_Files/MappingFile_DES_37.txt -f
/media/Seagate/QIIME/16s/Fastaqual_output/DES_37_Joined.fna -q
/media/Seagate/QIIME/16s/Fastaqual_output/DES_37_Joined.qual -b 0 -z
truncate_remove -o
/media/Seagate/QIIME/16s/split_libraries_output/split_library_37;split_libraries.py -m
/media/Seagate/QIIME/16s/Mapping_Files/MappingFile_DES_38.txt -f
/media/Seagate/QIIME/16s/Fastaqual_output/DES_38_Joined.fna -q
/media/Seagate/QIIME/16s/Fastaqual_output/DES_38_Joined.qual -b 0 -z
truncate_remove -o
/media/Seagate/QIIME/16s/split_libraries_output/split_library_38;split_libraries.py -m
/media/Seagate/QIIME/16s/Mapping_Files/MappingFile_DES_39.txt -f
/media/Seagate/QIIME/16s/Fastaqual_output/DES_39_Joined.fna -q
/media/Seagate/QIIME/16s/Fastaqual_output/DES_39_Joined.qual -b 0 -z

```

```

truncate_remove -o
/media/Seagate/QIIME/16s/split_libraries_output/split_library_39;split_libraries.py -m
/media/Seagate/QIIME/16s/Mapping_Files/MappingFile_DES_40.txt -f
/media/Seagate/QIIME/16s/Fastaqual_output/DES_40_Joined.fna -q
/media/Seagate/QIIME/16s/Fastaqual_output/DES_40_Joined.qual -b 0 -z
truncate_remove -o
/media/Seagate/QIIME/16s/split_libraries_output/split_library_40;split_libraries.py -m
/media/Seagate/QIIME/16s/Mapping_Files/MappingFile_DES_41.txt -f
/media/Seagate/QIIME/16s/Fastaqual_output/DES_41_Joined.fna -q
/media/Seagate/QIIME/16s/Fastaqual_output/DES_41_Joined.qual -b 0 -z
truncate_remove -o
/media/Seagate/QIIME/16s/split_libraries_output/split_library_41;split_libraries.py -m
/media/Seagate/QIIME/16s/Mapping_Files/MappingFile_DES_42.txt -f
/media/Seagate/QIIME/16s/Fastaqual_output/DES_42_Joined.fna -q
/media/Seagate/QIIME/16s/Fastaqual_output/DES_42_Joined.qual -b 0 -z
truncate_remove -o
/media/Seagate/QIIME/16s/split_libraries_output/split_library_42;split_libraries.py -m
/media/Seagate/QIIME/16s/Mapping_Files/MappingFile_DES_43.txt -f
/media/Seagate/QIIME/16s/Fastaqual_output/DES_43_Joined.fna -q
/media/Seagate/QIIME/16s/Fastaqual_output/DES_43_Joined.qual -b 0 -z
truncate_remove -o
/media/Seagate/QIIME/16s/split_libraries_output/split_library_43;split_libraries.py -m
/media/Seagate/QIIME/16s/Mapping_Files/MappingFile_DES_44.txt -f
/media/Seagate/QIIME/16s/Fastaqual_output/DES_44_Joined.fna -q
/media/Seagate/QIIME/16s/Fastaqual_output/DES_44_Joined.qual -b 0 -z
truncate_remove -o
/media/Seagate/QIIME/16s/split_libraries_output/split_library_44;split_libraries.py -m
/media/Seagate/QIIME/16s/Mapping_Files/MappingFile_DES_45.txt -f
/media/Seagate/QIIME/16s/Fastaqual_output/DES_45_Joined.fna -q
/media/Seagate/QIIME/16s/Fastaqual_output/DES_45_Joined.qual -b 0 -z
truncate_remove -o
/media/Seagate/QIIME/16s/split_libraries_output/split_library_45;split_libraries.py -m
/media/Seagate/QIIME/16s/Mapping_Files/MappingFile_DES_46.txt -f
/media/Seagate/QIIME/16s/Fastaqual_output/DES_46_Joined.fna -q
/media/Seagate/QIIME/16s/Fastaqual_output/DES_46_Joined.qual -b 0 -z
truncate_remove -o
/media/Seagate/QIIME/16s/split_libraries_output/split_library_46;split_libraries.py -m
/media/Seagate/QIIME/16s/Mapping_Files/MappingFile_DES_47.txt -f
/media/Seagate/QIIME/16s/Fastaqual_output/DES_47_Joined.fna -q
/media/Seagate/QIIME/16s/Fastaqual_output/DES_47_Joined.qual -b 0 -z
truncate_remove -o
/media/Seagate/QIIME/16s/split_libraries_output/split_library_47;split_libraries.py -m
/media/Seagate/QIIME/16s/Mapping_Files/MappingFile_DES_48.txt -f
/media/Seagate/QIIME/16s/Fastaqual_output/DES_48_Joined.fna -q
/media/Seagate/QIIME/16s/Fastaqual_output/DES_48_Joined.qual -b 0 -z
truncate_remove -o

```

```

/media/Seagate/QIIME/16s/split_libraries_output/split_library_48;split_libraries.py -m
/media/Seagate/QIIME/16s/Mapping_Files/MappingFile_DES_49.txt -f
/media/Seagate/QIIME/16s/Fastaqual_output/DES_49_Joined.fna -q
/media/Seagate/QIIME/16s/Fastaqual_output/DES_49_Joined.qual -b 0 -z
truncate_remove -o
/media/Seagate/QIIME/16s/split_libraries_output/split_library_49;split_libraries.py -m
/media/Seagate/QIIME/16s/Mapping_Files/MappingFile_DES_50.txt -f
/media/Seagate/QIIME/16s/Fastaqual_output/DES_50_Joined.fna -q
/media/Seagate/QIIME/16s/Fastaqual_output/DES_50_Joined.qual -b 0 -z
truncate_remove -o
/media/Seagate/QIIME/16s/split_libraries_output/split_library_50;split_libraries.py -m
/media/Seagate/QIIME/16s/Mapping_Files/MappingFile_DES_51.txt -f
/media/Seagate/QIIME/16s/Fastaqual_output/DES_51_Joined.fna -q
/media/Seagate/QIIME/16s/Fastaqual_output/DES_51_Joined.qual -b 0 -z
truncate_remove -o
/media/Seagate/QIIME/16s/split_libraries_output/split_library_51;split_libraries.py -m
/media/Seagate/QIIME/16s/Mapping_Files/MappingFile_DES_52.txt -f
/media/Seagate/QIIME/16s/Fastaqual_output/DES_52_Joined.fna -q
/media/Seagate/QIIME/16s/Fastaqual_output/DES_52_Joined.qual -b 0 -z
truncate_remove -o
/media/Seagate/QIIME/16s/split_libraries_output/split_library_52;split_libraries.py -m
/media/Seagate/QIIME/16s/Mapping_Files/MappingFile_DES_53.txt -f
/media/Seagate/QIIME/16s/Fastaqual_output/DES_53_Joined.fna -q
/media/Seagate/QIIME/16s/Fastaqual_output/DES_53_Joined.qual -b 0 -z
truncate_remove -o
/media/Seagate/QIIME/16s/split_libraries_output/split_library_53;split_libraries.py -m
/media/Seagate/QIIME/16s/Mapping_Files/MappingFile_DES_54.txt -f
/media/Seagate/QIIME/16s/Fastaqual_output/DES_54_Joined.fna -q
/media/Seagate/QIIME/16s/Fastaqual_output/DES_54_Joined.qual -b 0 -z
truncate_remove -o
/media/Seagate/QIIME/16s/split_libraries_output/split_library_54;split_libraries.py -m
/media/Seagate/QIIME/16s/Mapping_Files/MappingFile_DES_55.txt -f
/media/Seagate/QIIME/16s/Fastaqual_output/DES_55_Joined.fna -q
/media/Seagate/QIIME/16s/Fastaqual_output/DES_55_Joined.qual -b 0 -z
truncate_remove -o
/media/Seagate/QIIME/16s/split_libraries_output/split_library_55;split_libraries.py -m
/media/Seagate/QIIME/16s/Mapping_Files/MappingFile_DES_56.txt -f
/media/Seagate/QIIME/16s/Fastaqual_output/DES_56_Joined.fna -q
/media/Seagate/QIIME/16s/Fastaqual_output/DES_56_Joined.qual -b 0 -z
truncate_remove -o
/media/Seagate/QIIME/16s/split_libraries_output/split_library_56;split_libraries.py -m
/media/Seagate/QIIME/16s/Mapping_Files/MappingFile_DES_57.txt -f
/media/Seagate/QIIME/16s/Fastaqual_output/DES_57_Joined.fna -q
/media/Seagate/QIIME/16s/Fastaqual_output/DES_57_Joined.qual -b 0 -z
truncate_remove -o
/media/Seagate/QIIME/16s/split_libraries_output/split_library_57;split_libraries.py -m

```

```
/media/Seagate/QIIME/16s/Mapping_Files/MappingFile_DES_58.txt -f
/media/Seagate/QIIME/16s/Fastaqual_output/DES_58_Joined.fna -q
/media/Seagate/QIIME/16s/Fastaqual_output/DES_58_Joined.qual -b 0 -z
truncate_remove -o
/media/Seagate/QIIME/16s/split_libraries_output/split_library_58;split_libraries.py -m
/media/Seagate/QIIME/16s/Mapping_Files/MappingFile_DES_59.txt -f
/media/Seagate/QIIME/16s/Fastaqual_output/DES_59_Joined.fna -q
/media/Seagate/QIIME/16s/Fastaqual_output/DES_59_Joined.qual -b 0 -z
truncate_remove -o
/media/Seagate/QIIME/16s/split_libraries_output/split_library_59;split_libraries.py -m
/media/Seagate/QIIME/16s/Mapping_Files/MappingFile_DES_60.txt -f
/media/Seagate/QIIME/16s/Fastaqual_output/DES_60_Joined.fna -q
/media/Seagate/QIIME/16s/Fastaqual_output/DES_60_Joined.qual -b 0 -z
truncate_remove -o
/media/Seagate/QIIME/16s/split_libraries_output/split_library_60;split_libraries.py -m
/media/Seagate/QIIME/16s/Mapping_Files/MappingFile_DES_61.txt -f
/media/Seagate/QIIME/16s/Fastaqual_output/DES_61_Joined.fna -q
/media/Seagate/QIIME/16s/Fastaqual_output/DES_61_Joined.qual -b 0 -z
truncate_remove -o
/media/Seagate/QIIME/16s/split_libraries_output/split_library_61;split_libraries.py -m
/media/Seagate/QIIME/16s/Mapping_Files/MappingFile_DES_62.txt -f
/media/Seagate/QIIME/16s/Fastaqual_output/DES_62_Joined.fna -q
/media/Seagate/QIIME/16s/Fastaqual_output/DES_62_Joined.qual -b 0 -z
truncate_remove -o
/media/Seagate/QIIME/16s/split_libraries_output/split_library_62;split_libraries.py -m
/media/Seagate/QIIME/16s/Mapping_Files/MappingFile_DES_63.txt -f
/media/Seagate/QIIME/16s/Fastaqual_output/DES_63_Joined.fna -q
/media/Seagate/QIIME/16s/Fastaqual_output/DES_63_Joined.qual -b 0 -z
truncate_remove -o
/media/Seagate/QIIME/16s/split_libraries_output/split_library_63;split_libraries.py -m
/media/Seagate/QIIME/16s/Mapping_Files/MappingFile_DES_64.txt -f
/media/Seagate/QIIME/16s/Fastaqual_output/DES_64_Joined.fna -q
/media/Seagate/QIIME/16s/Fastaqual_output/DES_64_Joined.qual -b 0 -z
truncate_remove -o
/media/Seagate/QIIME/16s/split_libraries_output/split_library_64;split_libraries.py -m
/media/Seagate/QIIME/16s/Mapping_Files/MappingFile_DES_65.txt -f
/media/Seagate/QIIME/16s/Fastaqual_output/DES_65_Joined.fna -q
/media/Seagate/QIIME/16s/Fastaqual_output/DES_65_Joined.qual -b 0 -z
truncate_remove -o
/media/Seagate/QIIME/16s/split_libraries_output/split_library_65;split_libraries.py -m
/media/Seagate/QIIME/16s/Mapping_Files/MappingFile_DES_66.txt -f
/media/Seagate/QIIME/16s/Fastaqual_output/DES_66_Joined.fna -q
/media/Seagate/QIIME/16s/Fastaqual_output/DES_66_Joined.qual -b 0 -z
truncate_remove -o
/media/Seagate/QIIME/16s/split_libraries_output/split_library_66;split_libraries.py -m
/media/Seagate/QIIME/16s/Mapping_Files/MappingFile_DES_67.txt -f
```



```
/media/Seagate/QIIME/16s/Fastaqual_output/DES_67_Joined.fna -q
/media/Seagate/QIIME/16s/Fastaqual_output/DES_67_Joined.qual -b 0 -z
truncate_remove -o
/media/Seagate/QIIME/16s/split_libraries_output/split_library_67;split_libraries.py -m
/media/Seagate/QIIME/16s/Mapping_Files/MappingFile_DES_68.txt -f
/media/Seagate/QIIME/16s/Fastaqual_output/DES_68_Joined.fna -q
/media/Seagate/QIIME/16s/Fastaqual_output/DES_68_Joined.qual -b 0 -z
truncate_remove -o
/media/Seagate/QIIME/16s/split_libraries_output/split_library_68;split_libraries.py -m
/media/Seagate/QIIME/16s/Mapping_Files/MappingFile_DES_69.txt -f
/media/Seagate/QIIME/16s/Fastaqual_output/DES_69_Joined.fna -q
/media/Seagate/QIIME/16s/Fastaqual_output/DES_69_Joined.qual -b 0 -z
truncate_remove -o
/media/Seagate/QIIME/16s/split_libraries_output/split_library_69;split_libraries.py -m
/media/Seagate/QIIME/16s/Mapping_Files/MappingFile_DES_70.txt -f
/media/Seagate/QIIME/16s/Fastaqual_output/DES_70_Joined.fna -q
/media/Seagate/QIIME/16s/Fastaqual_output/DES_70_Joined.qual -b 0 -z
truncate_remove -o
/media/Seagate/QIIME/16s/split_libraries_output/split_library_70;split_libraries.py -m
/media/Seagate/QIIME/16s/Mapping_Files/MappingFile_DES_71.txt -f
/media/Seagate/QIIME/16s/Fastaqual_output/DES_71_Joined.fna -q
/media/Seagate/QIIME/16s/Fastaqual_output/DES_71_Joined.qual -b 0 -z
truncate_remove -o
/media/Seagate/QIIME/16s/split_libraries_output/split_library_71;split_libraries.py -m
/media/Seagate/QIIME/16s/Mapping_Files/MappingFile_DES_72.txt -f
/media/Seagate/QIIME/16s/Fastaqual_output/DES_72_Joined.fna -q
/media/Seagate/QIIME/16s/Fastaqual_output/DES_72_Joined.qual -b 0 -z
truncate_remove -o
/media/Seagate/QIIME/16s/split_libraries_output/split_library_72;split_libraries.py -m
/media/Seagate/QIIME/16s/Mapping_Files/MappingFile_DES_73.txt -f
/media/Seagate/QIIME/16s/Fastaqual_output/DES_73_Joined.fna -q
/media/Seagate/QIIME/16s/Fastaqual_output/DES_73_Joined.qual -b 0 -z
truncate_remove -o
/media/Seagate/QIIME/16s/split_libraries_output/split_library_73;split_libraries.py -m
/media/Seagate/QIIME/16s/Mapping_Files/MappingFile_DES_74.txt -f
/media/Seagate/QIIME/16s/Fastaqual_output/DES_74_Joined.fna -q
/media/Seagate/QIIME/16s/Fastaqual_output/DES_74_Joined.qual -b 0 -z
truncate_remove -o
/media/Seagate/QIIME/16s/split_libraries_output/split_library_74;split_libraries.py -m
/media/Seagate/QIIME/16s/Mapping_Files/MappingFile_DES_75.txt -f
/media/Seagate/QIIME/16s/Fastaqual_output/DES_75_Joined.fna -q
/media/Seagate/QIIME/16s/Fastaqual_output/DES_75_Joined.qual -b 0 -z
truncate_remove -o
/media/Seagate/QIIME/16s/split_libraries_output/split_library_75;split_libraries.py -m
/media/Seagate/QIIME/16s/Mapping_Files/MappingFile_DES_76.txt -f
/media/Seagate/QIIME/16s/Fastaqual_output/DES_76_Joined.fna -q
```

```

/media/Seagate/QIIME/16s/Fastaqual_output/DES_76_Joined.qual -b 0 -z
truncate_remove -o
/media/Seagate/QIIME/16s/split_libraries_output/split_library_76;split_libraries.py -m
/media/Seagate/QIIME/16s/Mapping_Files/MappingFile_DES_77.txt -f
/media/Seagate/QIIME/16s/Fastaqual_output/DES_77_Joined.fna -q
/media/Seagate/QIIME/16s/Fastaqual_output/DES_77_Joined.qual -b 0 -z
truncate_remove -o
/media/Seagate/QIIME/16s/split_libraries_output/split_library_77;split_libraries.py -m
/media/Seagate/QIIME/16s/Mapping_Files/MappingFile_DES_78.txt -f
/media/Seagate/QIIME/16s/Fastaqual_output/DES_78_Joined.fna -q
/media/Seagate/QIIME/16s/Fastaqual_output/DES_78_Joined.qual -b 0 -z
truncate_remove -o
/media/Seagate/QIIME/16s/split_libraries_output/split_library_78;split_libraries.py -m
/media/Seagate/QIIME/16s/Mapping_Files/MappingFile_DES_79.txt -f
/media/Seagate/QIIME/16s/Fastaqual_output/DES_79_Joined.fna -q
/media/Seagate/QIIME/16s/Fastaqual_output/DES_79_Joined.qual -b 0 -z
truncate_remove -o
/media/Seagate/QIIME/16s/split_libraries_output/split_library_79;split_libraries.py -m
/media/Seagate/QIIME/16s/Mapping_Files/MappingFile_DES_80.txt -f
/media/Seagate/QIIME/16s/Fastaqual_output/DES_80_Joined.fna -q
/media/Seagate/QIIME/16s/Fastaqual_output/DES_80_Joined.qual -b 0 -z
truncate_remove -o
/media/Seagate/QIIME/16s/split_libraries_output/split_library_80;split_libraries.py -m
/media/Seagate/QIIME/16s/Mapping_Files/MappingFile_DES_81.txt -f
/media/Seagate/QIIME/16s/Fastaqual_output/DES_81_Joined.fna -q
/media/Seagate/QIIME/16s/Fastaqual_output/DES_81_Joined.qual -b 0 -z
truncate_remove -o
/media/Seagate/QIIME/16s/split_libraries_output/split_library_81;split_libraries.py -m
/media/Seagate/QIIME/16s/Mapping_Files/MappingFile_DES_82.txt -f
/media/Seagate/QIIME/16s/Fastaqual_output/DES_82_Joined.fna -q
/media/Seagate/QIIME/16s/Fastaqual_output/DES_82_Joined.qual -b 0 -z
truncate_remove -o
/media/Seagate/QIIME/16s/split_libraries_output/split_library_82;split_libraries.py -m
/media/Seagate/QIIME/16s/Mapping_Files/MappingFile_DES_83.txt -f
/media/Seagate/QIIME/16s/Fastaqual_output/DES_83_Joined.fna -q
/media/Seagate/QIIME/16s/Fastaqual_output/DES_83_Joined.qual -b 0 -z
truncate_remove -o
/media/Seagate/QIIME/16s/split_libraries_output/split_library_83;split_libraries.py -m
/media/Seagate/QIIME/16s/Mapping_Files/MappingFile_DES_84.txt -f
/media/Seagate/QIIME/16s/Fastaqual_output/DES_84_Joined.fna -q
/media/Seagate/QIIME/16s/Fastaqual_output/DES_84_Joined.qual -b 0 -z
truncate_remove -o
/media/Seagate/QIIME/16s/split_libraries_output/split_library_84;split_libraries.py -m
/media/Seagate/QIIME/16s/Mapping_Files/MappingFile_DES_85.txt -f
/media/Seagate/QIIME/16s/Fastaqual_output/DES_85_Joined.fna -q
/media/Seagate/QIIME/16s/Fastaqual_output/DES_85_Joined.qual -b 0 -z

```

```

truncate_remove -o
/media/Seagate/QIIME/16s/split_libraries_output/split_library_85;split_libraries.py -m
/media/Seagate/QIIME/16s/Mapping_Files/MappingFile_DES_86.txt -f
/media/Seagate/QIIME/16s/Fastaqual_output/DES_86_Joined.fna -q
/media/Seagate/QIIME/16s/Fastaqual_output/DES_86_Joined.qual -b 0 -z
truncate_remove -o
/media/Seagate/QIIME/16s/split_libraries_output/split_library_86;split_libraries.py -m
/media/Seagate/QIIME/16s/Mapping_Files/MappingFile_DES_87.txt -f
/media/Seagate/QIIME/16s/Fastaqual_output/DES_87_Joined.fna -q
/media/Seagate/QIIME/16s/Fastaqual_output/DES_87_Joined.qual -b 0 -z
truncate_remove -o
/media/Seagate/QIIME/16s/split_libraries_output/split_library_87;split_libraries.py -m
/media/Seagate/QIIME/16s/Mapping_Files/MappingFile_DES_88.txt -f
/media/Seagate/QIIME/16s/Fastaqual_output/DES_88_Joined.fna -q
/media/Seagate/QIIME/16s/Fastaqual_output/DES_88_Joined.qual -b 0 -z
truncate_remove -o
/media/Seagate/QIIME/16s/split_libraries_output/split_library_88;split_libraries.py -m
/media/Seagate/QIIME/16s/Mapping_Files/MappingFile_DES_89.txt -f
/media/Seagate/QIIME/16s/Fastaqual_output/DES_89_Joined.fna -q
/media/Seagate/QIIME/16s/Fastaqual_output/DES_89_Joined.qual -b 0 -z
truncate_remove -o
/media/Seagate/QIIME/16s/split_libraries_output/split_library_89;split_libraries.py -m
/media/Seagate/QIIME/16s/Mapping_Files/MappingFile_DES_90.txt -f
/media/Seagate/QIIME/16s/Fastaqual_output/DES_90_Joined.fna -q
/media/Seagate/QIIME/16s/Fastaqual_output/DES_90_Joined.qual -b 0 -z
truncate_remove -o
/media/Seagate/QIIME/16s/split_libraries_output/split_library_90;split_libraries.py -m
/media/Seagate/QIIME/16s/Mapping_Files/MappingFile_DES_91.txt -f
/media/Seagate/QIIME/16s/Fastaqual_output/DES_91_Joined.fna -q
/media/Seagate/QIIME/16s/Fastaqual_output/DES_91_Joined.qual -b 0 -z
truncate_remove -o
/media/Seagate/QIIME/16s/split_libraries_output/split_library_91;split_libraries.py -m
/media/Seagate/QIIME/16s/Mapping_Files/MappingFile_DES_92.txt -f
/media/Seagate/QIIME/16s/Fastaqual_output/DES_92_Joined.fna -q
/media/Seagate/QIIME/16s/Fastaqual_output/DES_92_Joined.qual -b 0 -z
truncate_remove -o
/media/Seagate/QIIME/16s/split_libraries_output/split_library_92;split_libraries.py -m
/media/Seagate/QIIME/16s/Mapping_Files/MappingFile_DES_93.txt -f
/media/Seagate/QIIME/16s/Fastaqual_output/DES_93_Joined.fna -q
/media/Seagate/QIIME/16s/Fastaqual_output/DES_93_Joined.qual -b 0 -z
truncate_remove -o
/media/Seagate/QIIME/16s/split_libraries_output/split_library_93;split_libraries.py -m
/media/Seagate/QIIME/16s/Mapping_Files/MappingFile_DES_94.txt -f
/media/Seagate/QIIME/16s/Fastaqual_output/DES_94_Joined.fna -q
/media/Seagate/QIIME/16s/Fastaqual_output/DES_94_Joined.qual -b 0 -z
truncate_remove -o

```

```

/media/Seagate/QIIME/16s/split_libraries_output/split_library_94;split_libraries.py -m
/media/Seagate/QIIME/16s/Mapping_Files/MappingFile_DES_95.txt -f
/media/Seagate/QIIME/16s/Fastaqual_output/DES_95_Joined.fna -q
/media/Seagate/QIIME/16s/Fastaqual_output/DES_95_Joined.qual -b 0 -z
truncate_remove -o
/media/Seagate/QIIME/16s/split_libraries_output/split_library_95;split_libraries.py -m
/media/Seagate/QIIME/16s/Mapping_Files/MappingFile_DES_96.txt -f
/media/Seagate/QIIME/16s/Fastaqual_output/DES_96_Joined.fna -q
/media/Seagate/QIIME/16s/Fastaqual_output/DES_96_Joined.qual -b 0 -z
truncate_remove -o
/media/Seagate/QIIME/16s/split_libraries_output/split_library_96;split_libraries.py -m
/media/Seagate/QIIME/16s/Mapping_Files/MappingFile_DES_97.txt -f
/media/Seagate/QIIME/16s/Fastaqual_output/DES_97_Joined.fna -q
/media/Seagate/QIIME/16s/Fastaqual_output/DES_97_Joined.qual -b 0 -z
truncate_remove -o
/media/Seagate/QIIME/16s/split_libraries_output/split_library_97;split_libraries.py -m
/media/Seagate/QIIME/16s/Mapping_Files/MappingFile_DES_98.txt -f
/media/Seagate/QIIME/16s/Fastaqual_output/DES_98_Joined.fna -q
/media/Seagate/QIIME/16s/Fastaqual_output/DES_98_Joined.qual -b 0 -z
truncate_remove -o
/media/Seagate/QIIME/16s/split_libraries_output/split_library_98;split_libraries.py -m
/media/Seagate/QIIME/16s/Mapping_Files/MappingFile_DES_99.txt -f
/media/Seagate/QIIME/16s/Fastaqual_output/DES_99_Joined.fna -q
/media/Seagate/QIIME/16s/Fastaqual_output/DES_99_Joined.qual -b 0 -z
truncate_remove -o
/media/Seagate/QIIME/16s/split_libraries_output/split_library_99;split_libraries.py -m
/media/Seagate/QIIME/16s/Mapping_Files/MappingFile_DES_100.txt -f
/media/Seagate/QIIME/16s/Fastaqual_output/DES_100_Joined.fna -q
/media/Seagate/QIIME/16s/Fastaqual_output/DES_100_Joined.qual -b 0 -z
truncate_remove -o /media/Seagate/QIIME/16s/split_libraries_output/split_library_100

```

```

cat /media/Seagate/QIIME/16s/split_libraries_output/split_library_1/seqs.fna
/media/Seagate/QIIME/16s/split_libraries_output/split_library_2/seqs.fna
/media/Seagate/QIIME/16s/split_libraries_output/split_library_3/seqs.fna
/media/Seagate/QIIME/16s/split_libraries_output/split_library_4/seqs.fna
/media/Seagate/QIIME/16s/split_libraries_output/split_library_5/seqs.fna
/media/Seagate/QIIME/16s/split_libraries_output/split_library_6/seqs.fna
/media/Seagate/QIIME/16s/split_libraries_output/split_library_7/seqs.fna
/media/Seagate/QIIME/16s/split_libraries_output/split_library_8/seqs.fna
/media/Seagate/QIIME/16s/split_libraries_output/split_library_9/seqs.fna
/media/Seagate/QIIME/16s/split_libraries_output/split_library_10/seqs.fna
/media/Seagate/QIIME/16s/split_libraries_output/split_library_11/seqs.fna
/media/Seagate/QIIME/16s/split_libraries_output/split_library_12/seqs.fna
/media/Seagate/QIIME/16s/split_libraries_output/split_library_13/seqs.fna

```





```
pick_otus.py -i
/media/Seagate/QIIME/16s/split_libraries_output/Combined_sequence_libraries_050115
.fna -o /media/Seagate/QIIME/16s/Pick_OTU_050115/
```

```
pick_closed_reference_otus.py -r Desktop/gg_13_8_otus/rep_set/97_otus.fasta -i
/media/Seagate/QIIME/16s/split_libraries_output/Combined_sequence_libraries_050115
.fna -o /media/Seagate/QIIME/16s/Pick_OTU_ClosedReference_091315/
```

```
pick_rep_set.py -i
/media/Seagate/QIIME/16s/Pick_OTU_050115/Combined_sequence_libraries_050115_
otus.txt -f
/media/Seagate/QIIME/16s/split_libraries_output/Combined_sequence_libraries_050115
.fna -o
/media/Seagate/QIIME/16s/Rep_set/Combined_sequence_libraries_050115_repset.fna
```

```
assign_taxonomy.py -i
/media/Seagate/QIIME/16s/Rep_set/Combined_sequence_libraries_050115_repset.fna -
o /media/Seagate/QIIME/16s/taxonomy_results/
```

```
make_otu_table.py -i
/media/Seagate/QIIME/16s/Pick_OTU_050115/Combined_sequence_libraries_050115_
otus.txt -t
/media/Seagate/QIIME/16s/taxonomy_results/Combined_sequence_libraries_050115_re
pset_tax_assignments.txt -o
/media/Seagate/QIIME/16s/OTU_Tables/Combined_sequence_libraries_050115_OTU_t
able.biom
```

```
biom convert -i
/media/Seagate/QIIME/16s/OTU_Tables/Combined_sequence_libraries_050115_OTU_t
able.biom -o /media/Seagate/QIIME/16s/OTU_Tables/OTU_Table_050115.txt -b --
header-key="taxonomy" --output-metadata-id="consensus_lineage";summarize_taxa.py -
i
/media/Seagate/QIIME/16s/OTU_Tables/Combined_sequence_libraries_050115_OTU_t
able.biom -o /media/Seagate/QIIME/16s/taxonomy_summaries/;align_seqs.py -i
/media/Seagate/QIIME/16s/Rep_set/Combined_sequence_libraries_050115_repset.fna -o
/media/Seagate/QIIME/16s/alignment/
```

## ITS Library:

fastq-join:

```
fastq-join -m 50 -r /media/Seagate/QIIME/ITS/Joinreport/DES_1_Joinreport
Illumina/Data/MiSeq_79_Sequences/files.cgrb.oregonstate.edu/Illumina/140512_M0149
8_0079_000000000-A86D3/BaseCalls/lane1-s008-indexN708-S501-CAGAGAGG-
TAGATCGC-8_S8_L001_R1_001.fastq.gz
Illumina/Data/MiSeq_79_Sequences/files.cgrb.oregonstate.edu/Illumina/140512_M0149
8_0079_000000000-A86D3/BaseCalls/lane1-s008-indexN708-S501-CAGAGAGG-
TAGATCGC-8_S8_L001_R2_001.fastq.gz -o
/media/Seagate/QIIME/ITS/Joined/Sample1_R1_Unpaired -o
/media/Seagate/QIIME/ITS/Joined/Sample1_R2_Unpaired -o
/media/Seagate/QIIME/ITS/Joined/Sample1_Joined;fastq-join -m 50 -r
/media/Seagate/QIIME/ITS/Joinreport/DES_2_Joinreport
Illumina/Data/MiSeq_79_Sequences/files.cgrb.oregonstate.edu/Illumina/140512_M0149
8_0079_000000000-A86D3/BaseCalls/lane1-s020-indexN708-S502-CAGAGAGG-
CTCTCTAT-20_S20_L001_R1_001.fastq.gz
Illumina/Data/MiSeq_79_Sequences/files.cgrb.oregonstate.edu/Illumina/140512_M0149
8_0079_000000000-A86D3/BaseCalls/lane1-s020-indexN708-S502-CAGAGAGG-
CTCTCTAT-20_S20_L001_R2_001.fastq.gz -o
/media/Seagate/QIIME/ITS/Joined/Sample2_R1_Unpaired -o
/media/Seagate/QIIME/ITS/Joined/Sample2_R2_Unpaired -o
/media/Seagate/QIIME/ITS/Joined/Sample2_Joined;fastq-join -m 50 -r
/media/Seagate/QIIME/ITS/Joinreport/DES_3_Joinreport
Illumina/Data/MiSeq_79_Sequences/files.cgrb.oregonstate.edu/Illumina/140512_M0149
8_0079_000000000-A86D3/BaseCalls/lane1-s032-indexN708-S503-CAGAGAGG-
TATCCTCT-32_S32_L001_R1_001.fastq.gz
Illumina/Data/MiSeq_79_Sequences/files.cgrb.oregonstate.edu/Illumina/140512_M0149
8_0079_000000000-A86D3/BaseCalls/lane1-s032-indexN708-S503-CAGAGAGG-
TATCCTCT-32_S32_L001_R2_001.fastq.gz -o
/media/Seagate/QIIME/ITS/Joined/Sample3_R1_Unpaired -o
/media/Seagate/QIIME/ITS/Joined/Sample3_R2_Unpaired -o
/media/Seagate/QIIME/ITS/Joined/Sample3_Joined;fastq-join -m 50 -r
/media/Seagate/QIIME/ITS/Joinreport/DES_4_Joinreport
Illumina/Data/MiSeq_79_Sequences/files.cgrb.oregonstate.edu/Illumina/140512_M0149
8_0079_000000000-A86D3/BaseCalls/lane1-s044-indexN708-S504-CAGAGAGG-
AGAGTAGA-44_S44_L001_R1_001.fastq.gz
Illumina/Data/MiSeq_79_Sequences/files.cgrb.oregonstate.edu/Illumina/140512_M0149
8_0079_000000000-A86D3/BaseCalls/lane1-s044-indexN708-S504-CAGAGAGG-
AGAGTAGA-44_S44_L001_R2_001.fastq.gz -o
/media/Seagate/QIIME/ITS/Joined/Sample4_R1_Unpaired -o
/media/Seagate/QIIME/ITS/Joined/Sample4_R2_Unpaired -o
/media/Seagate/QIIME/ITS/Joined/Sample4_Joined;fastq-join -m 50 -r
/media/Seagate/QIIME/ITS/Joinreport/DES_5_Joinreport
Illumina/Data/MiSeq_79_Sequences/files.cgrb.oregonstate.edu/Illumina/140512_M0149
8_0079_000000000-A86D3/BaseCalls/lane1-s056-indexN708-S505-CAGAGAGG-
GTAAGGAG-56_S56_L001_R1_001.fastq.gz
```



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/media/Seagate/Illumina\_Run151/files.cgrb.oregonstate.edu/Illumina/150422\_M01498\_0151\_000000000-AF1EJ/BaseCalls/[lane1-s055-indexN707-S505-CTCTCTAC-GTAAGGAG-55 S55 L001 R2 001.fastq.gz](#) -o  
/media/Seagate/QIIME/ITS/Joined/DES\_100b\_Unpaired\_R1 -o  
/media/Seagate/QIIME/ITS/Joined/DES\_100b\_Unpaired\_R2 -o

```
/media/Seagate/QIIME/ITS/Joined/DES_100b_Joined;fastq-join -m 50 -r  
/media/Seagate/QIIME/ITS/Joinreport/DES_29_Joinreport  
/media/Seagate/Illumina_Run151/files.cgrb.oregonstate.edu/Illumina/150422_M01498_0151_  
000000000-AF1EJ/BaseCalls/lane1-s056-indexN708-S505-CAGAGAGG-  
GTAAGGAG-56\_S56\_L001\_R1\_001.fastq.gz  
/media/Seagate/Illumina_Run151/files.cgrb.oregonstate.edu/Illumina/150422_M01498_0151_  
000000000-AF1EJ/BaseCalls/lane1-s056-indexN708-S505-CAGAGAGG-  
GTAAGGAG-56\_S56\_L001\_R2\_001.fastq.gz -o  
/media/Seagate/QIIME/ITS/Joined/DES_29_Unpaired_R1 -o  
/media/Seagate/QIIME/ITS/Joined/DES_29_Unpaired_R2 -o  
/media/Seagate/QIIME/ITS/Joined/DES_29_Joined;
```

```
fastq-join -m 50 -r /media/Seagate/QIIME/ITS/Joinreport/Joinreport_26  
Illumina/Data/MiSeq_110_Sequences/files.cgrb.oregonstate.edu/Illumina/141022_M014  
98_0110_000000000-ABM1R/BaseCalls/lane1-s004-indexN704-S517-TCCTGAGC-  
GCGTAAGA-4_S4_L001_R1_001.fastq.gz  
Illumina/Data/MiSeq_110_Sequences/files.cgrb.oregonstate.edu/Illumina/141022_M01  
498_0110_000000000-ABM1R/BaseCalls/lane1-s004-indexN704-S517-TCCTGAGC-  
GCGTAAGA-4_S4_L001_R2_001.fastq.gz -o  
GLUSEEN/QIIME/Publication_Data/ITS/110/Sample26_R1_Unpaired -o  
GLUSEEN/QIIME/Publication_Data/ITS/110/Sample26_R2_Unpaired -o  
GLUSEEN/QIIME/Publication_Data/ITS/110/Sample26_Joined
```

```
fastq-join -m 50 -r /media/Seagate/QIIME/ITS/Joinreport/Joinreport_28  
Illumina/Data/MiSeq_110_Sequences/files.cgrb.oregonstate.edu/Illumina/141022_M014  
98_0110_000000000-ABM1R/BaseCalls/lane1-s006-indexN706-S517-TAGGCATG-  
GCGTAAGA-6_S6_L001_R1_001.fastq.gz  
Illumina/Data/MiSeq_110_Sequences/files.cgrb.oregonstate.edu/Illumina/141022_M01  
498_0110_000000000-ABM1R/BaseCalls/lane1-s006-indexN706-S517-TAGGCATG-  
GCGTAAGA-6_S6_L001_R2_001.fastq.gz -o  
GLUSEEN/QIIME/Publication_Data/ITS/110/Sample28_R1_Unpaired -o  
GLUSEEN/QIIME/Publication_Data/ITS/110/Sample28_R2_Unpaired -o  
GLUSEEN/QIIME/Publication_Data/ITS/110/Sample28_Joined
```

```
fastq-join -m 50 -r /media/Seagate/QIIME/ITS/Joinreport/Joinreport/Joinreport_35  
Illumina/Data/MiSeq_110_Sequences/files.cgrb.oregonstate.edu/Illumina/141022_M014  
98_0110_000000000-ABM1R/BaseCalls/lane1-s013-indexN701-S502-TAAGGCGA-  
CTCTCTAT-13_S13_L001_R1_001.fastq.gz  
Illumina/Data/MiSeq_110_Sequences/files.cgrb.oregonstate.edu/Illumina/141022_M01  
498_0110_000000000-ABM1R/BaseCalls/lane1-s013-indexN701-S502-TAAGGCGA-  
CTCTCTAT-13_S13_L001_R2_001.fastq.gz -o  
GLUSEEN/QIIME/Publication_Data/ITS/110/Sample35_R1_Unpaired -o
```

GLUSEEN/QIIME/Publication\_Data/ITS/110/Sample35\_R2\_Unpaired -o  
GLUSEEN/QIIME/Publication\_Data/ITS/110/Sample35\_Joined

fastq-join -m 50 -r /media/Seagate/QIIME/ITS/Joinreport/Joinreport/Joinreport\_38  
Illumina/Data/MiSeq\_110\_Sequences/files.cgrb.oregonstate.edu/Illumina/141022\_M014  
98\_0110\_000000000-ABM1R/BaseCalls/lane1-s016-indexN704-S502-TCCTGAGC-  
CTCTCTAT-16\_S16\_L001\_R1\_001.fastq.gz  
Illumina/Data/MiSeq\_110\_Sequences/files.cgrb.oregonstate.edu/Illumina/141022\_M01  
498\_0110\_000000000-ABM1R/BaseCalls/lane1-s016-indexN704-S502-TCCTGAGC-  
CTCTCTAT-16\_S16\_L001\_R2\_001.fastq.gz -o  
GLUSEEN/QIIME/Publication\_Data/ITS/110/Sample38\_R1\_Unpaired -o  
GLUSEEN/QIIME/Publication\_Data/ITS/110/Sample38\_R2\_Unpaired -o  
GLUSEEN/QIIME/Publication\_Data/ITS/110/Sample38\_Joined

fastq-join -m 50 -r /media/Seagate/QIIME/ITS/Joinreport/Joinreport\_40  
Illumina/Data/MiSeq\_110\_Sequences/files.cgrb.oregonstate.edu/Illumina/141022\_M014  
98\_0110\_000000000-ABM1R/BaseCalls/lane1-s018-indexN706-S502-TAGGCATG-  
CTCTCTAT-18\_S18\_L001\_R1\_001.fastq.gz  
Illumina/Data/MiSeq\_110\_Sequences/files.cgrb.oregonstate.edu/Illumina/141022\_M01  
498\_0110\_000000000-ABM1R/BaseCalls/lane1-s018-indexN706-S502-TAGGCATG-  
CTCTCTAT-18\_S18\_L001\_R2\_001.fastq.gz -o  
GLUSEEN/QIIME/Publication\_Data/ITS/110/Sample40\_R1\_Unpaired -o  
GLUSEEN/QIIME/Publication\_Data/ITS/110/Sample40\_R2\_Unpaired -o  
GLUSEEN/QIIME/Publication\_Data/ITS/110/Sample40\_Joined;

fastq-join -m 50 -r /media/Seagate/QIIME/ITS/Joinreport/Joinreport\_41  
Illumina/Data/MiSeq\_110\_Sequences/files.cgrb.oregonstate.edu/Illumina/141022\_M014  
98\_0110\_000000000-ABM1R/BaseCalls/lane1-s019-indexN707-S502-CTCTCTAC-  
CTCTCTAT-19\_S19\_L001\_R1\_001.fastq.gz  
Illumina/Data/MiSeq\_110\_Sequences/files.cgrb.oregonstate.edu/Illumina/141022\_M01  
498\_0110\_000000000-ABM1R/BaseCalls/lane1-s019-indexN707-S502-CTCTCTAC-  
CTCTCTAT-19\_S19\_L001\_R2\_001.fastq.gz -o  
GLUSEEN/QIIME/Publication\_Data/ITS/110/Sample41\_R1\_Unpaired -o  
GLUSEEN/QIIME/Publication\_Data/ITS/110/Sample41\_R2\_Unpaired -o  
GLUSEEN/QIIME/Publication\_Data/ITS/110/Sample41\_Joined

fastq-join -m 50 -r /media/Seagate/QIIME/ITS/Joinreport/Joinreport/Joinreport\_43  
Illumina/Data/MiSeq\_110\_Sequences/files.cgrb.oregonstate.edu/Illumina/141022\_M014  
98\_0110\_000000000-ABM1R/BaseCalls/lane1-s021-indexN709-S502-GCTACGCT-  
CTCTCTAT-21\_S21\_L001\_R1\_001.fastq.gz  
Illumina/Data/MiSeq\_110\_Sequences/files.cgrb.oregonstate.edu/Illumina/141022\_M01  
498\_0110\_000000000-ABM1R/BaseCalls/lane1-s021-indexN709-S502-GCTACGCT-  
CTCTCTAT-21\_S21\_L001\_R2\_001.fastq.gz -o  
GLUSEEN/QIIME/Publication\_Data/ITS/110/Sample43\_R1\_Unpaired -o  
GLUSEEN/QIIME/Publication\_Data/ITS/110/Sample43\_R2\_Unpaired -o

GLUSEEN/QIIME/Publication\_Data/ITS/110/Sample43\_Joined

```
fastq-join -m 50 -r /media/Seagate/QIIME/ITS/Joinreport/Joinreport/Joinreport_45  
Illumina/Data/MiSeq_110_Sequences/files.cgrb.oregonstate.edu/Illumina/141022_M014  
98_0110_000000000-ABM1R/BaseCalls/lane1-s023-indexN711-S502-AAGAGGCA-  
CTCTCTAT-23_S23_L001_R1_001.fastq.gz  
Illumina/Data/MiSeq_110_Sequences/files.cgrb.oregonstate.edu/Illumina/141022_M01  
498_0110_000000000-ABM1R/BaseCalls/lane1-s023-indexN711-S502-AAGAGGCA-  
CTCTCTAT-23_S23_L001_R2_001.fastq.gz -o  
GLUSEEN/QIIME/Publication_Data/ITS/110/Sample45_R1_Unpaired -o  
GLUSEEN/QIIME/Publication_Data/ITS/110/Sample45_R2_Unpaired -o  
GLUSEEN/QIIME/Publication_Data/ITS/110/Sample45_Joined
```

```
fastq-join -m 50 -r /media/Seagate/QIIME/ITS/Joinreport/Joinreport/Joinreport_49  
Illumina/Data/MiSeq_110_Sequences/files.cgrb.oregonstate.edu/Illumina/141022_M014  
98_0110_000000000-ABM1R/BaseCalls/lane1-s027-indexN703-S503-AGGCAGAA-  
TATCCTCT-27_S27_L001_R1_001.fastq.gz  
Illumina/Data/MiSeq_110_Sequences/files.cgrb.oregonstate.edu/Illumina/141022_M01  
498_0110_000000000-ABM1R/BaseCalls/lane1-s027-indexN703-S503-AGGCAGAA-  
TATCCTCT-27_S27_L001_R2_001.fastq.gz -o  
GLUSEEN/QIIME/Publication_Data/ITS/110/Sample49_R1_Unpaired -o  
GLUSEEN/QIIME/Publication_Data/ITS/110/Sample49_R2_Unpaired -o  
GLUSEEN/QIIME/Publication_Data/ITS/110/Sample49_Joined
```

```
fastq-join -m 50 -r /media/Seagate/QIIME/ITS/Joinreport/Joinreport/Joinreport_53  
Illumina/Data/MiSeq_110_Sequences/files.cgrb.oregonstate.edu/Illumina/141022_M014  
98_0110_000000000-ABM1R/BaseCalls/lane1-s031-indexN707-S503-CTCTCTAC-  
TATCCTCT-31_S31_L001_R1_001.fastq.gz  
Illumina/Data/MiSeq_110_Sequences/files.cgrb.oregonstate.edu/Illumina/141022_M01  
498_0110_000000000-ABM1R/BaseCalls/lane1-s031-indexN707-S503-CTCTCTAC-  
TATCCTCT-31_S31_L001_R2_001.fastq.gz -o  
GLUSEEN/QIIME/Publication_Data/ITS/110/Sample53_R1_Unpaired -o  
GLUSEEN/QIIME/Publication_Data/ITS/110/Sample53_R2_Unpaired -o  
GLUSEEN/QIIME/Publication_Data/ITS/110/Sample53_Joined
```

```
fastq-join -m 50 -r /media/Seagate/QIIME/ITS/Joinreport/Joinreport_55  
Illumina/Data/MiSeq_110_Sequences/files.cgrb.oregonstate.edu/Illumina/141022_M014  
98_0110_000000000-ABM1R/BaseCalls/lane1-s033-indexN709-S503-GCTACGCT-  
TATCCTCT-33_S33_L001_R1_001.fastq.gz  
Illumina/Data/MiSeq_110_Sequences/files.cgrb.oregonstate.edu/Illumina/141022_M01  
498_0110_000000000-ABM1R/BaseCalls/lane1-s033-indexN709-S503-GCTACGCT-  
TATCCTCT-33_S33_L001_R2_001.fastq.gz -o  
GLUSEEN/QIIME/Publication_Data/ITS/110/Sample55_R1_Unpaired -o  
GLUSEEN/QIIME/Publication_Data/ITS/110/Sample55_R2_Unpaired -o  
GLUSEEN/QIIME/Publication_Data/ITS/110/Sample55_Joined;
```

```
fastq-join -m 50 -r /media/Seagate/QIIME/ITS/Joinreport/Joinreport_56
Illumina/Data/MiSeq_110_Sequences/files.cgrb.oregonstate.edu/Illumina/141022_M014
98_0110_000000000-ABM1R/BaseCalls/lane1-s034-indexN710-S503-CGAGGCTG-
TATCCTCT-34_S34_L001_R1_001.fastq.gz
Illumina/Data/MiSeq_110_Sequences/files.cgrb.oregonstate.edu/Illumina/141022_M01
498_0110_000000000-ABM1R/BaseCalls/lane1-s034-indexN710-S503-CGAGGCTG-
TATCCTCT-34_S34_L001_R2_001.fastq.gz -o
GLUSEEN/QIIME/Publication_Data/ITS/110/Sample56_R1_Unpaired -o
GLUSEEN/QIIME/Publication_Data/ITS/110/Sample56_R2_Unpaired -o
GLUSEEN/QIIME/Publication_Data/ITS/110/Sample56_Joined;
```

```
fastq-join -m 50 -r /media/Seagate/QIIME/ITS/Joinreport/Joinreport_57
Illumina/Data/MiSeq_110_Sequences/files.cgrb.oregonstate.edu/Illumina/141022_M014
98_0110_000000000-ABM1R/BaseCalls/lane1-s035-indexN711-S503-AAGAGGCA-
TATCCTCT-35_S35_L001_R1_001.fastq.gz
Illumina/Data/MiSeq_110_Sequences/files.cgrb.oregonstate.edu/Illumina/141022_M01
498_0110_000000000-ABM1R/BaseCalls/lane1-s035-indexN711-S503-AAGAGGCA-
TATCCTCT-35_S35_L001_R2_001.fastq.gz -o
GLUSEEN/QIIME/Publication_Data/ITS/110/Sample57_R1_Unpaired -o
GLUSEEN/QIIME/Publication_Data/ITS/110/Sample57_R2_Unpaired -o
GLUSEEN/QIIME/Publication_Data/ITS/110/Sample57_Joined
```

```
fastq-join -m 50 -r /media/Seagate/QIIME/ITS/Joinreport/Joinreport_62
Illumina/Data/MiSeq_110_Sequences/files.cgrb.oregonstate.edu/Illumina/141022_M014
98_0110_000000000-ABM1R/BaseCalls/lane1-s040-indexN704-S504-TCCTGAGC-
AGAGTAGA-40_S40_L001_R1_001.fastq.gz
Illumina/Data/MiSeq_110_Sequences/files.cgrb.oregonstate.edu/Illumina/141022_M01
498_0110_000000000-ABM1R/BaseCalls/lane1-s040-indexN704-S504-TCCTGAGC-
AGAGTAGA-40_S40_L001_R2_001.fastq.gz -o
GLUSEEN/QIIME/Publication_Data/ITS/110/Sample62_R1_Unpaired -o
GLUSEEN/QIIME/Publication_Data/ITS/110/Sample62_R2_Unpaired -o
GLUSEEN/QIIME/Publication_Data/ITS/110/Sample62_Joined
```

```
fastq-join -m 50 -r /media/Seagate/QIIME/ITS/Joinreport/Joinreport_76
Illumina/Data/MiSeq_110_Sequences/files.cgrb.oregonstate.edu/Illumina/141022_M014
98_0110_000000000-ABM1R/BaseCalls/lane1-s054-indexN706-S505-TAGGCATG-
GTAAGGAG-54_S54_L001_R1_001.fastq.gz
Illumina/Data/MiSeq_110_Sequences/files.cgrb.oregonstate.edu/Illumina/141022_M01
498_0110_000000000-ABM1R/BaseCalls/lane1-s054-indexN706-S505-TAGGCATG-
GTAAGGAG-54_S54_L001_R2_001.fastq.gz -o
GLUSEEN/QIIME/Publication_Data/ITS/110/Sample76_R1_Unpaired -o
GLUSEEN/QIIME/Publication_Data/ITS/110/Sample76_R2_Unpaired -o
GLUSEEN/QIIME/Publication_Data/ITS/110/Sample76_Joined
```



```
fastq-join -m 50 -r /media/Seagate/QIIME/ITS/Joinreport/Joinreport_80
Illumina/Data/MiSeq_110_Sequences/files.cgrb.oregonstate.edu/Illumina/141022_M014
98_0110_000000000-ABM1R/BaseCalls/lane1-s058-indexN710-S505-CGAGGCTG-
GTAAGGAG-58_S58_L001_R1_001.fastq.gz
Illumina/Data/MiSeq_110_Sequences/files.cgrb.oregonstate.edu/Illumina/141022_M01
498_0110_000000000-ABM1R/BaseCalls/lane1-s058-indexN710-S505-CGAGGCTG-
GTAAGGAG-58_S58_L001_R2_001.fastq.gz -o
GLUSEEN/QIIME/Publication_Data/ITS/110/Sample80_R1_Unpaired -o
GLUSEEN/QIIME/Publication_Data/ITS/110/Sample80_R2_Unpaired -o
GLUSEEN/QIIME/Publication_Data/ITS/110/Sample80_Joined
```

```
fastq-join -m 50 -r /media/Seagate/QIIME/ITS/Joinreport/Joinreport_81
Illumina/Data/MiSeq_110_Sequences/files.cgrb.oregonstate.edu/Illumina/141022_M014
98_0110_000000000-ABM1R/BaseCalls/lane1-s059-indexN711-S505-AAGAGGCA-
GTAAGGAG-59_S59_L001_R1_001.fastq.gz
Illumina/Data/MiSeq_110_Sequences/files.cgrb.oregonstate.edu/Illumina/141022_M01
498_0110_000000000-ABM1R/BaseCalls/lane1-s059-indexN711-S505-AAGAGGCA-
GTAAGGAG-59_S59_L001_R2_001.fastq.gz -o
GLUSEEN/QIIME/Publication_Data/ITS/110/Sample81_R1_Unpaired -o
GLUSEEN/QIIME/Publication_Data/ITS/110/Sample81_R2_Unpaired -o
GLUSEEN/QIIME/Publication_Data/ITS/110/Sample81_Joined
```

```
fastq-join -m 50 -r /media/Seagate/QIIME/ITS/Joinreport/Joinreport_83
Illumina/Data/MiSeq_110_Sequences/files.cgrb.oregonstate.edu/Illumina/141022_M014
98_0110_000000000-ABM1R/BaseCalls/lane1-s061-indexN701-S506-TAAGGCGA-
ACTGCATA-61_S61_L001_R1_001.fastq.gz
Illumina/Data/MiSeq_110_Sequences/files.cgrb.oregonstate.edu/Illumina/141022_M01
498_0110_000000000-ABM1R/BaseCalls/lane1-s061-indexN701-S506-TAAGGCGA-
ACTGCATA-61_S61_L001_R2_001.fastq.gz -o
GLUSEEN/QIIME/Publication_Data/ITS/110/Sample83_R1_Unpaired -o
GLUSEEN/QIIME/Publication_Data/ITS/110/Sample83_R2_Unpaired -o
GLUSEEN/QIIME/Publication_Data/ITS/110/Sample83_Joined
```

```
fastq-join -m 50 -r /media/Seagate/QIIME/ITS/Joinreport/Joinreport_85
Illumina/Data/MiSeq_110_Sequences/files.cgrb.oregonstate.edu/Illumina/141022_M014
98_0110_000000000-ABM1R/BaseCalls/lane1-s063-indexN703-S506-AGGCAGAA-
ACTGCATA-63_S63_L001_R1_001.fastq.gz
Illumina/Data/MiSeq_110_Sequences/files.cgrb.oregonstate.edu/Illumina/141022_M01
498_0110_000000000-ABM1R/BaseCalls/lane1-s063-indexN703-S506-AGGCAGAA-
ACTGCATA-63_S63_L001_R2_001.fastq.gz -o
GLUSEEN/QIIME/Publication_Data/ITS/110/Sample85_R1_Unpaired -o
GLUSEEN/QIIME/Publication_Data/ITS/110/Sample85_R2_Unpaired -o
GLUSEEN/QIIME/Publication_Data/ITS/110/Sample85_Joined;
```

```
fastq-join -m 50 -r /media/Seagate/QIIME/ITS/Joinreport/Joinreport_86
```

```

Illumina/Data/MiSeq_110_Sequences/files.cgrb.oregonstate.edu/Illumina/141022_M014
98_0110_000000000-ABM1R/BaseCalls/lane1-s064-indexN704-S506-TCCTGAGC-
ACTGCATA-64_S64_L001_R1_001.fastq.gz
Illumina/Data/MiSeq_110_Sequences/files.cgrb.oregonstate.edu/Illumina/141022_M01
498_0110_000000000-ABM1R/BaseCalls/lane1-s064-indexN704-S506-TCCTGAGC-
ACTGCATA-64_S64_L001_R2_001.fastq.gz -o
GLUSEEN/QIIME/Publication_Data/ITS/110/Sample86_R1_Unpaired -o
GLUSEEN/QIIME/Publication_Data/ITS/110/Sample86_R2_Unpaired -o
GLUSEEN/QIIME/Publication_Data/ITS/110/Sample86_Joined

```

```

fastq-join -m 50 -r /media/Seagate/QIIME/ITS/Joinreport/Joinreport_93
Illumina/Data/MiSeq_110_Sequences/files.cgrb.oregonstate.edu/Illumina/141022_M014
98_0110_000000000-ABM1R/BaseCalls/lane1-s071-indexN711-S506-AAGAGGCA-
ACTGCATA-71_S71_L001_R1_001.fastq.gz
Illumina/Data/MiSeq_110_Sequences/files.cgrb.oregonstate.edu/Illumina/141022_M01
498_0110_000000000-ABM1R/BaseCalls/lane1-s071-indexN711-S506-AAGAGGCA-
ACTGCATA-71_S71_L001_R2_001.fastq.gz -o
GLUSEEN/QIIME/Publication_Data/ITS/110/Sample93_R1_Unpaired -o
GLUSEEN/QIIME/Publication_Data/ITS/110/Sample93_R2_Unpaired -o
GLUSEEN/QIIME/Publication_Data/ITS/110/Sample93_Joined;

```

```

fastq-join -m 50 -r /media/Seagate/QIIME/ITS/Joinreport/Joinreport_99
Illumina/Data/MiSeq_110_Sequences/files.cgrb.oregonstate.edu/Illumina/141022_M014
98_0110_000000000-ABM1R/BaseCalls/lane1-s077-indexN705-S507-GGACTCCT-
AAGGAGTA-77_S77_L001_R1_001.fastq.gz
Illumina/Data/MiSeq_110_Sequences/files.cgrb.oregonstate.edu/Illumina/141022_M01
498_0110_000000000-ABM1R/BaseCalls/lane1-s077-indexN705-S507-GGACTCCT-
AAGGAGTA-77_S77_L001_R2_001.fastq.gz -o
GLUSEEN/QIIME/Publication_Data/ITS/110/Sample99_R1_Unpaired -o
GLUSEEN/QIIME/Publication_Data/ITS/110/Sample99_R2_Unpaired -o
GLUSEEN/QIIME/Publication_Data/ITS/110/Sample99_Joined

```

```

cat /media/Seagate/QIIME/ITS/Joined/DES_23b_Joined
/media/Seagate/QIIME/ITS/Joined/DES_23a_Joined >
/media/Seagate/QIIME/ITS/Joined/DES_23_Joined;cat
/media/Seagate/QIIME/ITS/Joined/DES_24b_Joined
/media/Seagate/QIIME/ITS/Joined/DES_24a_Joined >
/media/Seagate/QIIME/ITS/Joined/DES_24_Joined;cat
/media/Seagate/QIIME/ITS/Joined/DES_25b_Joined
/media/Seagate/QIIME/ITS/Joined/DES_25a_Joined >
/media/Seagate/QIIME/ITS/Joined/DES_25_Joined;cat
/media/Seagate/QIIME/ITS/Joined/DES_27b_Joined
/media/Seagate/QIIME/ITS/Joined/DES_27a_Joined >
/media/Seagate/QIIME/ITS/Joined/DES_27_Joined;cat
/media/Seagate/QIIME/ITS/Joined/DES_30b_Joined

```

/media/Seagate/QIIME/ITS/Joined/DES\_30a\_Joined >  
/media/Seagate/QIIME/ITS/Joined/DES\_30\_Joined;cat  
/media/Seagate/QIIME/ITS/Joined/DES\_31b\_Joined  
/media/Seagate/QIIME/ITS/Joined/DES\_31a\_Joined >  
/media/Seagate/QIIME/ITS/Joined/DES\_31\_Joined;cat  
/media/Seagate/QIIME/ITS/Joined/DES\_32b\_Joined  
/media/Seagate/QIIME/ITS/Joined/DES\_32a\_Joined >  
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```
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fastq_to_fastaqual -f /media/Seagate/QIIME/ITS/Joined/DES_84_Joined -o
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fastq_to_fastaqual -f /media/Seagate/QIIME/ITS/Joined/DES_85_Joined -o
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fastq_to_fastaqual -f /media/Seagate/QIIME/ITS/Joined/DES_87_Joined -o
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fastq_to_fastaqual -f /media/Seagate/QIIME/ITS/Joined/DES_89_Joined -o
/media/Seagate/QIIME/ITS/Joined/Fastaqual_output/;convert_fastaqual_fastq.py -c
fastq_to_fastaqual -f /media/Seagate/QIIME/ITS/Joined/DES_90_Joined -o
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fastq_to_fastaqual -f /media/Seagate/QIIME/ITS/Joined/DES_91_Joined -o
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fastq_to_fastaqual -f /media/Seagate/QIIME/ITS/Joined/DES_92_Joined -o
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fastq_to_fastaqual -f /media/Seagate/QIIME/ITS/Joined/DES_93_Joined -o
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fastq_to_fastaqual -f /media/Seagate/QIIME/ITS/Joined/DES_95_Joined -o
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fastq_to_fastaqual -f /media/Seagate/QIIME/ITS/Joined/DES_96_Joined -o
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fastq_to_fastaqual -f /media/Seagate/QIIME/ITS/Joined/DES_97_Joined -o
/media/Seagate/QIIME/ITS/Joined/Fastaqual_output/;convert_fastaqual_fastq.py -c
fastq_to_fastaqual -f /media/Seagate/QIIME/ITS/Joined/DES_98_Joined -o
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fastq_to_fastaqual -f /media/Seagate/QIIME/ITS/Joined/DES_99_Joined -o
/media/Seagate/QIIME/ITS/Joined/Fastaqual_output/;convert_fastaqual_fastq.py -c
fastq_to_fastaqual -f /media/Seagate/QIIME/ITS/Joined/DES_100_Joined -o
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```

```
split_libraries.py -H 8 -m
/media/Seagate/QIIME/ITS/Mapping_Files_ITS/MappingFile_DES_1.txt -f
/media/Seagate/QIIME/ITS/Fastaqual_output/Sample1_Joined.fna -q
/media/Seagate/QIIME/ITS/Fastaqual_output/Sample1_Joined.qual -b 0 -z
truncate_remove -o
/media/Seagate/QIIME/ITS/split_libraries_output/split_library_1;split_libraries.py -H 8 -m
/media/Seagate/QIIME/ITS/Mapping_Files_ITS/MappingFile_DES_2.txt -f
/media/Seagate/QIIME/ITS/Fastaqual_output/Sample2_Joined.fna -q
```

```

/media/Seagate/QIIME/ITS/Fastaqual_output/Sample2_Joined.qual -b 0 -z
truncate_remove -o
/media/Seagate/QIIME/ITS/split_libraries_output/split_library_2;split_libraries.py -H 8 -m
/media/Seagate/QIIME/ITS/Mapping_Files_ITS/MappingFile_DES_3.txt -f
/media/Seagate/QIIME/ITS/Fastaqual_output/Sample3_Joined.fna -q
/media/Seagate/QIIME/ITS/Fastaqual_output/Sample3_Joined.qual -b 0 -z
truncate_remove -o
/media/Seagate/QIIME/ITS/split_libraries_output/split_library_3;split_libraries.py -H 8 -m
/media/Seagate/QIIME/ITS/Mapping_Files_ITS/MappingFile_DES_4.txt -f
/media/Seagate/QIIME/ITS/Fastaqual_output/Sample4_Joined.fna -q
/media/Seagate/QIIME/ITS/Fastaqual_output/Sample4_Joined.qual -b 0 -z
truncate_remove -o
/media/Seagate/QIIME/ITS/split_libraries_output/split_library_4;split_libraries.py -H 8 -m
/media/Seagate/QIIME/ITS/Mapping_Files_ITS/MappingFile_DES_5.txt -f
/media/Seagate/QIIME/ITS/Fastaqual_output/Sample5_Joined.fna -q
/media/Seagate/QIIME/ITS/Fastaqual_output/Sample5_Joined.qual -b 0 -z
truncate_remove -o
/media/Seagate/QIIME/ITS/split_libraries_output/split_library_5;split_libraries.py -H 8 -m
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/media/Seagate/QIIME/ITS/Fastaqual_output/Sample6_Joined.fna -q
/media/Seagate/QIIME/ITS/Fastaqual_output/Sample6_Joined.qual -b 0 -z
truncate_remove -o
/media/Seagate/QIIME/ITS/split_libraries_output/split_library_6;split_libraries.py -H 8 -m
/media/Seagate/QIIME/ITS/Mapping_Files_ITS/MappingFile_DES_7.txt -f
/media/Seagate/QIIME/ITS/Fastaqual_output/Sample7_Joined.fna -q
/media/Seagate/QIIME/ITS/Fastaqual_output/Sample7_Joined.qual -b 0 -z
truncate_remove -o
/media/Seagate/QIIME/ITS/split_libraries_output/split_library_7;split_libraries.py -H 8 -m
/media/Seagate/QIIME/ITS/Mapping_Files_ITS/MappingFile_DES_8.txt -f
/media/Seagate/QIIME/ITS/Fastaqual_output/Sample8_Joined.fna -q
/media/Seagate/QIIME/ITS/Fastaqual_output/Sample8_Joined.qual -b 0 -z
truncate_remove -o
/media/Seagate/QIIME/ITS/split_libraries_output/split_library_8;split_libraries.py -H 8 -m
/media/Seagate/QIIME/ITS/Mapping_Files_ITS/MappingFile_DES_9.txt -f
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/media/Seagate/QIIME/ITS/Fastaqual_output/Sample9_Joined.qual -b 0 -z
truncate_remove -o
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/media/Seagate/QIIME/ITS/Mapping_Files_ITS/MappingFile_DES_10.txt -f
/media/Seagate/QIIME/ITS/Fastaqual_output/Sample10_Joined.fna -q
/media/Seagate/QIIME/ITS/Fastaqual_output/Sample10_Joined.qual -b 0 -z
truncate_remove -o
/media/Seagate/QIIME/ITS/split_libraries_output/split_library_10;split_libraries.py -H 8 -
m /media/Seagate/QIIME/ITS/Mapping_Files_ITS/MappingFile_DES_11.txt -f
/media/Seagate/QIIME/ITS/Fastaqual_output/Sample11_Joined.fna -q
/media/Seagate/QIIME/ITS/Fastaqual_output/Sample11_Joined.qual -b 0 -z
truncate_remove -o
/media/Seagate/QIIME/ITS/split_libraries_output/split_library_11;split_libraries.py -H 8 -
m /media/Seagate/QIIME/ITS/Mapping_Files_ITS/MappingFile_DES_12.txt -f

```

```

/media/Seagate/QIIME/ITS/Fastaqual_output/Sample12_Joined.fna -q
/media/Seagate/QIIME/ITS/Fastaqual_output/Sample12_Joined.qual -b 0 -z
truncate_remove -o
/media/Seagate/QIIME/ITS/split_libraries_output/split_library_12;split_libraries.py -H 8 -
m /media/Seagate/QIIME/ITS/Mapping_Files_ITS/MappingFile_DES_13.txt -f
/media/Seagate/QIIME/ITS/Fastaqual_output/Sample13_Joined.fna -q
/media/Seagate/QIIME/ITS/Fastaqual_output/Sample13_Joined.qual -b 0 -z
truncate_remove -o
/media/Seagate/QIIME/ITS/split_libraries_output/split_library_13;split_libraries.py -H 8 -
m /media/Seagate/QIIME/ITS/Mapping_Files_ITS/MappingFile_DES_14.txt -f
/media/Seagate/QIIME/ITS/Fastaqual_output/Sample14_Joined.fna -q
/media/Seagate/QIIME/ITS/Fastaqual_output/Sample14_Joined.qual -b 0 -z
truncate_remove -o
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m /media/Seagate/QIIME/ITS/Mapping_Files_ITS/MappingFile_DES_15.txt -f
/media/Seagate/QIIME/ITS/Fastaqual_output/Sample15_Joined.fna -q
/media/Seagate/QIIME/ITS/Fastaqual_output/Sample15_Joined.qual -b 0 -z
truncate_remove -o
/media/Seagate/QIIME/ITS/split_libraries_output/split_library_15;split_libraries.py -H 8 -
m /media/Seagate/QIIME/ITS/Mapping_Files_ITS/MappingFile_DES_16.txt -f
/media/Seagate/QIIME/ITS/Fastaqual_output/Sample16_Joined.fna -q
/media/Seagate/QIIME/ITS/Fastaqual_output/Sample16_Joined.qual -b 0 -z
truncate_remove -o
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/media/Seagate/QIIME/ITS/Fastaqual_output/Sample17_Joined.qual -b 0 -z
truncate_remove -o
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m /media/Seagate/QIIME/ITS/Mapping_Files_ITS/MappingFile_DES_18.txt -f
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/media/Seagate/QIIME/ITS/Fastaqual_output/Sample18_Joined.qual -b 0 -z
truncate_remove -o
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m /media/Seagate/QIIME/ITS/Mapping_Files_ITS/MappingFile_DES_19.txt -f
/media/Seagate/QIIME/ITS/Fastaqual_output/Sample19_Joined.fna -q
/media/Seagate/QIIME/ITS/Fastaqual_output/Sample19_Joined.qual -b 0 -z
truncate_remove -o
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m /media/Seagate/QIIME/ITS/Mapping_Files_ITS/MappingFile_DES_20.txt -f
/media/Seagate/QIIME/ITS/Fastaqual_output/Sample20_Joined.fna -q
/media/Seagate/QIIME/ITS/Fastaqual_output/Sample20_Joined.qual -b 0 -z
truncate_remove -o
/media/Seagate/QIIME/ITS/split_libraries_output/split_library_20;split_libraries.py -H 8 -
m /media/Seagate/QIIME/ITS/Mapping_Files_ITS/MappingFile_DES_21.txt -f
/media/Seagate/QIIME/ITS/Fastaqual_output/Sample21_Joined.fna -q
/media/Seagate/QIIME/ITS/Fastaqual_output/Sample21_Joined.qual -b 0 -z
truncate_remove -o
/media/Seagate/QIIME/ITS/split_libraries_output/split_library_21;split_libraries.py -H 8 -

```

```

m /media/Seagate/QIIME/ITS/Mapping_Files_ITS/MappingFile_DES_22.txt -f
/media/Seagate/QIIME/ITS/Fastaqual_output/Sample22_Joined.fna -q
/media/Seagate/QIIME/ITS/Fastaqual_output/Sample22_Joined.qual -b 0 -z
truncate_remove -o
/media/Seagate/QIIME/ITS/split_libraries_output/split_library_22;split_libraries.py -H 8 -
m /media/Seagate/QIIME/ITS/Mapping_Files_ITS/MappingFile_DES_23.txt -f
/media/Seagate/QIIME/ITS/Fastaqual_output/DES_23_Joined.fna -q
/media/Seagate/QIIME/ITS/Fastaqual_output/DES_23_Joined.qual -b 0 -z
truncate_remove -o
/media/Seagate/QIIME/ITS/split_libraries_output/split_library_23;split_libraries.py -H 8 -
m /media/Seagate/QIIME/ITS/Mapping_Files_ITS/MappingFile_DES_24.txt -f
/media/Seagate/QIIME/ITS/Fastaqual_output/DES_24_Joined.fna -q
/media/Seagate/QIIME/ITS/Fastaqual_output/DES_24_Joined.qual -b 0 -z
truncate_remove -o
/media/Seagate/QIIME/ITS/split_libraries_output/split_library_24;split_libraries.py -H 8 -
m /media/Seagate/QIIME/ITS/Mapping_Files_ITS/MappingFile_DES_25.txt -f
/media/Seagate/QIIME/ITS/Fastaqual_output/DES_25_Joined.fna -q
/media/Seagate/QIIME/ITS/Fastaqual_output/DES_25_Joined.qual -b 0 -z
truncate_remove -o
/media/Seagate/QIIME/ITS/split_libraries_output/split_library_25;split_libraries.py -H 8 -
m /media/Seagate/QIIME/ITS/Mapping_Files_ITS/MappingFile_DES_26.txt -f
/media/Seagate/QIIME/ITS/Fastaqual_output/DES_26_Joined.fna -q
/media/Seagate/QIIME/ITS/Fastaqual_output/DES_26_Joined.qual -b 0 -z
truncate_remove -o
/media/Seagate/QIIME/ITS/split_libraries_output/split_library_26;split_libraries.py -H 8 -
m /media/Seagate/QIIME/ITS/Mapping_Files_ITS/MappingFile_DES_27.txt -f
/media/Seagate/QIIME/ITS/Fastaqual_output/DES_27_Joined.fna -q
/media/Seagate/QIIME/ITS/Fastaqual_output/DES_27_Joined.qual -b 0 -z
truncate_remove -o
/media/Seagate/QIIME/ITS/split_libraries_output/split_library_27;split_libraries.py -H 8 -
m /media/Seagate/QIIME/ITS/Mapping_Files_ITS/MappingFile_DES_28.txt -f
/media/Seagate/QIIME/ITS/Fastaqual_output/DES_28_Joined.fna -q
/media/Seagate/QIIME/ITS/Fastaqual_output/DES_28_Joined.qual -b 0 -z
truncate_remove -o
/media/Seagate/QIIME/ITS/split_libraries_output/split_library_28;split_libraries.py -H 8 -
m /media/Seagate/QIIME/ITS/Mapping_Files_ITS/MappingFile_DES_29.txt -f
/media/Seagate/QIIME/ITS/Fastaqual_output/DES_29_Joined.fna -q
/media/Seagate/QIIME/ITS/Fastaqual_output/DES_29_Joined.qual -b 0 -z
truncate_remove -o
/media/Seagate/QIIME/ITS/split_libraries_output/split_library_29;split_libraries.py -H 8 -
m /media/Seagate/QIIME/ITS/Mapping_Files_ITS/MappingFile_DES_30.txt -f
/media/Seagate/QIIME/ITS/Fastaqual_output/DES_30_Joined.fna -q
/media/Seagate/QIIME/ITS/Fastaqual_output/DES_30_Joined.qual -b 0 -z
truncate_remove -o
/media/Seagate/QIIME/ITS/split_libraries_output/split_library_30;split_libraries.py -H 8 -
m /media/Seagate/QIIME/ITS/Mapping_Files_ITS/MappingFile_DES_31.txt -f
/media/Seagate/QIIME/ITS/Fastaqual_output/DES_31_Joined.fna -q
/media/Seagate/QIIME/ITS/Fastaqual_output/DES_31_Joined.qual -b 0 -z
truncate_remove -o

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/media/Seagate/QIIME/ITS/split_libraries_output/split_library_31;split_libraries.py -H 8 -
m /media/Seagate/QIIME/ITS/Mapping_Files_ITS/MappingFile_DES_32.txt -f
/media/Seagate/QIIME/ITS/Fastaqual_output/DES_32_Joined.fna -q
/media/Seagate/QIIME/ITS/Fastaqual_output/DES_32_Joined.qual -b 0 -z
truncate_remove -o
/media/Seagate/QIIME/ITS/split_libraries_output/split_library_32;split_libraries.py -H 8 -
m /media/Seagate/QIIME/ITS/Mapping_Files_ITS/MappingFile_DES_33.txt -f
/media/Seagate/QIIME/ITS/Fastaqual_output/DES_33_Joined.fna -q
/media/Seagate/QIIME/ITS/Fastaqual_output/DES_33_Joined.qual -b 0 -z
truncate_remove -o
/media/Seagate/QIIME/ITS/split_libraries_output/split_library_33;split_libraries.py -H 8 -
m /media/Seagate/QIIME/ITS/Mapping_Files_ITS/MappingFile_DES_34.txt -f
/media/Seagate/QIIME/ITS/Fastaqual_output/DES_34_Joined.fna -q
/media/Seagate/QIIME/ITS/Fastaqual_output/DES_34_Joined.qual -b 0 -z
truncate_remove -o
/media/Seagate/QIIME/ITS/split_libraries_output/split_library_34;split_libraries.py -H 8 -
m /media/Seagate/QIIME/ITS/Mapping_Files_ITS/MappingFile_DES_35.txt -f
/media/Seagate/QIIME/ITS/Fastaqual_output/DES_35_Joined.fna -q
/media/Seagate/QIIME/ITS/Fastaqual_output/DES_35_Joined.qual -b 0 -z
truncate_remove -o
/media/Seagate/QIIME/ITS/split_libraries_output/split_library_35;split_libraries.py -H 8 -
m /media/Seagate/QIIME/ITS/Mapping_Files_ITS/MappingFile_DES_36.txt -f
/media/Seagate/QIIME/ITS/Fastaqual_output/DES_36_Joined.fna -q
/media/Seagate/QIIME/ITS/Fastaqual_output/DES_36_Joined.qual -b 0 -z
truncate_remove -o
/media/Seagate/QIIME/ITS/split_libraries_output/split_library_36;split_libraries.py -H 8 -
m /media/Seagate/QIIME/ITS/Mapping_Files_ITS/MappingFile_DES_37.txt -f
/media/Seagate/QIIME/ITS/Fastaqual_output/DES_37_Joined.fna -q
/media/Seagate/QIIME/ITS/Fastaqual_output/DES_37_Joined.qual -b 0 -z
truncate_remove -o
/media/Seagate/QIIME/ITS/split_libraries_output/split_library_37;split_libraries.py -H 8 -
m /media/Seagate/QIIME/ITS/Mapping_Files_ITS/MappingFile_DES_38.txt -f
/media/Seagate/QIIME/ITS/Fastaqual_output/DES_38_Joined.fna -q
/media/Seagate/QIIME/ITS/Fastaqual_output/DES_38_Joined.qual -b 0 -z
truncate_remove -o
/media/Seagate/QIIME/ITS/split_libraries_output/split_library_38;split_libraries.py -H 8 -
m /media/Seagate/QIIME/ITS/Mapping_Files_ITS/MappingFile_DES_39.txt -f
/media/Seagate/QIIME/ITS/Fastaqual_output/DES_39_Joined.fna -q
/media/Seagate/QIIME/ITS/Fastaqual_output/DES_39_Joined.qual -b 0 -z
truncate_remove -o
/media/Seagate/QIIME/ITS/split_libraries_output/split_library_39;split_libraries.py -H 8 -
m /media/Seagate/QIIME/ITS/Mapping_Files_ITS/MappingFile_DES_40.txt -f
/media/Seagate/QIIME/ITS/Fastaqual_output/DES_40_Joined.fna -q
/media/Seagate/QIIME/ITS/Fastaqual_output/DES_40_Joined.qual -b 0 -z
truncate_remove -o
/media/Seagate/QIIME/ITS/split_libraries_output/split_library_40;split_libraries.py -H 8 -
m /media/Seagate/QIIME/ITS/Mapping_Files_ITS/MappingFile_DES_41.txt -f
/media/Seagate/QIIME/ITS/Fastaqual_output/DES_41_Joined.fna -q
/media/Seagate/QIIME/ITS/Fastaqual_output/DES_41_Joined.qual -b 0 -z

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truncate_remove -o
/media/Seagate/QIIME/ITS/split_libraries_output/split_library_41;split_libraries.py -H 8 -
m /media/Seagate/QIIME/ITS/Mapping_Files_ITS/MappingFile_DES_42.txt -f
/media/Seagate/QIIME/ITS/Fastaqual_output/DES_42_Joined.fna -q
/media/Seagate/QIIME/ITS/Fastaqual_output/DES_42_Joined.qual -b 0 -z
truncate_remove -o
/media/Seagate/QIIME/ITS/split_libraries_output/split_library_42;split_libraries.py -H 8 -
m /media/Seagate/QIIME/ITS/Mapping_Files_ITS/MappingFile_DES_43.txt -f
/media/Seagate/QIIME/ITS/Fastaqual_output/DES_43_Joined.fna -q
/media/Seagate/QIIME/ITS/Fastaqual_output/DES_43_Joined.qual -b 0 -z
truncate_remove -o
/media/Seagate/QIIME/ITS/split_libraries_output/split_library_43;split_libraries.py -H 8 -
m /media/Seagate/QIIME/ITS/Mapping_Files_ITS/MappingFile_DES_44.txt -f
/media/Seagate/QIIME/ITS/Fastaqual_output/DES_44_Joined.fna -q
/media/Seagate/QIIME/ITS/Fastaqual_output/DES_44_Joined.qual -b 0 -z
truncate_remove -o
/media/Seagate/QIIME/ITS/split_libraries_output/split_library_44;split_libraries.py -H 8 -
m /media/Seagate/QIIME/ITS/Mapping_Files_ITS/MappingFile_DES_45.txt -f
/media/Seagate/QIIME/ITS/Fastaqual_output/DES_45_Joined.fna -q
/media/Seagate/QIIME/ITS/Fastaqual_output/DES_45_Joined.qual -b 0 -z
truncate_remove -o
/media/Seagate/QIIME/ITS/split_libraries_output/split_library_45;split_libraries.py -H 8 -
m /media/Seagate/QIIME/ITS/Mapping_Files_ITS/MappingFile_DES_46.txt -f
/media/Seagate/QIIME/ITS/Fastaqual_output/DES_46_Joined.fna -q
/media/Seagate/QIIME/ITS/Fastaqual_output/DES_46_Joined.qual -b 0 -z
truncate_remove -o
/media/Seagate/QIIME/ITS/split_libraries_output/split_library_46;split_libraries.py -H 8 -
m /media/Seagate/QIIME/ITS/Mapping_Files_ITS/MappingFile_DES_47.txt -f
/media/Seagate/QIIME/ITS/Fastaqual_output/DES_47_Joined.fna -q
/media/Seagate/QIIME/ITS/Fastaqual_output/DES_47_Joined.qual -b 0 -z
truncate_remove -o
/media/Seagate/QIIME/ITS/split_libraries_output/split_library_47;split_libraries.py -H 8 -
m /media/Seagate/QIIME/ITS/Mapping_Files_ITS/MappingFile_DES_48.txt -f
/media/Seagate/QIIME/ITS/Fastaqual_output/DES_48_Joined.fna -q
/media/Seagate/QIIME/ITS/Fastaqual_output/DES_48_Joined.qual -b 0 -z
truncate_remove -o
/media/Seagate/QIIME/ITS/split_libraries_output/split_library_48;split_libraries.py -H 8 -
m /media/Seagate/QIIME/ITS/Mapping_Files_ITS/MappingFile_DES_49.txt -f
/media/Seagate/QIIME/ITS/Fastaqual_output/DES_49_Joined.fna -q
/media/Seagate/QIIME/ITS/Fastaqual_output/DES_49_Joined.qual -b 0 -z
truncate_remove -o
/media/Seagate/QIIME/ITS/split_libraries_output/split_library_49;split_libraries.py -H 8 -
m /media/Seagate/QIIME/ITS/Mapping_Files_ITS/MappingFile_DES_50.txt -f
/media/Seagate/QIIME/ITS/Fastaqual_output/DES_50_Joined.fna -q
/media/Seagate/QIIME/ITS/Fastaqual_output/DES_50_Joined.qual -b 0 -z
truncate_remove -o
/media/Seagate/QIIME/ITS/split_libraries_output/split_library_50;split_libraries.py -H 8 -
m /media/Seagate/QIIME/ITS/Mapping_Files_ITS/MappingFile_DES_51.txt -f
/media/Seagate/QIIME/ITS/Fastaqual_output/DES_51_Joined.fna -q

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/media/Seagate/QIIME/ITS/Fastaqual_output/DES_51_Joined.qual -b 0 -z
truncate_remove -o
/media/Seagate/QIIME/ITS/split_libraries_output/split_library_51;split_libraries.py -H 8 -
m /media/Seagate/QIIME/ITS/Mapping_Files_ITS/MappingFile_DES_52.txt -f
/media/Seagate/QIIME/ITS/Fastaqual_output/DES_52_Joined.fna -q
/media/Seagate/QIIME/ITS/Fastaqual_output/DES_52_Joined.qual -b 0 -z
truncate_remove -o
/media/Seagate/QIIME/ITS/split_libraries_output/split_library_52;split_libraries.py -H 8 -
m /media/Seagate/QIIME/ITS/Mapping_Files_ITS/MappingFile_DES_53.txt -f
/media/Seagate/QIIME/ITS/Fastaqual_output/DES_53_Joined.fna -q
/media/Seagate/QIIME/ITS/Fastaqual_output/DES_53_Joined.qual -b 0 -z
truncate_remove -o
/media/Seagate/QIIME/ITS/split_libraries_output/split_library_53;split_libraries.py -H 8 -
m /media/Seagate/QIIME/ITS/Mapping_Files_ITS/MappingFile_DES_54.txt -f
/media/Seagate/QIIME/ITS/Fastaqual_output/DES_54_Joined.fna -q
/media/Seagate/QIIME/ITS/Fastaqual_output/DES_54_Joined.qual -b 0 -z
truncate_remove -o
/media/Seagate/QIIME/ITS/split_libraries_output/split_library_54;split_libraries.py -H 8 -
m /media/Seagate/QIIME/ITS/Mapping_Files_ITS/MappingFile_DES_55.txt -f
/media/Seagate/QIIME/ITS/Fastaqual_output/DES_55_Joined.fna -q
/media/Seagate/QIIME/ITS/Fastaqual_output/DES_55_Joined.qual -b 0 -z
truncate_remove -o
/media/Seagate/QIIME/ITS/split_libraries_output/split_library_55;split_libraries.py -H 8 -
m /media/Seagate/QIIME/ITS/Mapping_Files_ITS/MappingFile_DES_56.txt -f
/media/Seagate/QIIME/ITS/Fastaqual_output/DES_56_Joined.fna -q
/media/Seagate/QIIME/ITS/Fastaqual_output/DES_56_Joined.qual -b 0 -z
truncate_remove -o
/media/Seagate/QIIME/ITS/split_libraries_output/split_library_56;split_libraries.py -H 8 -
m /media/Seagate/QIIME/ITS/Mapping_Files_ITS/MappingFile_DES_57.txt -f
/media/Seagate/QIIME/ITS/Fastaqual_output/DES_57_Joined.fna -q
/media/Seagate/QIIME/ITS/Fastaqual_output/DES_57_Joined.qual -b 0 -z
truncate_remove -o
/media/Seagate/QIIME/ITS/split_libraries_output/split_library_57;split_libraries.py -H 8 -
m /media/Seagate/QIIME/ITS/Mapping_Files_ITS/MappingFile_DES_58.txt -f
/media/Seagate/QIIME/ITS/Fastaqual_output/DES_58_Joined.fna -q
/media/Seagate/QIIME/ITS/Fastaqual_output/DES_58_Joined.qual -b 0 -z
truncate_remove -o
/media/Seagate/QIIME/ITS/split_libraries_output/split_library_58;split_libraries.py -H 8 -
m /media/Seagate/QIIME/ITS/Mapping_Files_ITS/MappingFile_DES_59.txt -f
/media/Seagate/QIIME/ITS/Fastaqual_output/DES_59_Joined.fna -q
/media/Seagate/QIIME/ITS/Fastaqual_output/DES_59_Joined.qual -b 0 -z
truncate_remove -o
/media/Seagate/QIIME/ITS/split_libraries_output/split_library_59;split_libraries.py -H 8 -
m /media/Seagate/QIIME/ITS/Mapping_Files_ITS/MappingFile_DES_60.txt -f
/media/Seagate/QIIME/ITS/Fastaqual_output/DES_60_Joined.fna -q
/media/Seagate/QIIME/ITS/Fastaqual_output/DES_60_Joined.qual -b 0 -z
truncate_remove -o
/media/Seagate/QIIME/ITS/split_libraries_output/split_library_60;split_libraries.py -H 8 -
m /media/Seagate/QIIME/ITS/Mapping_Files_ITS/MappingFile_DES_61.txt -f

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/media/Seagate/QIIME/ITS/Fastaqual_output/DES_61_Joined.fna -q
/media/Seagate/QIIME/ITS/Fastaqual_output/DES_61_Joined.qual -b 0 -z
truncate_remove -o
/media/Seagate/QIIME/ITS/split_libraries_output/split_library_61;split_libraries.py -H 8 -
m /media/Seagate/QIIME/ITS/Mapping_Files_ITS/MappingFile_DES_62.txt -f
/media/Seagate/QIIME/ITS/Fastaqual_output/DES_62_Joined.fna -q
/media/Seagate/QIIME/ITS/Fastaqual_output/DES_62_Joined.qual -b 0 -z
truncate_remove -o
/media/Seagate/QIIME/ITS/split_libraries_output/split_library_62;split_libraries.py -H 8 -
m /media/Seagate/QIIME/ITS/Mapping_Files_ITS/MappingFile_DES_63.txt -f
/media/Seagate/QIIME/ITS/Fastaqual_output/DES_63_Joined.fna -q
/media/Seagate/QIIME/ITS/Fastaqual_output/DES_63_Joined.qual -b 0 -z
truncate_remove -o
/media/Seagate/QIIME/ITS/split_libraries_output/split_library_63;split_libraries.py -H 8 -
m /media/Seagate/QIIME/ITS/Mapping_Files_ITS/MappingFile_DES_64.txt -f
/media/Seagate/QIIME/ITS/Fastaqual_output/DES_64_Joined.fna -q
/media/Seagate/QIIME/ITS/Fastaqual_output/DES_64_Joined.qual -b 0 -z
truncate_remove -o
/media/Seagate/QIIME/ITS/split_libraries_output/split_library_64;split_libraries.py -H 8 -
m /media/Seagate/QIIME/ITS/Mapping_Files_ITS/MappingFile_DES_65.txt -f
/media/Seagate/QIIME/ITS/Fastaqual_output/DES_65_Joined.fna -q
/media/Seagate/QIIME/ITS/Fastaqual_output/DES_65_Joined.qual -b 0 -z
truncate_remove -o
/media/Seagate/QIIME/ITS/split_libraries_output/split_library_65;split_libraries.py -H 8 -
m /media/Seagate/QIIME/ITS/Mapping_Files_ITS/MappingFile_DES_66.txt -f
/media/Seagate/QIIME/ITS/Fastaqual_output/DES_66_Joined.fna -q
/media/Seagate/QIIME/ITS/Fastaqual_output/DES_66_Joined.qual -b 0 -z
truncate_remove -o
/media/Seagate/QIIME/ITS/split_libraries_output/split_library_66;split_libraries.py -H 8 -
m /media/Seagate/QIIME/ITS/Mapping_Files_ITS/MappingFile_DES_67.txt -f
/media/Seagate/QIIME/ITS/Fastaqual_output/DES_67_Joined.fna -q
/media/Seagate/QIIME/ITS/Fastaqual_output/DES_67_Joined.qual -b 0 -z
truncate_remove -o
/media/Seagate/QIIME/ITS/split_libraries_output/split_library_67;split_libraries.py -H 8 -
m /media/Seagate/QIIME/ITS/Mapping_Files_ITS/MappingFile_DES_68.txt -f
/media/Seagate/QIIME/ITS/Fastaqual_output/DES_68_Joined.fna -q
/media/Seagate/QIIME/ITS/Fastaqual_output/DES_68_Joined.qual -b 0 -z
truncate_remove -o
/media/Seagate/QIIME/ITS/split_libraries_output/split_library_68;split_libraries.py -H 8 -
m /media/Seagate/QIIME/ITS/Mapping_Files_ITS/MappingFile_DES_69.txt -f
/media/Seagate/QIIME/ITS/Fastaqual_output/DES_69_Joined.fna -q
/media/Seagate/QIIME/ITS/Fastaqual_output/DES_69_Joined.qual -b 0 -z
truncate_remove -o
/media/Seagate/QIIME/ITS/split_libraries_output/split_library_69;split_libraries.py -H 8 -
m /media/Seagate/QIIME/ITS/Mapping_Files_ITS/MappingFile_DES_70.txt -f
/media/Seagate/QIIME/ITS/Fastaqual_output/DES_70_Joined.fna -q
/media/Seagate/QIIME/ITS/Fastaqual_output/DES_70_Joined.qual -b 0 -z
truncate_remove -o
/media/Seagate/QIIME/ITS/split_libraries_output/split_library_70;split_libraries.py -H 8 -

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m /media/Seagate/QIIME/ITS/Mapping_Files_ITS/MappingFile_DES_71.txt -f
/media/Seagate/QIIME/ITS/Fastaqual_output/DES_71_Joined.fna -q
/media/Seagate/QIIME/ITS/Fastaqual_output/DES_71_Joined.qual -b 0 -z
truncate_remove -o
/media/Seagate/QIIME/ITS/split_libraries_output/split_library_71;split_libraries.py -H 8 -
m /media/Seagate/QIIME/ITS/Mapping_Files_ITS/MappingFile_DES_72.txt -f
/media/Seagate/QIIME/ITS/Fastaqual_output/DES_72_Joined.fna -q
/media/Seagate/QIIME/ITS/Fastaqual_output/DES_72_Joined.qual -b 0 -z
truncate_remove -o
/media/Seagate/QIIME/ITS/split_libraries_output/split_library_72;split_libraries.py -H 8 -
m /media/Seagate/QIIME/ITS/Mapping_Files_ITS/MappingFile_DES_73.txt -f
/media/Seagate/QIIME/ITS/Fastaqual_output/DES_73_Joined.fna -q
/media/Seagate/QIIME/ITS/Fastaqual_output/DES_73_Joined.qual -b 0 -z
truncate_remove -o
/media/Seagate/QIIME/ITS/split_libraries_output/split_library_73;split_libraries.py -H 8 -
m /media/Seagate/QIIME/ITS/Mapping_Files_ITS/MappingFile_DES_74.txt -f
/media/Seagate/QIIME/ITS/Fastaqual_output/DES_74_Joined.fna -q
/media/Seagate/QIIME/ITS/Fastaqual_output/DES_74_Joined.qual -b 0 -z
truncate_remove -o
/media/Seagate/QIIME/ITS/split_libraries_output/split_library_74;split_libraries.py -H 8 -
m /media/Seagate/QIIME/ITS/Mapping_Files_ITS/MappingFile_DES_75.txt -f
/media/Seagate/QIIME/ITS/Fastaqual_output/DES_75_Joined.fna -q
/media/Seagate/QIIME/ITS/Fastaqual_output/DES_75_Joined.qual -b 0 -z
truncate_remove -o
/media/Seagate/QIIME/ITS/split_libraries_output/split_library_75;split_libraries.py -H 8 -
m /media/Seagate/QIIME/ITS/Mapping_Files_ITS/MappingFile_DES_76.txt -f
/media/Seagate/QIIME/ITS/Fastaqual_output/DES_76_Joined.fna -q
/media/Seagate/QIIME/ITS/Fastaqual_output/DES_76_Joined.qual -b 0 -z
truncate_remove -o
/media/Seagate/QIIME/ITS/split_libraries_output/split_library_76;split_libraries.py -H 8 -
m /media/Seagate/QIIME/ITS/Mapping_Files_ITS/MappingFile_DES_77.txt -f
/media/Seagate/QIIME/ITS/Fastaqual_output/DES_77_Joined.fna -q
/media/Seagate/QIIME/ITS/Fastaqual_output/DES_77_Joined.qual -b 0 -z
truncate_remove -o
/media/Seagate/QIIME/ITS/split_libraries_output/split_library_77;split_libraries.py -H 8 -
m /media/Seagate/QIIME/ITS/Mapping_Files_ITS/MappingFile_DES_78.txt -f
/media/Seagate/QIIME/ITS/Fastaqual_output/DES_78_Joined.fna -q
/media/Seagate/QIIME/ITS/Fastaqual_output/DES_78_Joined.qual -b 0 -z
truncate_remove -o
/media/Seagate/QIIME/ITS/split_libraries_output/split_library_78;split_libraries.py -H 8 -
m /media/Seagate/QIIME/ITS/Mapping_Files_ITS/MappingFile_DES_79.txt -f
/media/Seagate/QIIME/ITS/Fastaqual_output/DES_79_Joined.fna -q
/media/Seagate/QIIME/ITS/Fastaqual_output/DES_79_Joined.qual -b 0 -z
truncate_remove -o
/media/Seagate/QIIME/ITS/split_libraries_output/split_library_79;split_libraries.py -H 8 -
m /media/Seagate/QIIME/ITS/Mapping_Files_ITS/MappingFile_DES_80.txt -f
/media/Seagate/QIIME/ITS/Fastaqual_output/DES_80_Joined.fna -q
/media/Seagate/QIIME/ITS/Fastaqual_output/DES_80_Joined.qual -b 0 -z
truncate_remove -o

```

```
/media/Seagate/QIIME/ITS/split_libraries_output/split_library_80;split_libraries.py -H 8 -
m /media/Seagate/QIIME/ITS/Mapping_Files_ITS/MappingFile_DES_81.txt -f
/media/Seagate/QIIME/ITS/Fastaqual_output/DES_81_Joined.fna -q
/media/Seagate/QIIME/ITS/Fastaqual_output/DES_81_Joined.qual -b 0 -z
truncate_remove -o
/media/Seagate/QIIME/ITS/split_libraries_output/split_library_81;split_libraries.py -H 8 -
m /media/Seagate/QIIME/ITS/Mapping_Files_ITS/MappingFile_DES_82.txt -f
/media/Seagate/QIIME/ITS/Fastaqual_output/DES_82_Joined.fna -q
/media/Seagate/QIIME/ITS/Fastaqual_output/DES_82_Joined.qual -b 0 -z
truncate_remove -o
/media/Seagate/QIIME/ITS/split_libraries_output/split_library_82;split_libraries.py -H 8 -
m /media/Seagate/QIIME/ITS/Mapping_Files_ITS/MappingFile_DES_83.txt -f
/media/Seagate/QIIME/ITS/Fastaqual_output/DES_83_Joined.fna -q
/media/Seagate/QIIME/ITS/Fastaqual_output/DES_83_Joined.qual -b 0 -z
truncate_remove -o
/media/Seagate/QIIME/ITS/split_libraries_output/split_library_83;split_libraries.py -H 8 -
m /media/Seagate/QIIME/ITS/Mapping_Files_ITS/MappingFile_DES_84.txt -f
/media/Seagate/QIIME/ITS/Fastaqual_output/DES_84_Joined.fna -q
/media/Seagate/QIIME/ITS/Fastaqual_output/DES_84_Joined.qual -b 0 -z
truncate_remove -o
/media/Seagate/QIIME/ITS/split_libraries_output/split_library_84;split_libraries.py -H 8 -
m /media/Seagate/QIIME/ITS/Mapping_Files_ITS/MappingFile_DES_85.txt -f
/media/Seagate/QIIME/ITS/Fastaqual_output/DES_85_Joined.fna -q
/media/Seagate/QIIME/ITS/Fastaqual_output/DES_85_Joined.qual -b 0 -z
truncate_remove -o
/media/Seagate/QIIME/ITS/split_libraries_output/split_library_85;split_libraries.py -H 8 -
m /media/Seagate/QIIME/ITS/Mapping_Files_ITS/MappingFile_DES_86.txt -f
/media/Seagate/QIIME/ITS/Fastaqual_output/DES_86_Joined.fna -q
/media/Seagate/QIIME/ITS/Fastaqual_output/DES_86_Joined.qual -b 0 -z
truncate_remove -o
/media/Seagate/QIIME/ITS/split_libraries_output/split_library_86;split_libraries.py -H 8 -
m /media/Seagate/QIIME/ITS/Mapping_Files_ITS/MappingFile_DES_87.txt -f
/media/Seagate/QIIME/ITS/Fastaqual_output/DES_87_Joined.fna -q
/media/Seagate/QIIME/ITS/Fastaqual_output/DES_87_Joined.qual -b 0 -z
truncate_remove -o
/media/Seagate/QIIME/ITS/split_libraries_output/split_library_87;split_libraries.py -H 8 -
m /media/Seagate/QIIME/ITS/Mapping_Files_ITS/MappingFile_DES_88.txt -f
/media/Seagate/QIIME/ITS/Fastaqual_output/DES_88_Joined.fna -q
/media/Seagate/QIIME/ITS/Fastaqual_output/DES_88_Joined.qual -b 0 -z
truncate_remove -o
/media/Seagate/QIIME/ITS/split_libraries_output/split_library_88;split_libraries.py -H 8 -
m /media/Seagate/QIIME/ITS/Mapping_Files_ITS/MappingFile_DES_89.txt -f
/media/Seagate/QIIME/ITS/Fastaqual_output/DES_89_Joined.fna -q
/media/Seagate/QIIME/ITS/Fastaqual_output/DES_89_Joined.qual -b 0 -z
truncate_remove -o
/media/Seagate/QIIME/ITS/split_libraries_output/split_library_89;split_libraries.py -H 8 -
m /media/Seagate/QIIME/ITS/Mapping_Files_ITS/MappingFile_DES_90.txt -f
/media/Seagate/QIIME/ITS/Fastaqual_output/DES_90_Joined.fna -q
/media/Seagate/QIIME/ITS/Fastaqual_output/DES_90_Joined.qual -b 0 -z
```

```

truncate_remove -o
/media/Seagate/QIIME/ITS/split_libraries_output/split_library_90;split_libraries.py -H 8 -
m /media/Seagate/QIIME/ITS/Mapping_Files_ITS/MappingFile_DES_91.txt -f
/media/Seagate/QIIME/ITS/Fastaqual_output/DES_91_Joined.fna -q
/media/Seagate/QIIME/ITS/Fastaqual_output/DES_91_Joined.qual -b 0 -z
truncate_remove -o
/media/Seagate/QIIME/ITS/split_libraries_output/split_library_91;split_libraries.py -H 8 -
m /media/Seagate/QIIME/ITS/Mapping_Files_ITS/MappingFile_DES_92.txt -f
/media/Seagate/QIIME/ITS/Fastaqual_output/DES_92_Joined.fna -q
/media/Seagate/QIIME/ITS/Fastaqual_output/DES_92_Joined.qual -b 0 -z
truncate_remove -o
/media/Seagate/QIIME/ITS/split_libraries_output/split_library_92;split_libraries.py -H 8 -
m /media/Seagate/QIIME/ITS/Mapping_Files_ITS/MappingFile_DES_93.txt -f
/media/Seagate/QIIME/ITS/Fastaqual_output/DES_93_Joined.fna -q
/media/Seagate/QIIME/ITS/Fastaqual_output/DES_93_Joined.qual -b 0 -z
truncate_remove -o
/media/Seagate/QIIME/ITS/split_libraries_output/split_library_93;split_libraries.py -H 8 -
m /media/Seagate/QIIME/ITS/Mapping_Files_ITS/MappingFile_DES_94.txt -f
/media/Seagate/QIIME/ITS/Fastaqual_output/DES_94_Joined.fna -q
/media/Seagate/QIIME/ITS/Fastaqual_output/DES_94_Joined.qual -b 0 -z
truncate_remove -o
/media/Seagate/QIIME/ITS/split_libraries_output/split_library_94;split_libraries.py -H 8 -
m /media/Seagate/QIIME/ITS/Mapping_Files_ITS/MappingFile_DES_95.txt -f
/media/Seagate/QIIME/ITS/Fastaqual_output/DES_95_Joined.fna -q
/media/Seagate/QIIME/ITS/Fastaqual_output/DES_95_Joined.qual -b 0 -z
truncate_remove -o
/media/Seagate/QIIME/ITS/split_libraries_output/split_library_95;split_libraries.py -H 8 -
m /media/Seagate/QIIME/ITS/Mapping_Files_ITS/MappingFile_DES_96.txt -f
/media/Seagate/QIIME/ITS/Fastaqual_output/DES_96_Joined.fna -q
/media/Seagate/QIIME/ITS/Fastaqual_output/DES_96_Joined.qual -b 0 -z
truncate_remove -o
/media/Seagate/QIIME/ITS/split_libraries_output/split_library_96;split_libraries.py -H 8 -
m /media/Seagate/QIIME/ITS/Mapping_Files_ITS/MappingFile_DES_97.txt -f
/media/Seagate/QIIME/ITS/Fastaqual_output/DES_97_Joined.fna -q
/media/Seagate/QIIME/ITS/Fastaqual_output/DES_97_Joined.qual -b 0 -z
truncate_remove -o
/media/Seagate/QIIME/ITS/split_libraries_output/split_library_97;split_libraries.py -H 8 -
m /media/Seagate/QIIME/ITS/Mapping_Files_ITS/MappingFile_DES_98.txt -f
/media/Seagate/QIIME/ITS/Fastaqual_output/DES_98_Joined.fna -q
/media/Seagate/QIIME/ITS/Fastaqual_output/DES_98_Joined.qual -b 0 -z
truncate_remove -o
/media/Seagate/QIIME/ITS/split_libraries_output/split_library_98;split_libraries.py -H 8 -
m /media/Seagate/QIIME/ITS/Mapping_Files_ITS/MappingFile_DES_99.txt -f
/media/Seagate/QIIME/ITS/Fastaqual_output/DES_99_Joined.fna -q
/media/Seagate/QIIME/ITS/Fastaqual_output/DES_99_Joined.qual -b 0 -z
truncate_remove -o
/media/Seagate/QIIME/ITS/split_libraries_output/split_library_99;split_libraries.py -H 8 -
m /media/Seagate/QIIME/ITS/Mapping_Files_ITS/MappingFile_DES_100.txt -f
/media/Seagate/QIIME/ITS/Fastaqual_output/DES_100_Joined.fna -q

```





```
/media/Seagate/QIIME/ITS/split_libraries_output/split_library_90/seqs.fna
/media/Seagate/QIIME/ITS/split_libraries_output/split_library_91/seqs.fna
/media/Seagate/QIIME/ITS/split_libraries_output/split_library_92/seqs.fna
/media/Seagate/QIIME/ITS/split_libraries_output/split_library_93/seqs.fna
/media/Seagate/QIIME/ITS/split_libraries_output/split_library_94/seqs.fna
/media/Seagate/QIIME/ITS/split_libraries_output/split_library_95/seqs.fna
/media/Seagate/QIIME/ITS/split_libraries_output/split_library_96/seqs.fna
/media/Seagate/QIIME/ITS/split_libraries_output/split_library_97/seqs.fna
/media/Seagate/QIIME/ITS/split_libraries_output/split_library_98/seqs.fna
/media/Seagate/QIIME/ITS/split_libraries_output/split_library_99/seqs.fna
/media/Seagate/QIIME/ITS/split_libraries_output/split_library_100/seqs.fna >
/media/Seagate/QIIME/ITS/split_libraries_output/Combined_sequence_libraries_050115
.fna
```

```
pick_otus.py -i
/media/Seagate/QIIME/ITS/split_libraries_output/Combined_sequence_libraries_050115
.fna -o /media/Seagate/QIIME/ITS/Pick_OTU_050115/
pick_rep_set
pick_rep_set.py -i
/media/Seagate/QIIME/ITS/Pick_OTU_050115/Combined_sequence_libraries_050115_
otus.txt -f
/media/Seagate/QIIME/ITS/split_libraries_output/Combined_sequence_libraries_050115
.fna -o
/media/Seagate/QIIME/ITS/Rep_set/Combined_sequence_libraries_050115_repset.fna
assign_taxonomy
assign_taxonomy.py -i
/media/Seagate/QIIME/ITS/Rep_set/Combined_sequence_libraries_050115_repset.fna -
t
/media/Seagate/QIIME/ITS/sh_qiime_release_10/sh_taxonomy_qiime_ver6_dynamic_1
0.09.2014.txt -r
/media/Seagate/QIIME/ITS/sh_qiime_release_10/sh_refs_qiime_ver6_dynamic_10.09.2
014.fasta -o /media/Seagate/QIIME/ITS/taxonomy_results/ -m rdp --rdp_max_memory
10000
```

```
make_otu_table.py -i
/media/Seagate/QIIME/ITS/Pick_OTU_050115/Combined_sequence_libraries_050115_o
tus.txt -t
/media/Seagate/QIIME/ITS/taxonomy_results/Combined_sequence_libraries_050115_rep
set_tax_assignments.txt -o
/media/Seagate/QIIME/ITS/otu_tables/combined_seqs_otu_table_050115.biom
```

```
summarize_taxa.py -i
/media/Seagate/QIIME/ITS/otu_tables/combined_seqs_otu_table_050115.biom -o
/media/Seagate/QIIME/ITS/taxonomy_summaries/
```

Appendix D: Funguilds:

```
python Guilds_v1.0.py -otu  
/Volumes/Seagate/FUNGuilds/Fungi_Indicators_Function.txt
```

```
python Guilds_v1.0.py -otu  
/Volumes/Seagate/FUNGuilds/TaxonomyAssignments.txt
```



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doi:10.1016/j.funeco.2015.06.006

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