EXAMINING THE POTENTIAL USE OF FUNGI IN FORENSIC SCIENCE

by

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Dedicated to my parents and friends who supported me through these two years.

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ABSTRACT

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Forensic science has gained popularity in the last few decades. Many new techniques are being studied and implemented. It includes a wide range of scientific disciplines, such as entomology, physics and biology. An important improvement to forensic science is the development of different DNA techniques which are implemented during an investigation, making DNA a gold standard for forensics. Most of the time DNA is mentioned it is in terms of human DNA, but there are microscopic organisms with useful DNA. In the last decade, with the development of next-generation sequencing (NGS), studies focusing on bacterial communities have been published, but fungal communities have not been extensively studied.

For this project, the potential of fungi in forensic science was investigated through three different studies. Human flora was looked at by NGS from thirty-seven human bodies with differing post-mortem intervals (PMIs). The communities were analyzed statistically and quantitatively, resulting in unique operational taxonomic units (OTUs) and genera which were only present in certain PMIs, and in some which were present through the entire PMI time scale. These attributes can help, not only to give a better view on human mycoflora during decomposition, but they can also help in determining fungal signatures during decomposition. These signatures can help in a PMI determination. Moreover, swine carcasses – the model animals for human forensic studies – were investigated as well to create a checklist of fungal flora after five months of winter decomposition in the West Lafayette, Indiana area. Furthermore, due to the increased

importance of wildlife forensics, a wildlife study was also conducted using four wildlife species (mute swan, red tailed hawk, river otter, bobcat). The fungal flora from these species were compared within species at the beginning of the study and at skeletonization stage to look at any indicator fungal species and to create a general checklist for wildlife studies in the West Lafayette, Indiana area for future studies. Additionally, the fungal communities were compared across species as well.

CHAPTER 1. INTRODUCTION AND LITERATURE REVIEW

1.1 What is forensic science?

Forensic science is the application of science to matters of law. Forensic scientists help investigators solve crimes by providing scientific information through the analysis of physical evidence (Forensic Sciences, n.d.). It uses different applications of other sciences such as biology, chemistry, medicine and physics. A major motivation, especially during the early ages of forensics, was death investigation. Since the beginning of times, people wanted to find answers to how and why a person died. This investigative mindset was recorded as early as 1247 CE, by T'zu Sung who authored The Washing Away of Wrong was written in China. In this book, differences between crime scenes in case of suicide versus homicide are explained in great detail (James & Norbdy, 2002).

1.2 Shaping of forensic science

During the 19th century forensics began to be recognized as a scientific discipline on its own. This could not have happened without the brilliant minds of many scientists who worked hard on different aspects of what was, at the time, a new science. The following seven people are major contributors to shaping forensics to become the discipline we know today.

Mathieu Orfila (1787-1853), who is considered the father of forensic toxicology, studied poisons – mainly arsenic, which was the most common poison at the time – and wrote multiple papers on the analysis of poisons in blood and other bodily fluids. Francis Galton (1822-1911) was studying fingerprints and developed a method for classifying them, which he published in his book, *Finger Prints*. Hans Gross (1847-1915) was the first person who wrote about how science can help

a criminal investigation and with detail explained in his book *Handbuch fur Untersuchungsrichter als System der Kriminalistik* (*Criminal Investigation*) how investigators can benefit from microscopy, chemistry, botany, and many other scientific disciplines which are still used today in a crime laboratory. Albert S. Osborn (1858-1946) developed another important tool in forensics; he set the base for questioned document examination, which is still the primary reference for questioned document examiners around the world. One of the most famous names in forensics is Edmond Locard (1877-1966), who is known for his theory called Locard's Exchange Principle, which is still a base for any criminal investigation, and the way scientists think about trace evidence. The idea behind this theory is the exchange of materials; no matter how careful one is a perpetrator will always leave some trace on a scene and some trace from that scene will leave with the perpetrator as well. Calvin Goddard (1891-1955) set the ground for analyzing bullets by examining bullets under a comparison microscope. Last, but not least, Walter McCrone (1916-2002) made a huge contribution to forensics with his microscopy work on trace evidence, specifically hair comparison analyses (James & Nordby, 2002).

The aforementioned people and many other scientists looking at application of science in a criminal investigation helped to make forensics a crucial part of any police investigation and helped to create the practices and disciplines in forensic science which are in use today. In the United States the American Academy of Forensic Sciences, one of the largest forensic organizations, recognizes the following categories: anthropology, criminalistics, digital and multimedia sciences, general, jurisprudence, odontology, pathology/biology, and psychiatry and behavioral science

1.3 Forensics and fungi

Several sub-disciplines of forensics are well known and researched, such as forensic entomology, the use of insects and arthropods that inhabit decomposing remains in a legal investigation (Hitosugi, 2006, Ishii, 2006, Tranchida, 2012). However, the potential use of other organisms, especially microbes that are invisible to the unaided eye, is an area that is almost completely unexplored. These microorganisms – bacteria, fungi, viruses, archaea – make up the microbiome.

To date, microbiome studies related to forensic science have focused on bacterial communities and looked at bacterial species abundance and species richness using high throughput metagenomics sequencing (Pechal, 2014). These studies were able to show some preliminary results that bacterial communities do change during decomposition, which can help to determine the post mortem interval (PMI), i.e., time passed since death (Pechal et al., 2013, Pechal et al., 2014). Even though research with bacteria in a forensic context is becoming more common, another large part of the microbiome – fungi – has not. It is known that bacteria and fungi have similar nutritional and environmental requirements (Bååth, 2003); hence fungi can potentially be as useful as bacteria during an investigation.

Fungi are a kingdom of eukaryotic organisms consisting of molds, yeasts, mushrooms and many others. Yeasts are single-celled organisms – like bacteria – while molds produce long branching thread-like filaments (hyphae) that form colonies. Mushrooms are also filamentous fungi that form large fruiting bodies during specific times in the life cycle which are often visible to the naked eye. There are many symbiotic and parasitic relationships known between fungi and animals. Some fungi are components of the normal flora in mammalian intestinal tracts, and some are parasitic inside the body and/or on skin tissues. Also, micro- and macro-fungi can be found in

the soil. Some of these soil fungi prefer ammonia- and lipid-rich soils, such as are produced by decomposing bodies (Sidrim et al., 2010).

The use of fungi during a forensic investigation is rather new, thus not many cases have used this type of biological evidence. However, it can be a source of versatile information, as it can be used as trace evidence. According to Dunhaime's Law Dictionary, trace evidence is defined as the following: "[f]ibers, hair and other such microscopic evidence which relates to the commission of a crime" – and can be used to determine time or manner of death. In the following section, cases where fungal evidence was used will be discussed.

1.4 Cases where fungi were used

1.4.1 Fungal spores used in a rape case in southern U.K.

Based on the knowledge on biotic and abiotic requirements for fungi to reproduce, it is known that by finding fungal remains or spores on evidence, one could make general conclusions the environment in which evidence was found. In this case mycological and botanical trace evidence – spores and pollen – were looked at to identify the location of a crime.

During this investigation a woman claimed that she was raped by her significant other. He walked her to a woody area between two roads. The boyfriend argued that they had consensual sexual relations on the turf of a park which was located about 200 m from the potential crime scene. Clothing and footwear were collected from both the suspect and the victim and they were examined and analyzed. Moreover, both sites – the wooded area and the park – were sampled and analyzed. The two sites had very different profiles as far as pollen, spores and dominant plant species are concerned, hence matching the profiles of the clothing and footwear evidence was possible. Some fungal species, e.g., *Epicoccum nigrum,* and *Melanospora* sp. were identified in the sample obtained from the park but were barely represented on the clothing. On the other hand, many fungal

traces – such as *Clasterosporium flexum* and *Endophragmiella fagicloa* – were found on the clothing of both the victim and the suspect which also appeared in the samples collected from the potential rape site. (Wiltshire, 2014)

In conclusion, the fugal evidence, as well as the other palynomorph analyses, were able to show that the activity happened not where the suspect said it but where the victim has showed it. This made a convincing case to the judge and jury that the victim was raped, and the suspect was sentenced to jail.

1.4.2 Fungal colony size determining minimum time since death

Fungi with filamentous growth can grow in colonies under favorable conditions. These circular colonies have recorded growth rates in artificial media which are available with species descriptions. This growth rate was used in a case in England where police had found a mutilated human body. The body was found with no blood around it, suggesting that it had been moved from one location to where it was eventually found. Officers found small colonies of non-sporulating *Mucor hiemalis* on the abdomen. Based on the sizes of the colonies, it was indicated that the growth was no more than 1-2 days old; however, this particular fungus is unable to grow on fresh skin and it does not appear until at least a week after death occurs on human bodies. This had shown that this body was at least 8-9 days old. Moreover, after looking at the colonies under a microscope, the good condition of the fungal mycelium (collection of hyphae) suggested that the body was not moved since the mold started to grow on it because these mycelia are very fragile and would have been broken into pieces in case the body was moved after they have developed (Hawksworth, 2010).

In another case from West Yorkshire, England, swabs were taken from a female body. She was found in her apartment. The police took samples from grey and bluish green fungal growths. After

the swab were taken the colony sizes were measured for the bluish green colonies (the gray colonies where too widely spread to measure them). The samples were identified as *Penicillium* sp. and *Mucor* sp.. After the genus level identification, these samples were grown in artificial media by a mycologist with the temperature that the body was found in as well as at room temperature. This experiment has determined that the body had been dead at least three to four weeks prior to the measurements taken on the body. This piece of evidence later helped in sentencing the suspect for life in prison (Hawksworth, 2015).

1.5 Previous Research

A handful of research experiments have been published in forensic science that have focused on fungi in order to answer forensic questions. Many of these were conducted in other countries including Brazil, the United Kingdom, and Argentina. Many have focused on methods to determine PMI in different ways. They have tested different methods with varying number of cadavers, but all concluded that further studies have to be done in the field of forensic mycology in order to establish a reliable method to determine PMI using fungi.

In northeastern Brazil, in a study by Sidrim (2009), 60 human samples were collected from the city morgue at different stages of decomposition. Three stages of decomposition were identified as bloating, putrefaction and skeletonization (explanation on how these stages were determined are not present in the study). Thirty-four, six, and twenty samples were collected from each of these stages, respectively. Collection sites consisted of the following: mouth, rectum, vagina, under foreskin, lungs, skin, scalp hair, clothing and surrounding area. Two samples were collected from each site, one for microscopic analysis and another one for culturing. Fungi were observed at 100X and 400X and were grown in Sabouraud agar with antibiotics to inhibit bacterial and airborne fungal growth for 20 days in the dark at room temperature, then identified

morphologically and through a series of biochemical tests. The most diverse samples based on isolated species were recovered from the bloated stage followed by putrefaction and skeletonization stages. The morphological and chemical analysis have focused mostly on *Aspergillus* and *Penicillium*. From their findings, it was concluded that identification by microscopic analysis alone was not useful due to abundant bacterial growth. Additionally, by limiting focus on just members of two genera, other species that may play a role as indicators may have been overlooked.

In another experiment done in the United Kingdom, a *Sus scrofa domesticus* (pig) leg was used as a human model instead of human samples. Animals were buried and soil samples from 3 depths were collected on days 0, 3, 28 and 77 post burial. The DNA was extracted from these soil samples and amplified at the 18S rRNA locus. The results of this study did not yield in significant differences between soil mycobiome without and with carcass at any of the decomposition stages (Chimutsa, 2015).

On the other hand, Tranchida et al. (2014) worked on one human cadaver in Argentina, where they collected soil sample from under the body once the body was discovered. They washed the soil samples and plated them onto potato dextrose agar and malt yeast extract agar containing antibiotics to inhibit bacterial growth. In this experiment, the fungal flora was significantly different in the decomposition island, where the body and the nutrients leaving the body were than in the control sample. Moreover, they have identified Eurotiomycetes as a group which, if it is present, could indicate PMI (Tranchida, 2014).

After looking at the exploratory work with fungi in a forensic context, the following questions were asked: how can fungi help solve problems in forensic science? Are there keystone species or communities that can be used to determine time of death? Are there indicator species of

fungi that can help in establishing manner of death? Can fungal communities provide trace evidence for determining the origin of transferred materials or bodies?

1.6 Objectives

I. To provide a baseline pan mycobiome during human and animal decomposition

II. To analyze fungal community changes during decomposition of human cadavers using next generation sequencing

III. To determine what fungal species grew on swine carcasses which were left at a field over the winter in Indiana based on culturing methods

IV. To provide insight into how fungal communities change between the initial (fresh) and the final (skeletal) stages of decomposition for different wildlife species based on culturing method

CHAPTER 2. POTENTIAL USE OF FUNGAL FLORA IN FORENSICS VIA NEXT GENERATION SEQUENCING

2.1 Abstract

Mycology in a criminal investigation could be a useful tool; yet, it is an understudied field. Fungi are a part of the natural human flora; also, they are abundant in the surrounding environment. These microscopic organisms could help with post-mortem interval (PMI) determination by looking at the mycoflora on the body during evidence collection. Abiotic factors, such as temperature and chemical environment changes during decomposition, can cause certain fungal species to thrive while creating a nonviable environment for other species. This could cause a change in fungal flora over time, but the potential use of this change has not yet been explored to its full potential. For this, studies where fungal DNA is collected from multiple bodies are needed. Here, thirty-seven DNA samples from bodies with differing PMIs are analyzed via NextGen Sequencing to look at the fungal communities and the changes of those fungal flora over time during decomposition in order to possibly identify fungal biomarkers for specific times of death.

2.2 Introduction

Forensic investigators often focus on macroscopic evidences at a crime scene. However, microscopic trace evidence can be instrumental to solve a case even though they are not visible to the naked eyes (Hawksworth, 2011). Now that DNA sequencing is readily available, testing for the presence of human DNA is a well-established protocol, but other DNA related research is still minimal. Microbes (primarily bacteria and fungi) are a ubiquitous part of the human flora and the environment, yet their forensic use is limited. In recent years, bacteria have become the focus of

new studies such as Pechal (2014, 2017), but fungi are still under-studied in terms of forensic relevance.

Fungi were used to determine location of a crime in several forensic cases (Wiltshire, 2014; Hawksworth, 2015; Hawksworth, 2011). Fungican be found in any environment; they are present in and on a body as normal mycoflora, can be parasitic and cause serious illnesses like pneumonia. The vast abundance of these microscopic organisms could give investigators a better picture about a crime. Different fungi have very different environmental and nutritional needs which makes them a good indicator of location, such as it is described by Wiltshire (2014), where a rape case was solved by comparing the mycoflora of two sites. Specific fungi that are decomposers of woods in a forest area will not be found in a de-forested field, or vice versa. In some cases, mycologists were asked to help in an investigation related to location and were successful.

One of the most important aspects in a forensic investigation of a body is to determine the post-mortem interval (PMI), the time that has passed since death. The two widely used methods to determine PMI is via forensic entomology – the use of insects and their successional pattern on a body at the time of discovery – and by a medical examiner. These methods are useful, but with the advances in science newer methods could give a better estimate or could give an estimate of PMI when entomology cannot be used (such as when there is a lack of insects or when there are many generations of maggots on the body).

The purpose of this study was to investigate whether fungi may be useful to help determine PMI. DNA swabs were collected from 37 human bodies by a medical examiner in Detroit. DNA was extracted and sequenced using Next Generation Sequencing and data was interpreted both statistically and visually to find possible fungal signatures during decomposition which can be

used to determine PMI in the future. An additional objective of this study was to provide baseline data on the human mycobiome during decomposition.

2.3 Materials and methods

2.3.1 Sample collection

Samples were collected at the Wayne County Medical Examiner Office in the manner as it is described in Pechal et al. (2014). Each sample was taken from a different individual for differing sample areas and at a different PMI determined by the medical examiner. Table 2.1 below summarizes the sample area and PMI of each samples.

Table 2.1 List of samples with their corresponding sample area and PMI

Sequence ID	Sample Area	Estimated PMI (h)
WCME_6	Ears	>24
WCME_19	Rectum	>24
WCME_48	Nose	>24
WCME_53	Eyes	>24
WCME_62	Mouth	<24
WCME_85	Rectum	<24
WCME_103	Nose	>24
WCME_127	Eyes	12
WCME_128	Nose	12
WCME_129	Mouth	12
WCME_131	Rectum	<24
WCME_163	Nose	<24
WCME_170	Umbilicus	>72
WCME_171	Ears	>72
WCME_177	Umbilicus	>72
WCME_180	Eyes	12
WCME_211	Rectum	>48
WCME_215	Mouth	>48
WCME_223	Umbilicus	>24
WCME_235	Nose	<24
WCME_239	Rectum	>72
WCME_237	Eyes	>72

Table 2.1 continued

WCME_238	Mouth	>24
WCME_245	Umbilicus	>24
WCME_274	Ears	>48
WCME_240	Eyes	>48
WCME_230	Nose	>48
WCME_234	Mouth	>48
WCME_422	Mouth	>72
WCME_425	Eyes	>72
WCME_427	Nose	>72
WCME_502	Nose	>72
WCME_365	Mouth	465
WCME_597	Eyes	>48
WCME_598	Nose	>48
WCME_1027	Ears	>48
WCME_822	Skull	>24

WCME - Wayne County Medical Examiner's Office's numbering system.

2.3.2 Molecular protocol

DNA samples were extracted based on Pechal et al. (2014). To amplify fungal internal transcribed spacer (ITS) region primers ITS1F (Gardes and Bruns 1993) and ITS2 (White et al. 1990) were used. The protocol for the thermocycler was as follows: denaturation step at 95 °C for 5 min, followed by 35 cycles at 94 °C for 30 s, annealing T at 45 °C for 45 s, 72 °C for 45 s, and a final extension step at 72 °C for 7 min. PCR products were electrophoresed in 1% agarose gel and all successful PCR products were sent to Purdue Genomic Core Facility (West Lafayette, IN) for Illumina MiSeq sequencing.

2.3.3 Sequence analysis and statistical methods

The raw data was trimmed and analyzed with PIPITS pipeline by loading the R package bioinfo PIPITS (Gweon et al., 2015). After OTU abundance table was established based on at least 97% similarity (Gweon et al. 2015), R version 3.5.3 under R Studio was used for data analysis and

visualization of the data. Fungal communities were analyzed at both OTU and genus level taxonomic classification.

To differentiate possible groups multidimensional scaling technique was used with Bray-Curtis dissimilarity matrix, which was chosen because it is not affected by null values in a dataset, which happens when a certain OTU or genus is present in one sample but not in another. These distances were than used to create principal component analysis (PCoA), by the vegan library in R, to visually look at the possible groups based on PMI and Sample Area. Non-metric multidimensional scaling (nMDS) was also preformed to get a real two-dimensional view of the data. PERMANOVA test was done to look at p-values and a homogeneity test was also done via adonis statistical package in R. Moreover, Simpson's diversity index was calculated as well to look at diversity accounting for both species present and relative abundance of those species for the different PMIs. After the statistical analysis was done, Venn diagrams – via VennDiagram library in R – were created to visualize differences in OTUs and genera that might not show significance during a statistical analysis but is a potential indicator of a certain PMI.

2.4 Results

449 OTUs were recovered via the PIPITS pipeline and the relative abundance of these OTUs are represented in Appendix 7. Thirty-seven samples were analyzed on an OTU and genus level taxonomic classification using R. First OTU level analysis was conducted (Figure 2.1-2.10). Figures 2.2 and 2.3 showed that there are 3 groups presented by the data which explained 20.6% of the total variability for this data set.

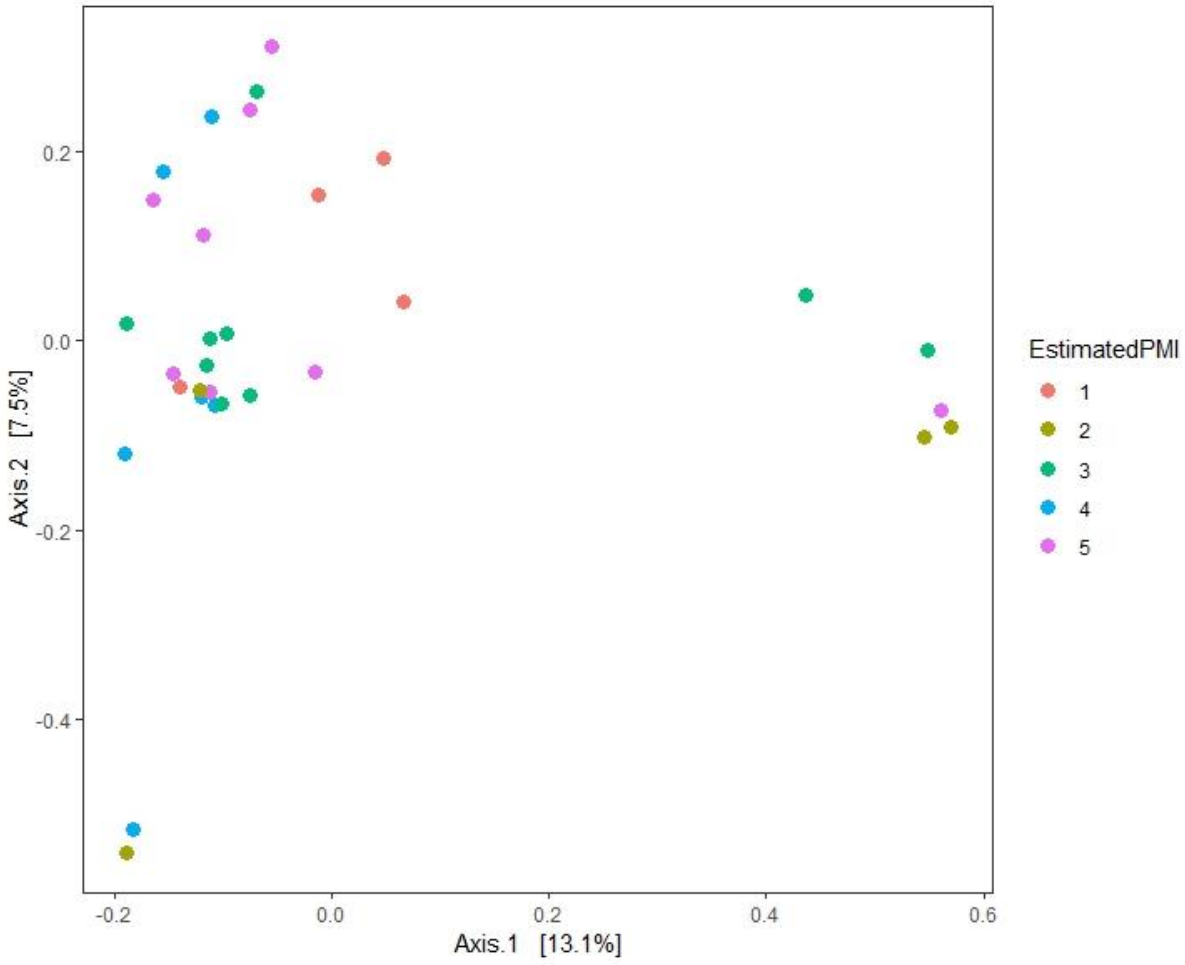


Figure 2.1 PCoA of PMI on an OTU level with the original PMIs from Table 2.1

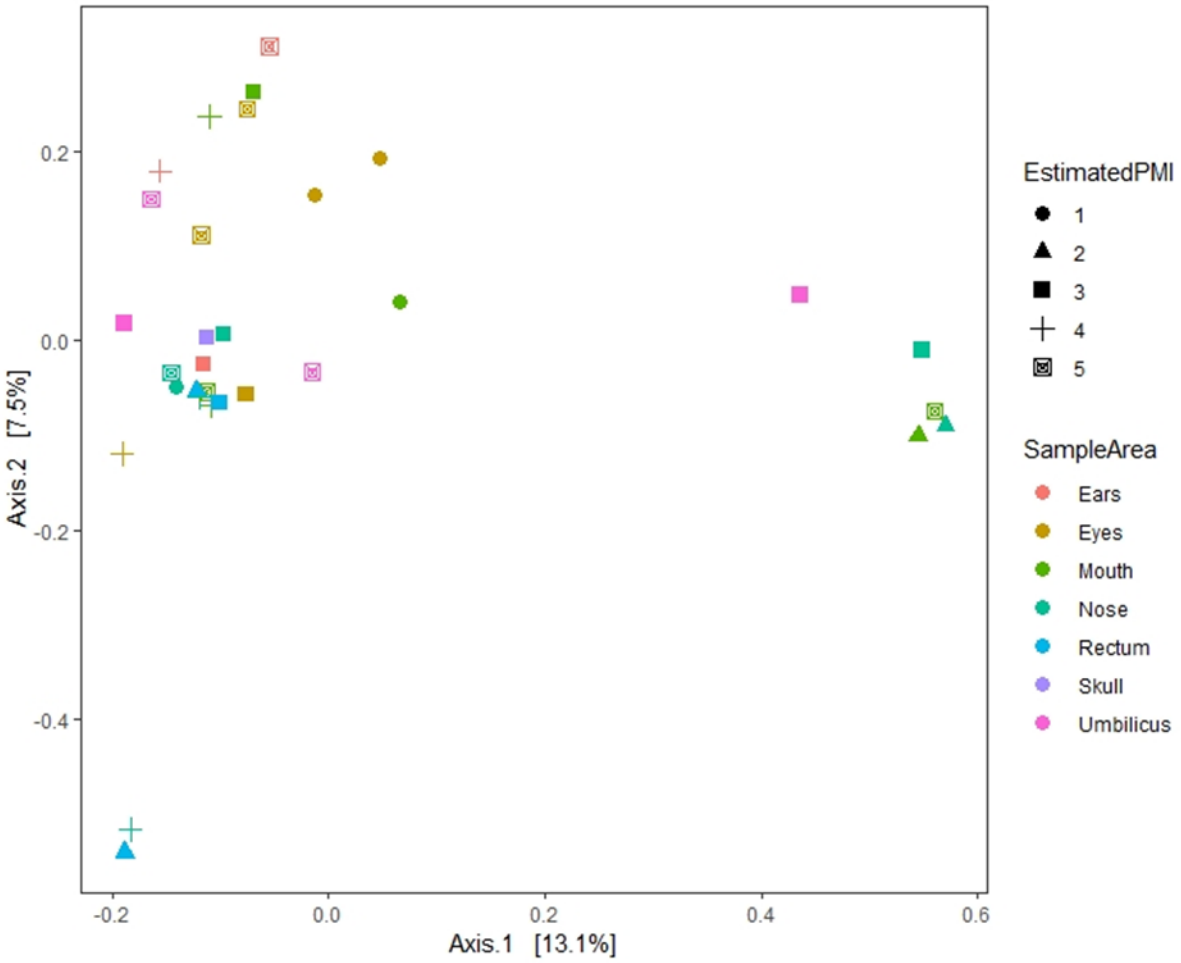


Figure 2.2 PCoA of sample area and PMI on an OTU level with the original sample areas and PMIs from Table 2.1

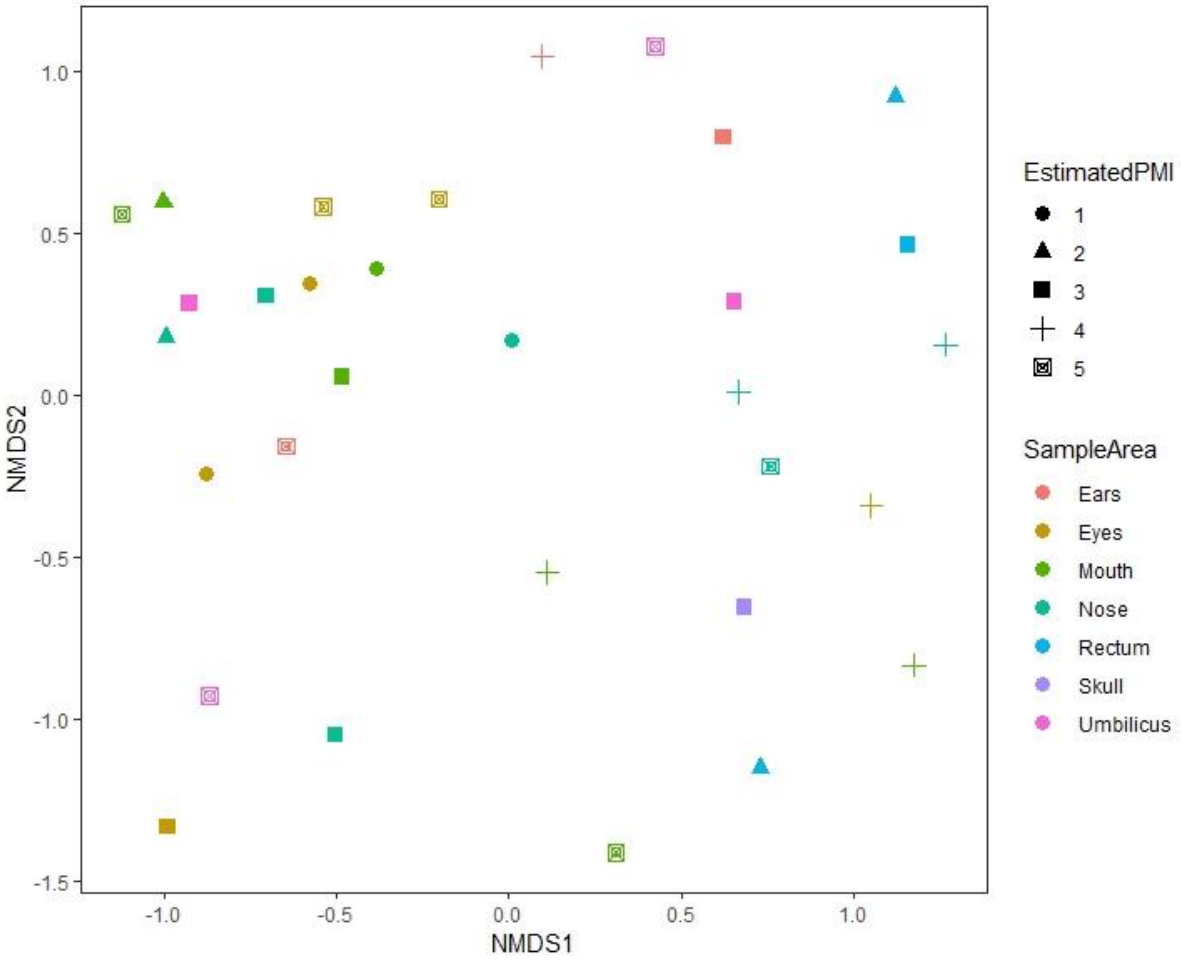


Figure 2.3 nMDS of sample area and PMI on an OTU level with the original sample areas and PMIs from Table 2.1

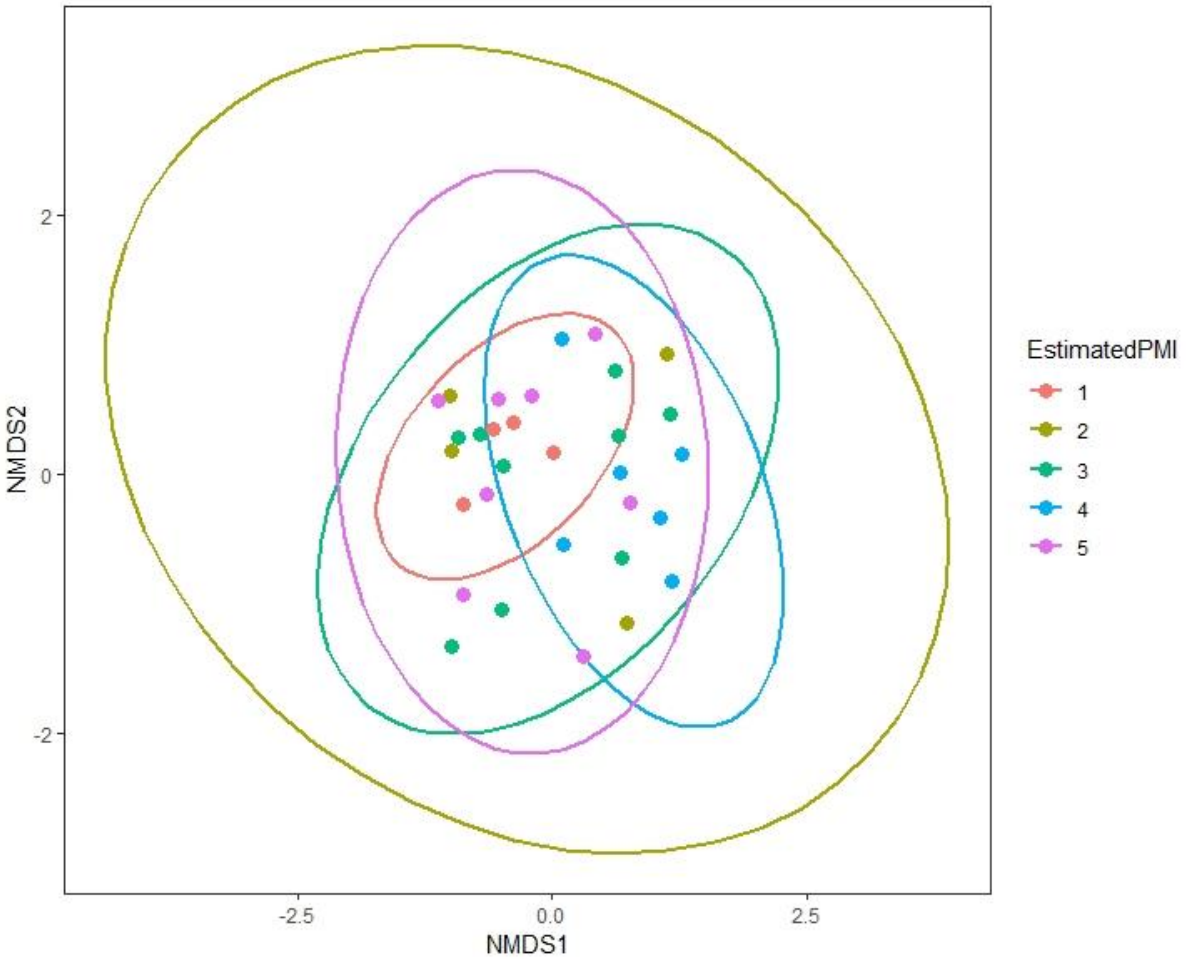


Figure 2.4 95% confidence interval ellipse based on nMDS of PMI on an OTU level with the original sample areas and PMIs from Table 2.1

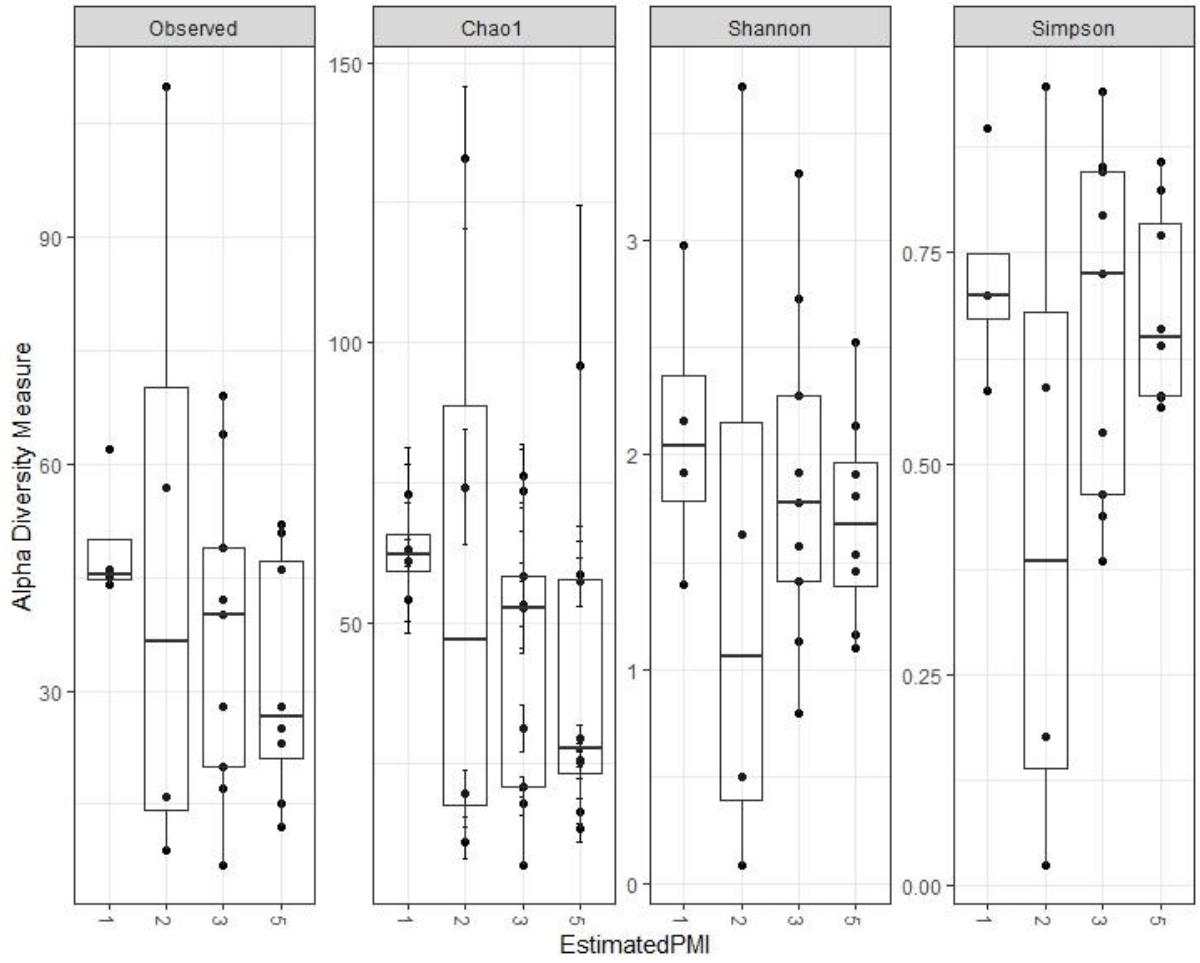


Figure 2.5 Whisker plots of alfa-diversity based on Chao1, Shannon and Simpson diversity indexes in OUT level classification in the 5 PMI stages

Due to the non-significant results by the original grouping, PMIs and sample areas were further grouped as in Table 2.2 and the same statistical analyses were run with the re-grouped data set.

Table 2.2 List of samples with their regrouped sample area and PMI

Sequence ID	Sample Area	Estimated PMI (h)
WCME_6	Head	>24
WCME_19	Body	>24
WCME_48	Head	>24
WCME_53	Head	>24
WCME_62	Head	<24
WCME_85	Body	<24
WCME_103	Head	>24
WCME_127	Head	<24
WCME_128	Head	<24
WCME_129	Head	<24
WCME_131	Body	<24
WCME_163	Head	<24
WCME_170	Body	>72
WCME_171	Head	>72
WCME_177	Body	>72
WCME_180	Head	<24
WCME_211	Body	>24
WCME_215	Head	>24
WCME_223	Body	>24
WCME_235	Head	<24
WCME_239	Body	>72
WCME_237	Eyes	>72
WCME_238	Mouth	>24
WCME_245	Umbilicus	>24
WCME_274	Ears	>24
WCME_240	Eyes	>24
WCME_230	Nose	>24
WCME_234	Mouth	>24
WCME_422	Mouth	>72
WCME_425	Eyes	>72
WCME_427	Nose	>72
WCME_502	Nose	>72
WCME_365	Mouth	>72
WCME_597	Eyes	>24
WCME_598	Nose	>24
WCME_1027	Ears	>24
WCME_822	Skull	>24

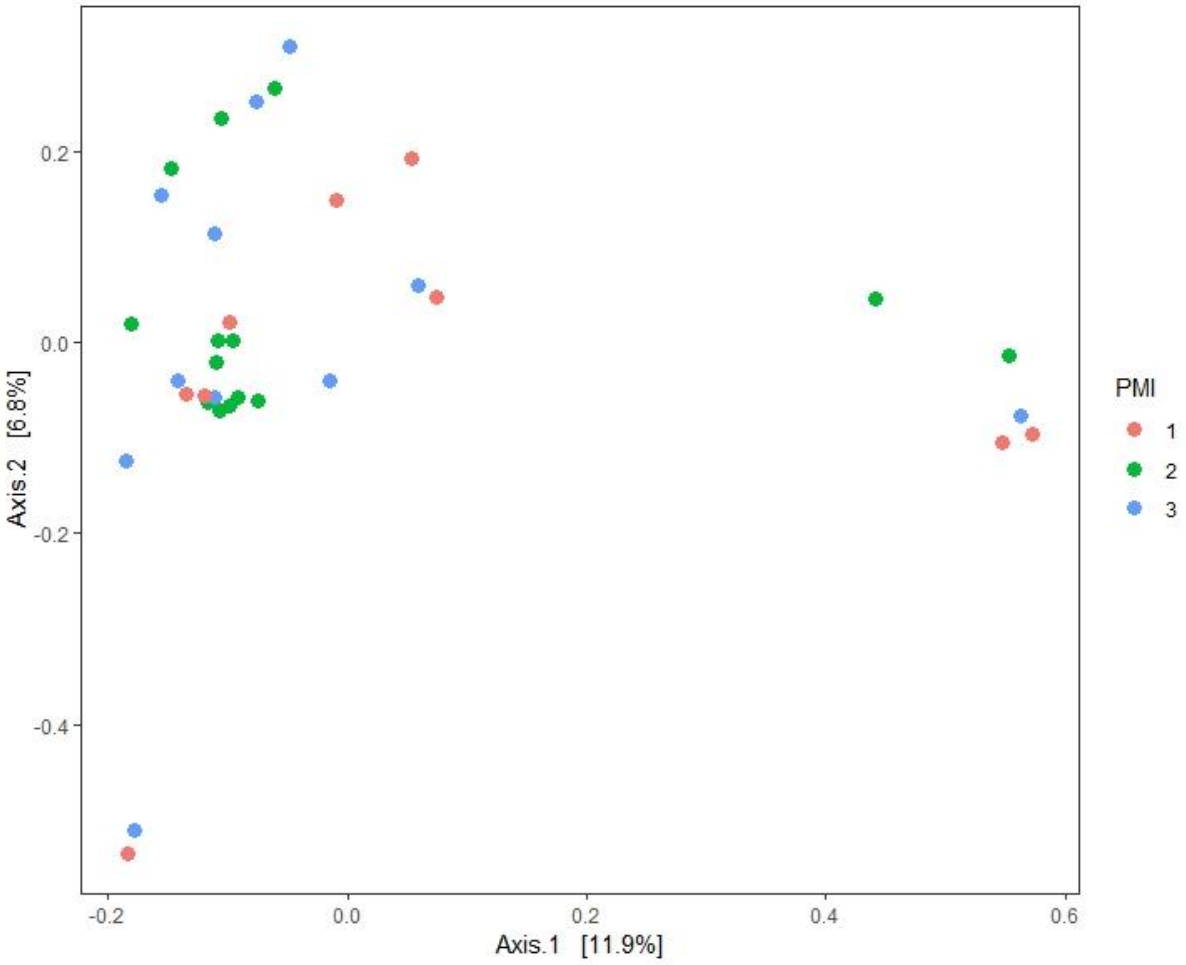


Figure 2.6 PCoA of sample area and PMI on an OTU level with the re-grouped PMIs from Table 2.2

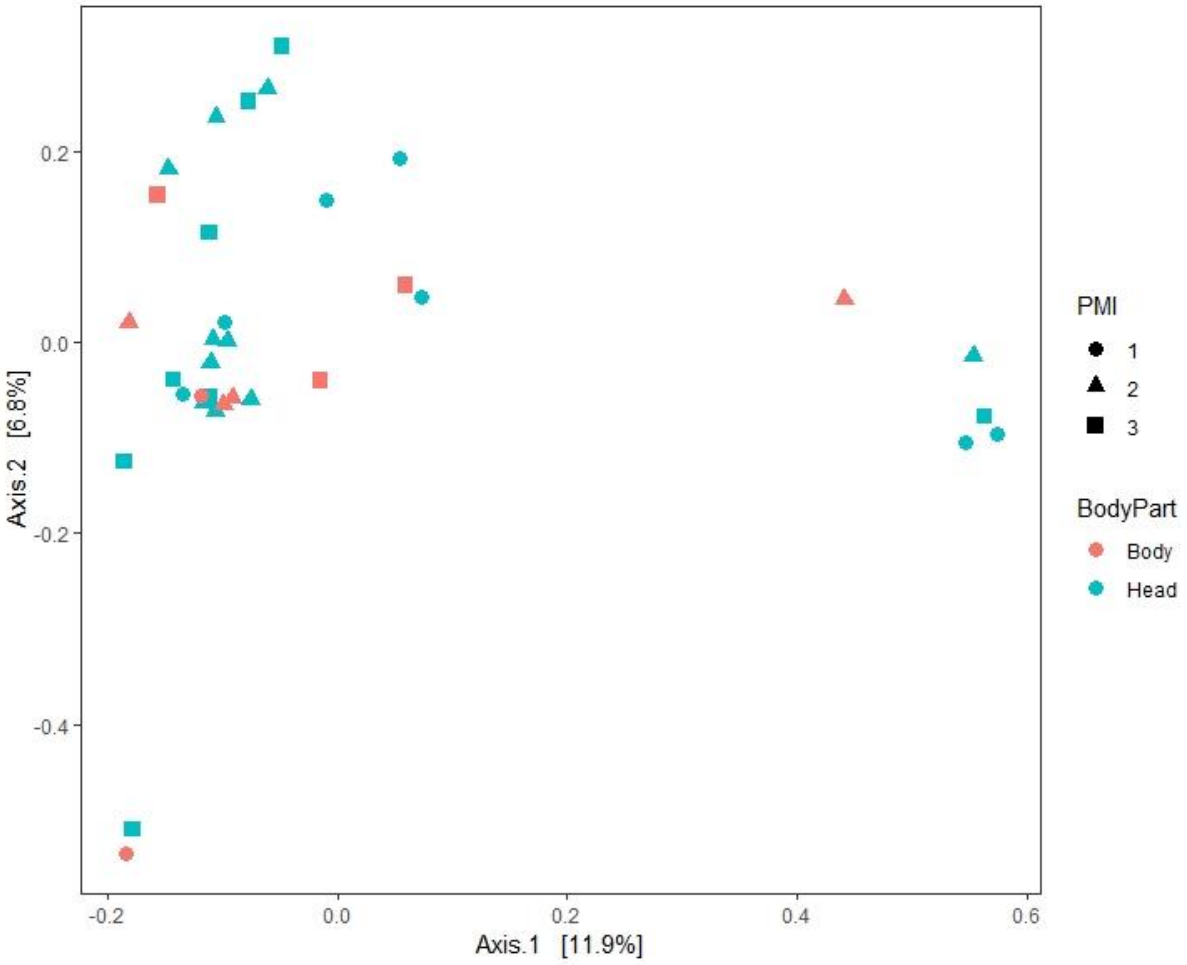


Figure 2.7 PCoA of sample area and PMI on an OTU level with the re-grouped sample areas and PMIs from Table 2.2

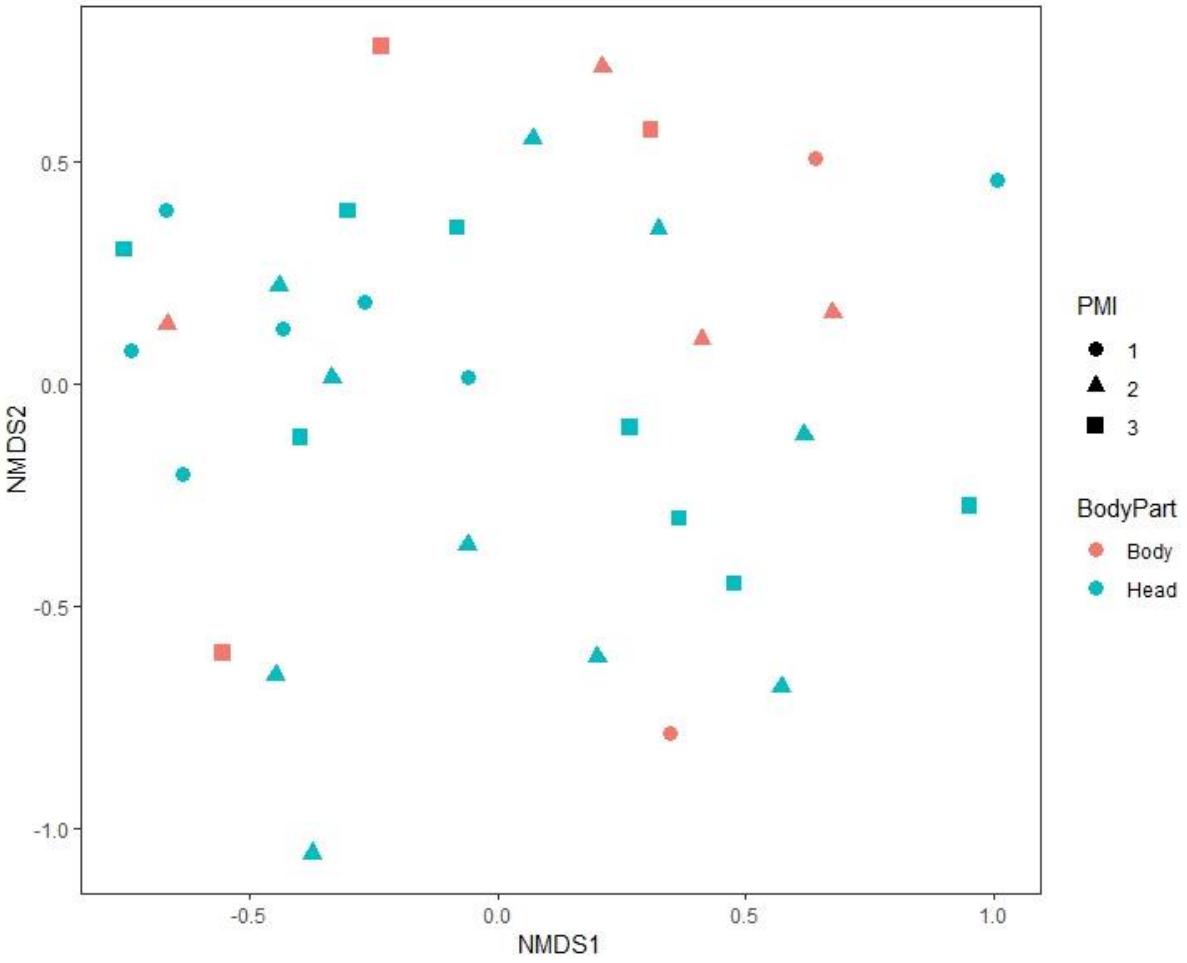


Figure 2.8 nMDS of sample area and PMI on an OTU level with the re-grouped sample areas and PMIs from Table 2.2

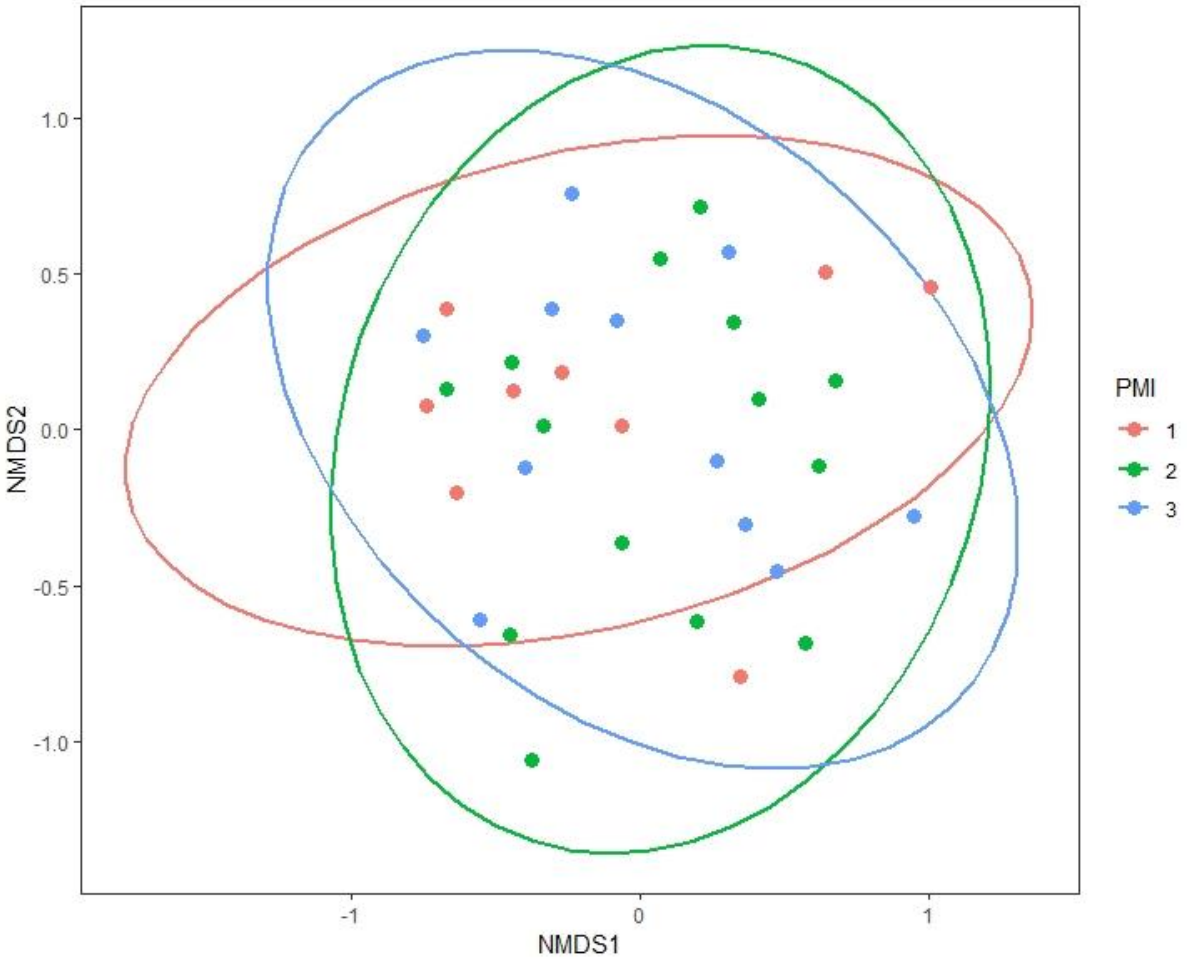


Figure 2.9 95% confidence interval ellipse based on nMDS of PMI on an OTU level with the re-grouped sample areas and PMIs from Table 2.2

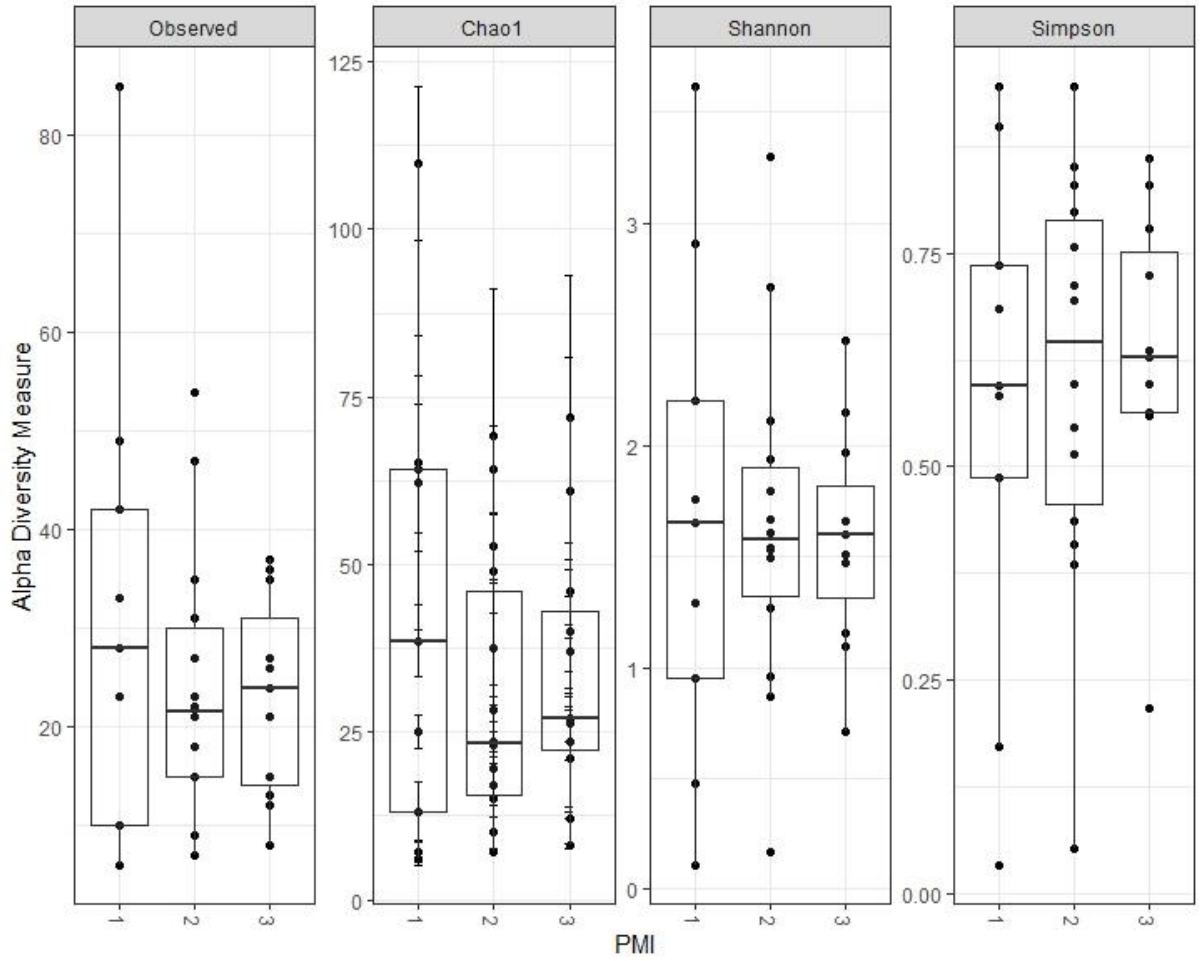


Figure 2.10 Whisker plots of alfa-diversity based on Chao1, Shannon and Simpson diversity indexes in OTU level classification in the 5 PMI stages

The same methods were repeated for genera present in the samples.

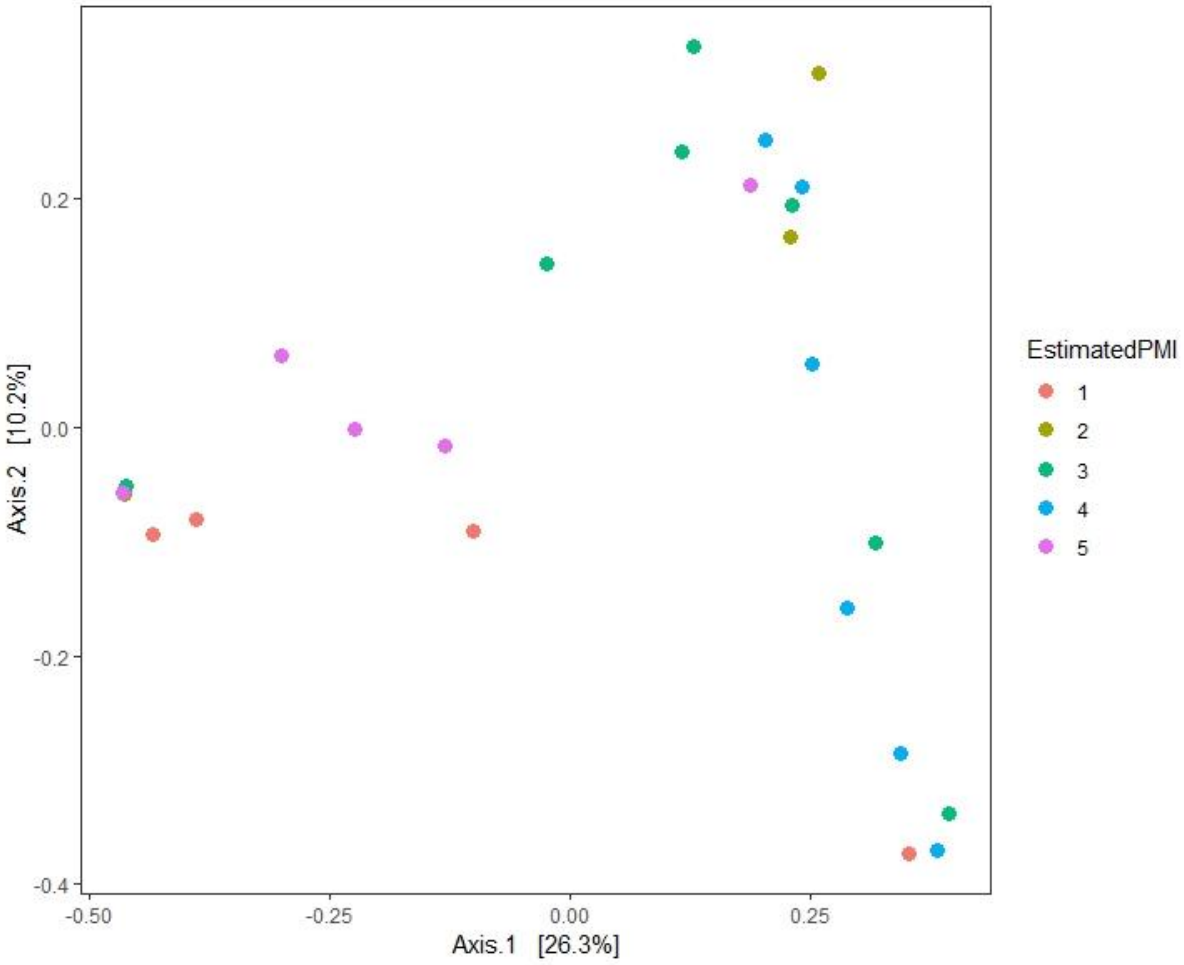


Figure 2.11 PCoA of sample area and PMI on genus level with the original PMIs from Table 2.2

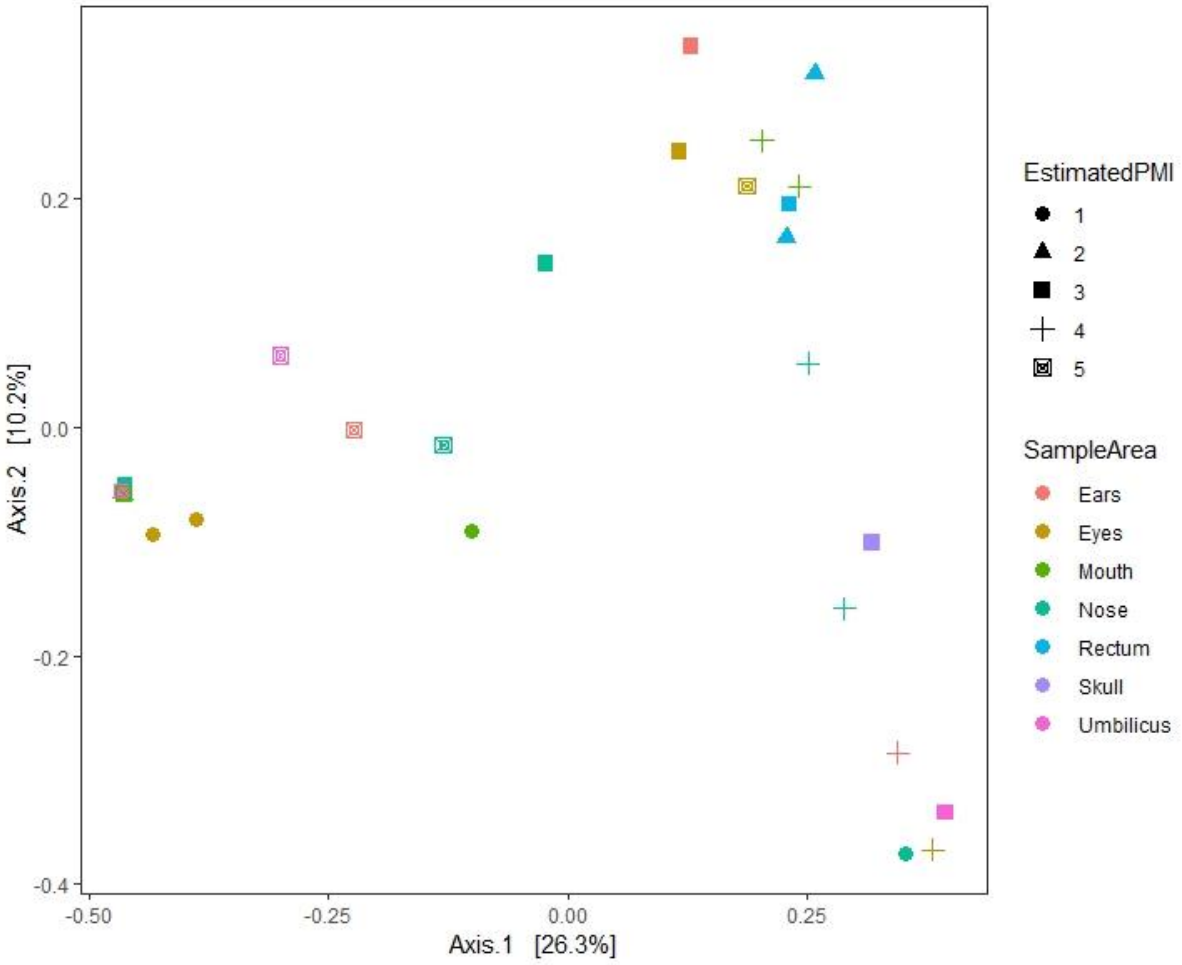


Figure 2.12 PCoA of sample area and PMI on genus level with the original sample areas and PMIs from Table 2.2

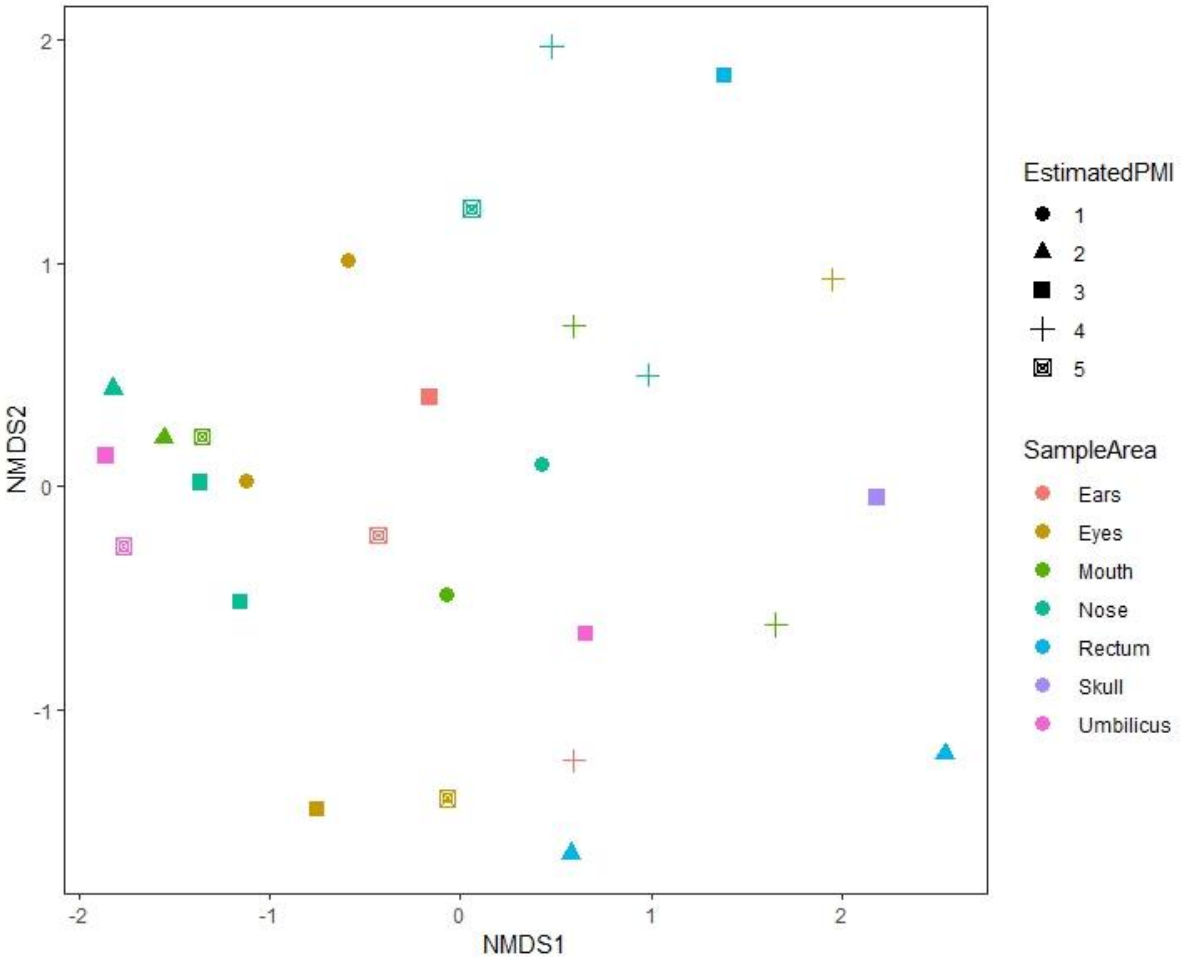


Figure 2.13 nMDS of sample area and PMI on genus level with the original sample areas and PMIs from Table 2.2

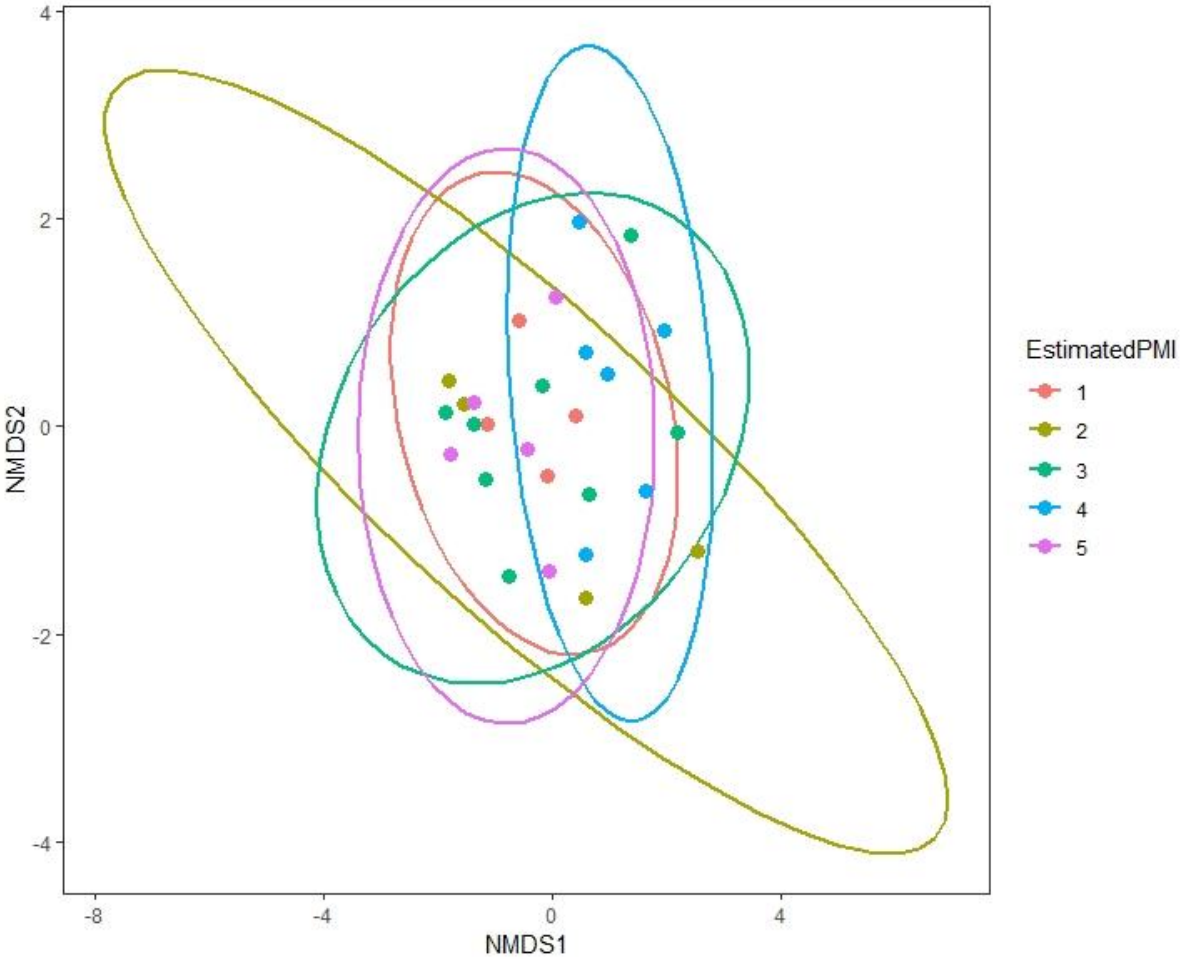


Figure 2.14 95% confidence interval ellipse based on nMDS of PMI on genus level with the original sample areas and PMIs from Table 2.2

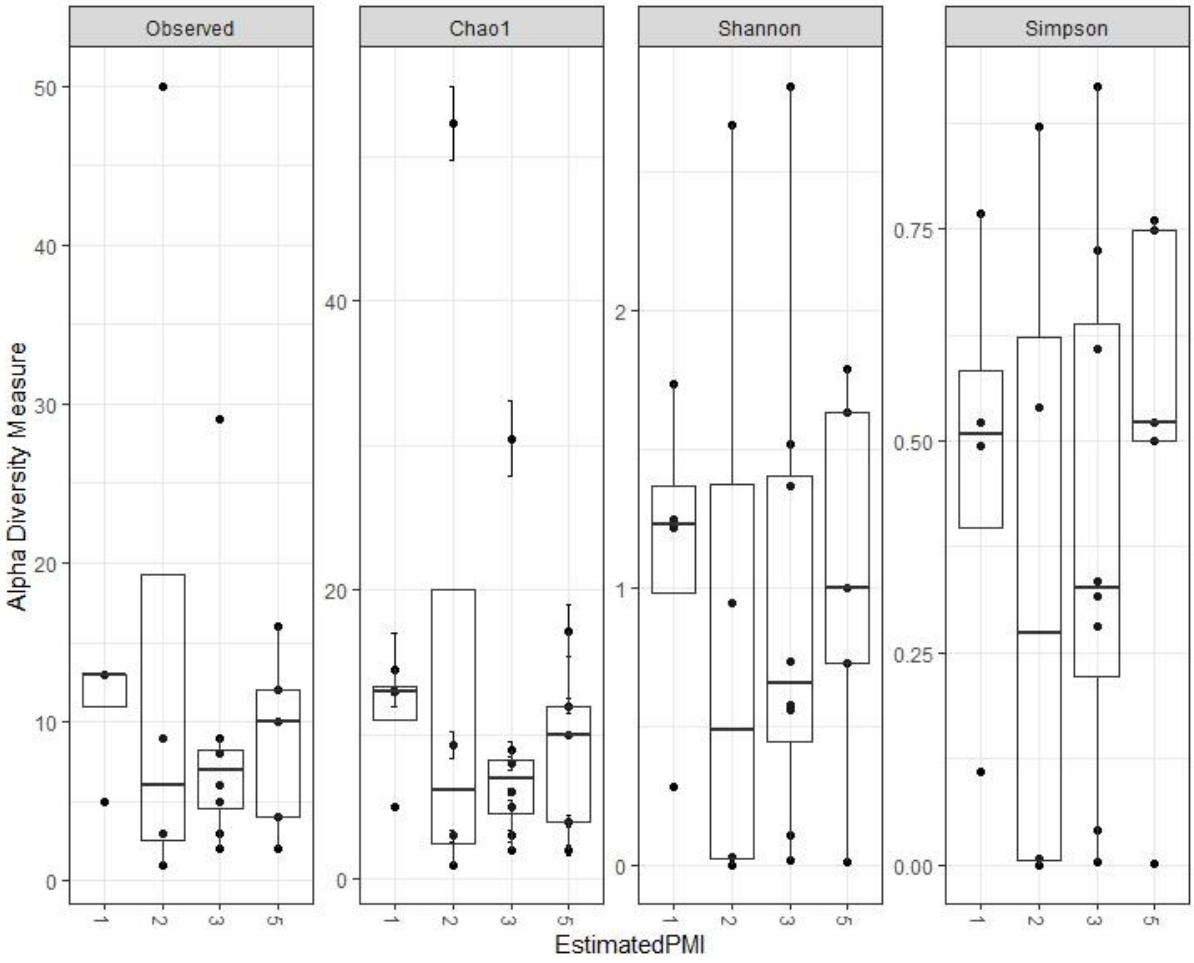


Figure 2.15 Whisker plots of alfa-diversity based on Chao1, Shannon and Simpson diversity indexes in genus level classification in the 5 PMI stages

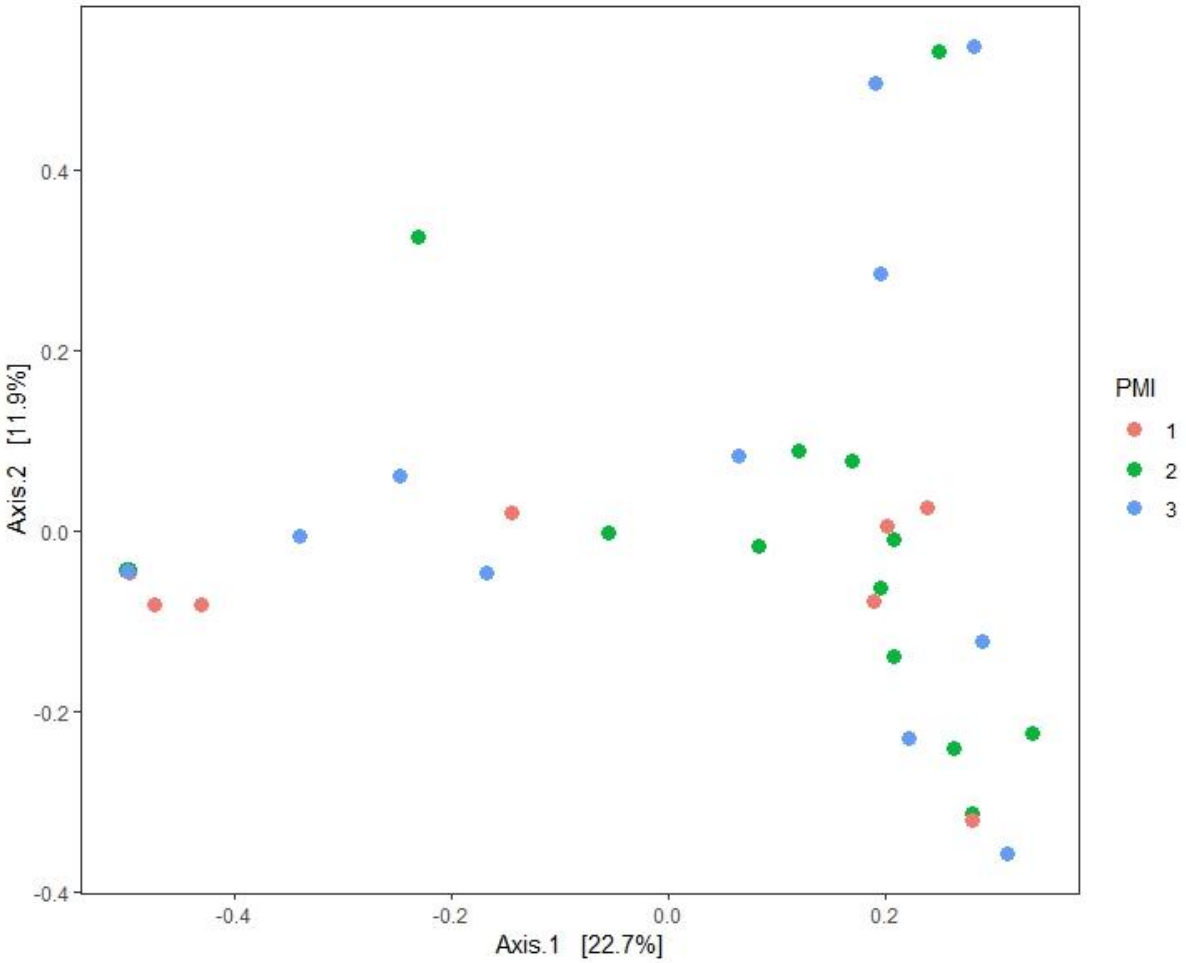


Figure 2.16 PCoA of sample area and PMI on genus level with the re-grouped PMIs from Table 2.2

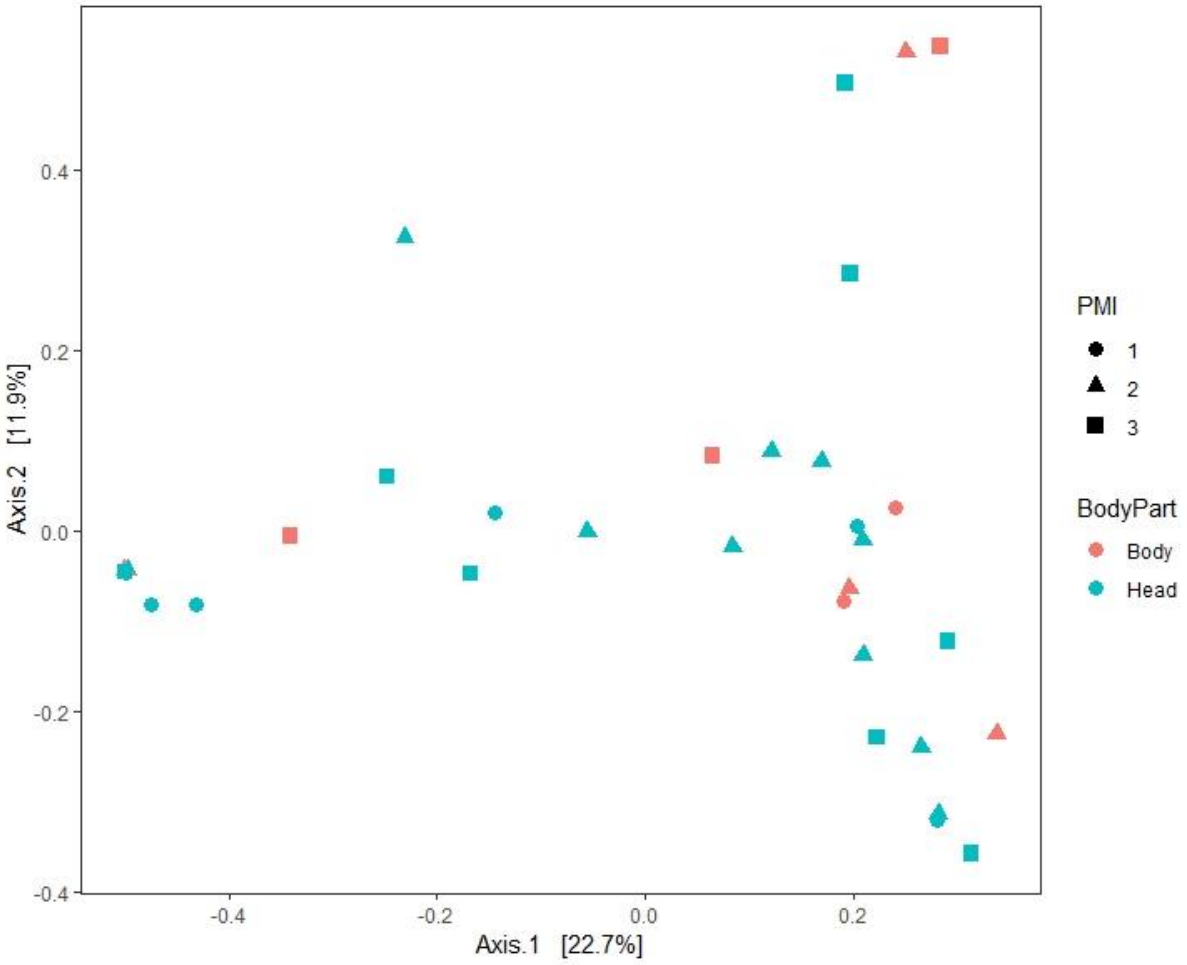


Figure 2.17 PCoA of sample area and PMI on genus level with the re-grouped sample areas and PMIs from Table 2.2

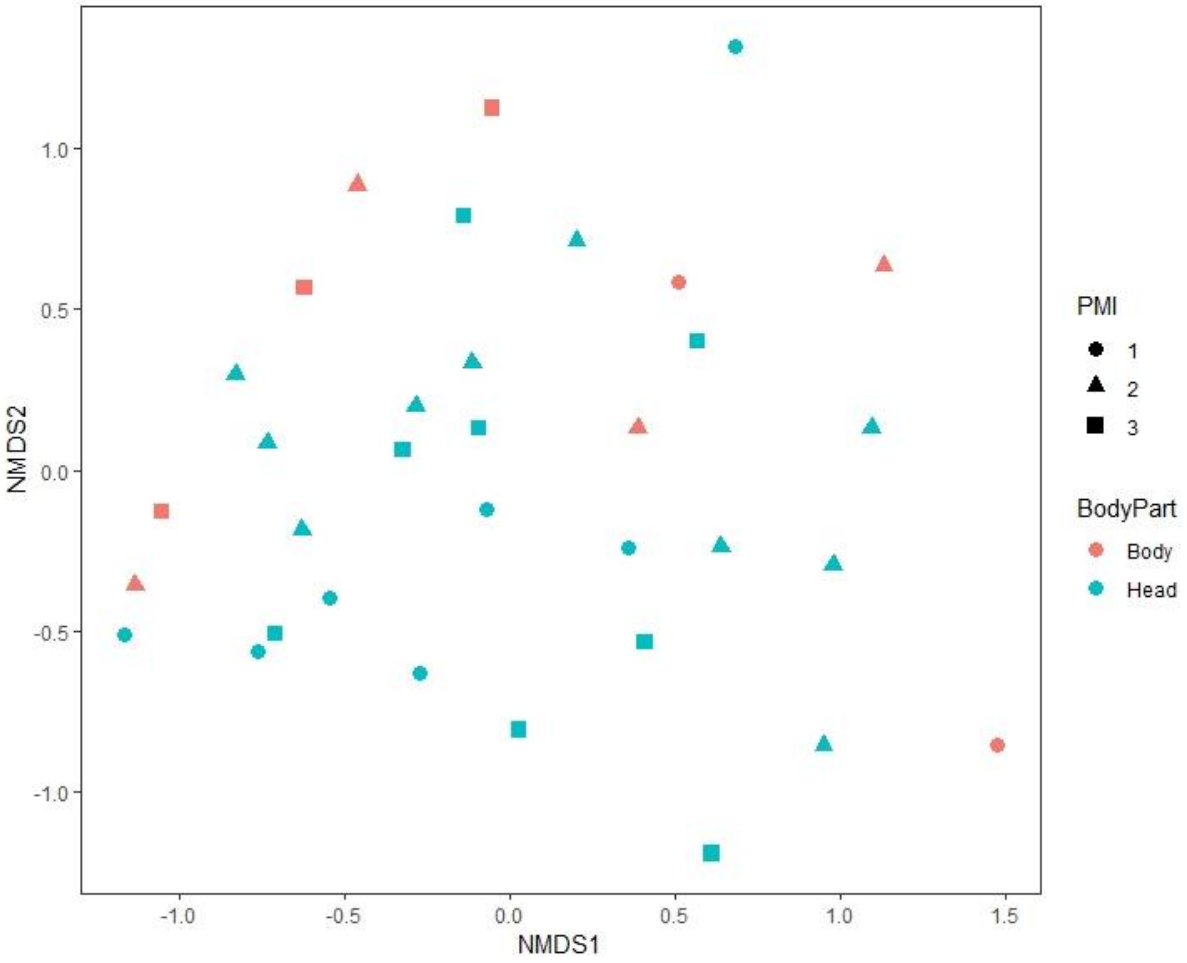


Figure 2.18 nMDS of sample area and PMI on genus level with the re-grouped sample areas and PMIs from Table 2.2

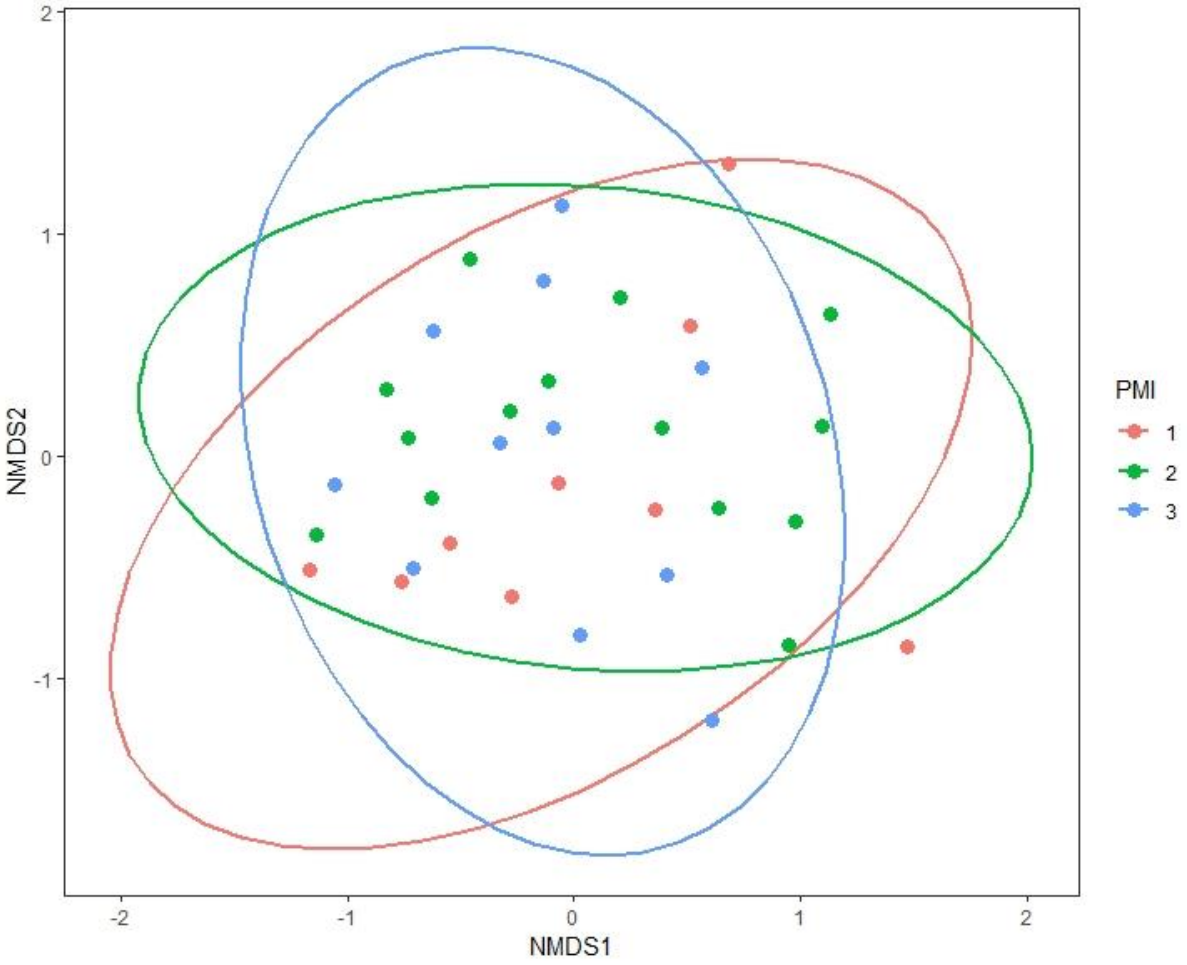


Figure 2.19 95% confidence interval ellipse based on nMDS of PMI on genus level with the re-grouped sample areas and PMIs from Table 2.2

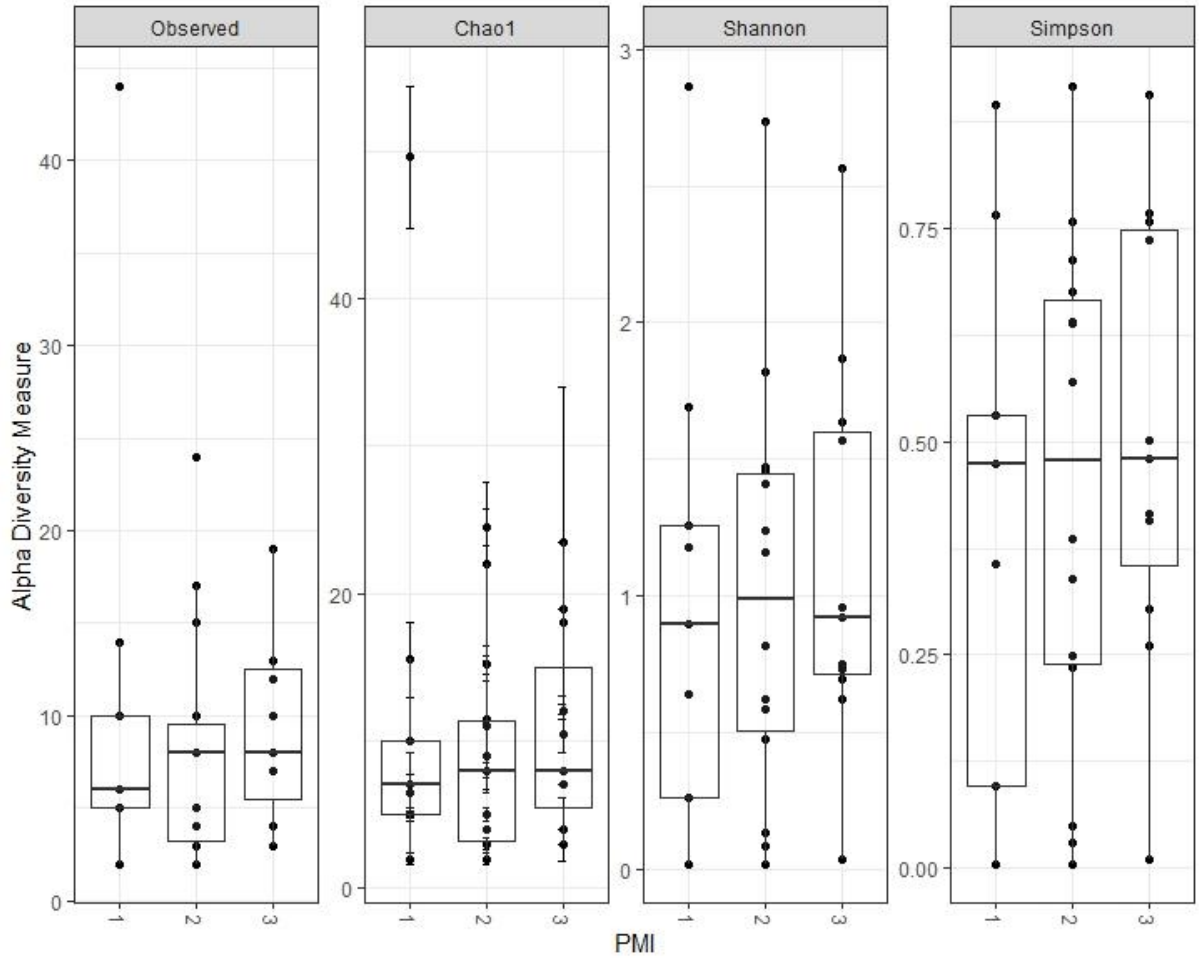


Figure 2.20 Whisker plots of alfa-diversity based on Chao1, Shannon and Simpson diversity indexes in genus level classification in the 3 PMI stages

After no significance was achieved, Venn diagrams were created to show OTUs and genera which is unique to a certain PMI from Table 2.1.

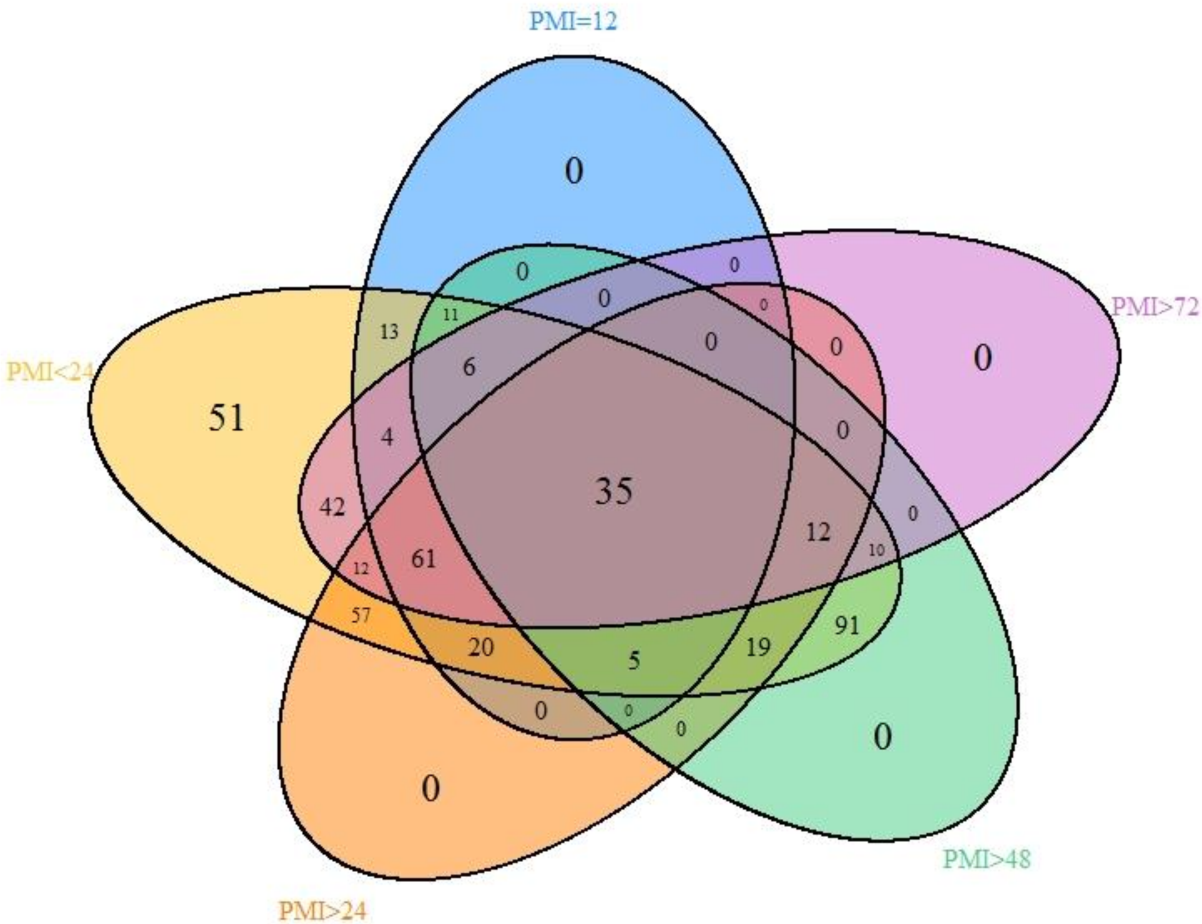


Figure 2.21 Venn Diagram showing the number of OTUs unique to a certain PMI or a certain set of PMIs based on the samples in Table 2.1

The list of OTUs for each section of the Venn Diagram is found in Appendix F.

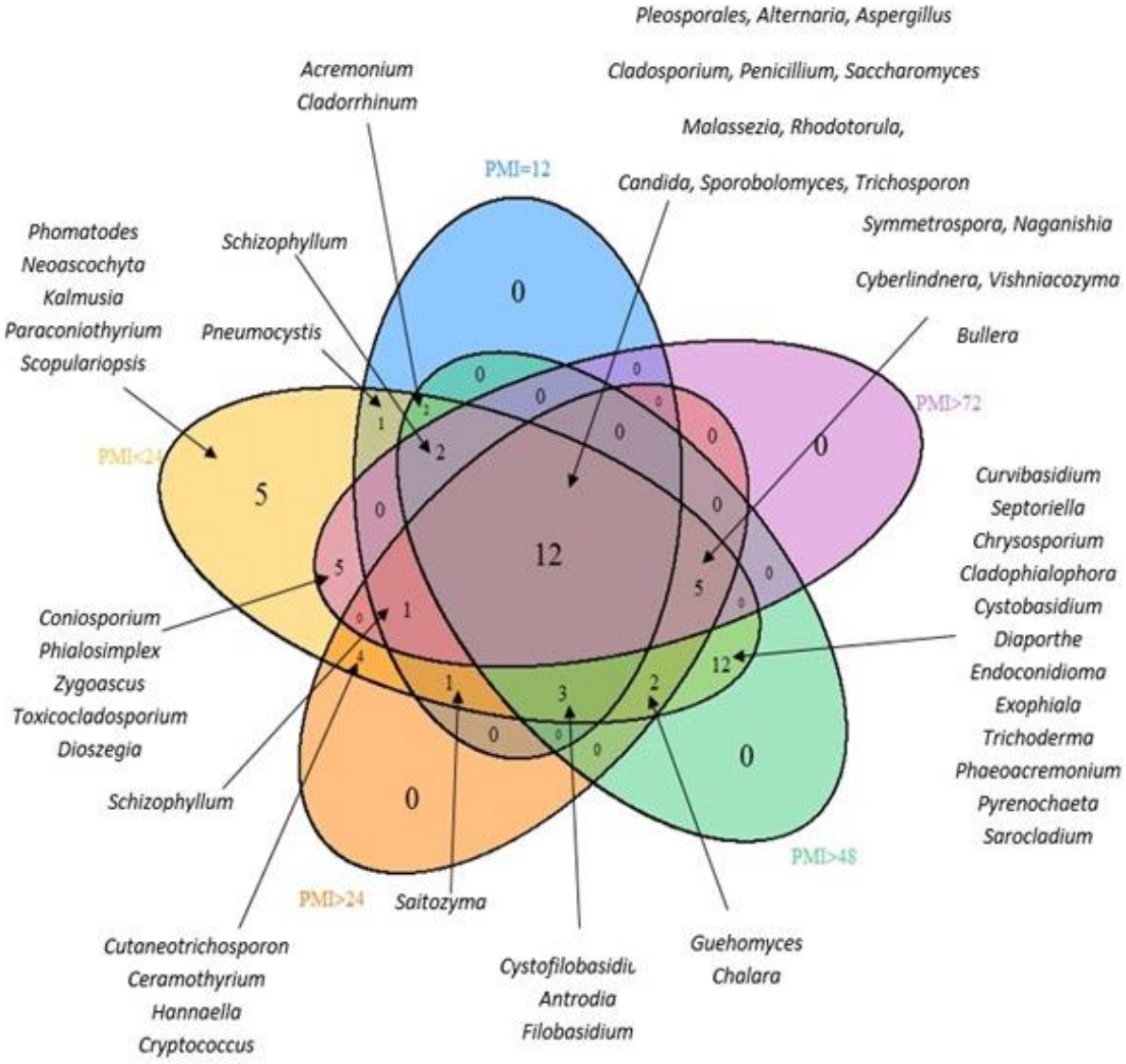


Figure 2.22 Venn Diagram showing the number and name of genera unique to a certain PMI or a certain set of PMIs based on the samples in Table 2.2

2.5 Discussion

2.5.1 Using fungi to establish PMI

Our results do not statistically support the presence of any PMI-specific fungal indicators based on PMI alone or PMI by sample area (Figures 2.1 & 2.2). The nMDS also confirms the non-grouping based on PMIs and sample area (Figure 2.3). Figure 2.4 shows ellipses of the 95%

confidence intervals based on nMDS. The distances between the samples in a given group is the largest for PM=less than 24 hrs. and the shortest distances were presented in samples with PMI=12 hrs. This means that the OTUs present in samples with PMI=12 hrs. are the most similar versus in the PMI=less than 24 hrs. where there is a large dissimilarity between the OTUs. The dissimilarity whisker plots (Figure 2.5) showing the differences between alpha-diversities also supports the data from Figure 2.4; however, one must keep it in mind that the samples by groups are not the same, hence it is a possible source for a bias. The same analysis was done after the PMIs and sample areas were re-grouped to see if any of the analysis show something more significant (Figure 2.6 to Figure 2.10), but the PCoA analysis for both PMI alone and PMI paired with sample area looked almost identical to the OTU level analysis. Differences can be seen with the distribution seen on the 95% confidence interval graph (Figure 2.9), showing that the distribution of OTUs are now more equally distributed than before. Figure 2.10 also supports the more equal distribution by looking at the similarity of the boxplots in any of the three dissimilarity index plots. After OTU level taxonomic data did not show any significant results, genus level taxonomic distribution was looked at for both the original (Table 2.1) and the re-grouped (Table 2.2) samples in the same manner as for OTUs. Establishing groups in PCoA and nMDS was still not possible based on PMI and PMI paired with sample areas for either the original and the re-grouped samples (Figure 2.11 to Figure 2.12 and Figure 2.16 to Figure 2.18) and distribution showed similar trend between the grouped samples (Figures 2.14, 2.15, 2.19 and 2.20). The statistical analyses have most likely failed to show significance due to the low sample sizes compared to the groups we have worked with.

Nonetheless, some patterns of fungal distribution by PMI can be observed (Figures 2.21 and 2.22). Based on our samples the most unique OTUs were present in the PMI=less than 24 hrs.

group with 51 unique OTUs in 5 genera namely, *Phomatodes*, *Neoascochyta*, *Kalmusia*, *Paraconiothyrium*, and *Scopulariopsis*. These genera can be opportunistic on humans; which means that they appear on an immunocompromised body which is a similar state as a body is in during the first 24 hrs. of decomposition, where the immune system is not responsive anymore, but the body temperature is still suitable for the fungi to grow. The other PMI intervals do not contain unique OTUs or genera, based on our study, which would not be present in other time intervals. Nineteen unique OTUs were identified in the time interval labeled as less than 24 hours and more than 48 hrs. Based on the data, these OTUs appear after the first 12 hours of decomposition and are not present later in decomposition. These OTUs can be further investigated to look for a smaller time interval of their presence on a body and with that smaller time interval they could be indicator species to determine a more refined range of PMIs than forensic entomology could provide. Also, there is not much similarity between the beginning of decomposition compared to the end. This supports the hypothesis that the fungal flora changes drastically over decomposition.

2.5.2 Checklist of human mycobiome during decomposition

449 OTUs were identified during this study which are a part of the human mycoflora during decomposition. There are 35 OTUs in 12 genera which are present in every time intervals set by the medical examiner, all of which are present as normal mycoflora of humans and have the possibility to become opportunistic pathogen; this is expected due to the loss of immune system as death occurs. These 35 OTUs would not be useful as indicators for decomposition due to their presence in all the time intervals, but they are a stable part of the human mycoflora as decomposition progresses. All the presented OTUs are a part of the decomposing mycoflora of the human body and they can serve as a checklist for further studies.

CHAPTER 3. ANNOTATED FUNGAL CHECKLIST OF SWINE CARCASSES AFTER FIVE MONTHS OF WINTER DECOMPOSITION IN WEST LAFAYETTE, INDIANA

3.1 Abstract

Studying decomposition on humans is very rare, only eight institutes – "Body Farms" – in the United Stated are allowed to conduct forensic decomposition experiments on human cadavers. Thus, swine carcasses are used by many scientists as models for human decomposition. With the advances in molecular methods, microbiology has become a crucial part of forensic research, but it tends to focus on bacteria, leaving a more diverse group of microorganisms – fungi – unrecorded. Many questions can be asked as a forensic investigator in relation to the mycobiome on a certain body. By looking at fungal communities, location of a crime and post-mortem interval (PMI) could be determined (Hawksworth, 2010). As fungi are understudied in terms of forensic relevance, a checklist is a way of gathering baseline information about what can be found on a carcass during decomposition. This provides information for future researchers on what they can expect to find during their study. In this chapter a checklist for decomposing swine carcasses in West Lafayette, IN. after five months of decomposition during the winter season is provided.

3.2 Introduction

The biodiversity of fungi is tremendous. According to Hawksworth & Luecking (2017), between 2.2 and 3.8 million species exist on earth but only eight percent of them are named at most. They can be found in any ecosystem; hence they are present during decomposition as well. The potential to use fungi in a forensic investigation is possible but is severely understudied. There is a need for more research which focuses on fungi in relation to decomposition to see if they can be used as a mean to determine post-mortem intervals (PMIs). In order to gain more knowledge,

it is useful to have a baseline of fungal communities that are present during decomposition. For this reason, checklists can be created. Checklists have been done by multiple researchers to look at the biodiversity of a certain region, such as it is described in Akulov et al. (2003). In forensics, multiple checklists have been created for entomologists for regions like Colorado (De Jeong, 1994) and Southeastern Brazil (Carvalho et al., 2000) to familiarize themselves with the possible biodiversity of insects in a given region.

The purpose of this study is to provide a checklist of fungal species on swine carcasses after five months of decomposition at a research site in West Lafayette, Indiana at the end of a winter season. This is the first checklist in Indiana for fungi isolated from decomposing swine carcasses. This checklist will be a useful source for future mycobiome studies in this region because it gives an insight on what kind of fungi can grow on the swine carcasses and highlight a few genera which could be used as indicators to determine PMI. Future researchers can build off this checklist to develop specialized collection and culturing techniques to maximize the effectiveness of their studies.

3.3 Material and methods

3.3.1 Sample collection

On March 21st, 2018, three pig (*Sus scrofa domesticus* L.) carcasses were sampled after they had been left to decompose over winter (October to March) at Purdue University's Forensic Entomology Research Compound in West Lafayette, IN (40° 25′36.0″ N, 86° 56′57.0″). This is an open field area surrounded by temperate forest. All three pigs were body swabbed by sterile wet and dry cotton swabs by a back and forth motion on the torso and limb area for approximately 30 seconds with each swab. Other visible fungal growths were also sampled by sterile dry and wet cotton swabs. These swabs samples were immediately transferred onto potato dextrose agar (PDA;

39 g/L, Livonia, Michigan) plates with chloramphenicol in the field then transferred to the laboratory.

3.3.2 Culturing

The streak plated samples on potato dextrose agar (PDA; 39 g/L, Livonia, Michigan) with chloramphenicol were grown at room temperature for minimum of 3 days and were re-evaluated for new growths at 5, 10, 15 and 30 days after field collection. Each morphologically different fungal species was subcultured from streak plates and grown in pure culture on PDA at room temperature. Pure cultures were maintained on slants at 4 °C for short-term storage and in 40% glycerol at -80 °C for long-term storage.

3.3.3 Molecular protocol

DNA was extracted from pure cultures grown on PDA. Colony PCR, a technique where fungal colonies are used in a PCR reaction without DNA extraction (Mirhendi, 2007), was done on yeasts by taking a small amount (a toothpick tip full) of a single colony and placing it into 200µl of nuclease free water. This dilution then served as the DNA template for the PRC reactions. For filamentous fungi, Wizard® Genomic DNA Purification kit (Promega Corp., Madison, Wisconsin) was used to extract DNA. To amplify the fungal internal transcribed spacer (ITS) region, primers ITS1F (Gardes and Bruns 1993) and ITS4 (White et al. 1990) were used. The protocol for the thermocycler was as follows: denaturation step at 95 °C for 5 min, followed by 35 cycles at 94 °C for 30 s, annealing T at 45 °C for 45 s, 72 °C for 45 s, and a final extension step at 72 °C for 7 min. The PCR products were electrophoresed in 1% agarose gel and all successful PCR products were sent to Beckman Coulter, Inc. (Indianapolis, IN., USA) for Sanger sequencing.

3.3.4 Sequencing analysis

Sequences were edited manually using Sequencher 5.2.3 (Gene Codes Corporation, Ann Arbor, Michigan, USA), then subjected to blast in the NCBI database to determine their closest relatives where 97% similarity was looked at when identified to a species level. Sequences with 94%-97% similarity were identified to genus level. Sequences with lower similarity were not included in the results.

3.4 Results

Seventeen swabs were made from three pigs. A total of fifty-four pure cultures were obtained, from which thirty-five cultures were identified to a species level, representing eleven species. Some species, such as *Rhodotorula mucilaginosa* and *Filobasidium magnum*, were abundant on all three pigs, while others, like *Mucor circinelloides* and *Vishniacozyma victoriae*, were rare. Yeasts were the most frequently cultured component of carcasses. Table 3.1 provides a list of all species recovered. For these species higher-level taxonomy is shown on Table 3.2 where the most abundant phylum is Basidiomycota. Also, some of the species from Table 1 belong to the same genus and found on the same pig. Nineteen cultures were only identified to a higher-level taxon – phylum or family – due to low similarity (less than 90% percent similarity) to an existing NCBI sequence and were removed from the data.

Table 3.1 Species found through culturing based on ITS region and at least 94% similarity to an NCBI record

Closest Match	Number of Cultures isolated	Hypothetical Role in Decomposition	NCBI Accession Number	ITS % Identity	Author of Sequence	Pig number
Cladosporium herbarum	2	Common mold found on various decomposing matters	MH863124.1	99.81	(Vu,D. et al., 2019)	1,2
Didymella keratinophila	1	Was previously recovered from a human superficial tissue specimen in the USA	NR_158275.1	99.8	(Valenzuela-Lopez,N.M.A. et al., 2018)	2
Epicoccum poea	4	Associated with Poa Annua grass, which is common in temperate climates	NR_158266.1	100	(Chen,Q. et al., 2017)	2,3
Filobasidium magnum	8	Colonizes fruits such as apples and pears, decomposer	KY103433.1	99.83-99.84	(Vu,D. et al., 2016)	1,2,3

Table 3.1 continued

Fusicolla septimanifiniscientiae	3	Found on decomposing plant matter, found in soil	MK069422.1	97.83-98.13	(Lombard,L., unpublished)	1,3
***Fulvifomes* sp.**	1	Fungi common to forests ecosystems	NR_154003.1	94.00	(Zhou,L.-W., 2015)	1
Leucosporidium golubevii	2	Common soil yeast	KY104019.1	99.47	(Vu,D. et al., 2016)	3
Mucor circinelloides	1	It can be found in soil and dung, some ff. ssp. can be pathogenic	NR_126116.1	99.68	(Schwarz,P. et al., 2006)	1
Rhodotorula dairenensis	1	Yeasts which are transient colonizers of wet skin, important for decomposition	AF444501.1	99.83	(Scorzetti,G., Fell,J.W., Fonseca,A. & Statzell-Tallman,A., 2002)	1
Rhodotorula mucilaginosa	11	Yeasts which are transient colonizers of wet skin, important for decomposition	KY104874.1	99.32-100	(Vu,D. et al., 2016)	1,2,3

Table 3.1 continued

Vishniacozyma tephrensis	1	Common in temperate forest's litter and soil communities	NR_144812.1	97.79	(Robert,V., Epping,W. and Boekhout,T., unpublished)	1
Vishniacozyma victoriae	1	Common in temperate forest's litter and soil communities	MH809977.1	99.4	(Rush,R.E., Lemons,A.R. and Green,B.J., unpublished)	3

Table 3.2 Higher level taxonomic organization of sequenced cultures

Phylum	Family	Genus	Number of Cultures Isolated	Pig
Ascomycota	Cladosporiaceae	*Cladosporium*	2	1,2
	Nectriaceae	*Fusicolla*	3	1,3
	Pleosporineae	*Didymella*	1	2
		Epicoccum	4	2,3
Basidiomycota	Bulleribasidiaceae	*Vishniacozyma*	2	1,3
	Filobasidiaceae	*Filobasidium*	8	1,2,3
	Hymenochaetaceae	*Fulvifomes*	1	1
	Leucosporidiaceae	*Leucosporidium*	2	3
	Sporidiobolaceae	*Rhodotorula*	11	1,2,3
Zygomycota	Mucoraceae	*Mucor*	1	1

3.5 Discussion

Thirty-five total cultures were identified to genus or species level from three swine carcasses. There are fungi representing the three different phyla: Ascomycota. Basidiomycota and Zygomycota. Most of the samples were in the Basidiomycota with 24 cultures. Yeasts and filamentous fungi were also found in these samples, but yeasts were more abundant compared to filamentous growths. Based on these data the most abundant fungal genera recovered were *Rhodotorula, Filobasidium, Epicoccum*, representing 31.24%, 22.86%, 11.43% of the total samples, respectively (Table 3.2). Based on Table 1, *Rhodotorula mucilaginosa* was the most abundant species with 11 cultures from all three pigs (Table 3.1).

These genera are all in the phylum of Basidiomycota which includes both yeasts and filamentous fungi. The genus *Rhodotorula,* which was the most abundant from the collected cultures, contains yeasts, which can be found during food spoilage (Deak, 2007; Stratford, 2006; Tournas, 2006). More importantly for our study, it was already recorded during human decomposition in several instances by medical examiners (Dosa, 1955; Lopez-Martinez et al., 2007); it is also found to be common on wet skin surfaces (Cooper, 2010). This genus has the potential to be further studied to use as a PMI indicator, due to its presence during decomposition on every pig we swabbed.

The second most common genus cultured was *Filobasidium*, another basidiomycete yeast genus. *Filobasidium* species are often found in soils (Botha, 2006). Most of the research has looked at its decomposing properties in relation to plant matter so far (Botha, 2006, and Sampaio 2006), but Li et al. (2018) has shown this genus in animal gut flora as well. *Filobasidium* has the potential to be another genus which could lead to PMI determination due to its presence on all the pigs we have swabbed.

Epicoccum was also recovered from two of the three pigs. This genus had appeared in a human oral mycobiome study (Dupuy et al., 2014). It is commonly found as an air pollutant in temperate regions and can cause allergies (Dunpuy, 2014). Even though this genus has not been associated with decomposition, it can be transmitted to decomposing bodies through air and is also a common soil fungus.

All the above-mentioned genera are a crucial part of the environment in which the carcasses were placed in. Some of them are involved in decomposition and lipid synthesis and are adapted to colder temperatures. However, from all the genera found, *Filobasidium* and *Rhodotorula* was found on all three pigs, which could indicate that they are a normal flora, or they can appear at a certain time during decomposition, hence they could be forensically important genera to further study.

CHAPTER 4. ANNOTATED FUNGAL CHECKLIST OF SEVERAL WILDLIFE SPECIES DURING DECOMPOSITION IN A TEMPERATE FOREST ECOSYSTEM DURING THE SUMMER IN INDIANA

4.1 Abstract

Wildlife forensics is a growing field that has become more important during the last several decades. The illegal trade of animals is growing every year and is worth over $20 billion US dollars every year (Alacs, 2010). There are methods in place, such as isotope testing and microsatellite studies, to determine the origin and movement of animals (Bowen, 2005, Maudet, 2002), but there are other methods that might be faster and less expensive, but are yet unexplored. A few microbiology studies have been done in human forensics (Pechal, 2014), but the methods have not been explored in wildlife forensics. This preliminary study was designed to see if there are any signature fungal species present on specific wildlife species that could help in identification during a forensic investigation. Moreover, this chapter includes a checklist based on culturing methods of fungal species found on animal carcasses in a temperate forest ecosystem in Indiana. This checklist can be used for further studies as a baseline data to develop specific collection and culturing methods for wildlife forensic research. In this study we have seen multiple species that have appeared later in decomposition such as *Trichoderma* sp. which could be used as an indicator for a late stage of decomposition on certain animal species.

4.2 Introduction

The need for new methods in wildlife forensics is growing every year due to the increase in illegal trafficking, but resources to control it are very limited (Brack, 2004; Wilson-Wilde, 2010). Due to the lack of funding, cost effective methods to collect and analyze samples are the best approach. Current techniques, such as microsatellite analysis, are an effective way to determine

origin of animals (Maudet, 2002), but studies focusing on determining post-mortem intervals are sparse and tend to focus on entomology, such as the studies done by Wilson et al. (2014) and by Rolo et al. (2013), leaving the microbes unstudied.

Microbes are found everywhere in nature; they are microscopic organisms and can be a part of natural flora, a pathogen, or just a trace of some previous contact. Fungi belong to this group of organisms and are abundant on and inside a body. There are fungi that live in animals as a part of a normal mycoflora. They can also be introduced via food source, such as *Fusarium*, a common genus of plant pathogenic fungi. Moreover, they can be mutualistic, parasitic or opportunistic.

This study provides a checklist of fungal species found on wildlife carcasses which can be used as a baseline for future studies in wildlife forensic. We have looked at mycoflora changes during decomposition to determine fungal species which could indicate PMI. Also, we compared the fungal community composition of four different wildlife species from both terrestrial and aquatic environments to see if there is any fungal indicator species for certain wildlife species which could help with identifying wildlife remains after advanced decomposition.

4.3 Materials and methods

4.3.1 Sample Collection

Four wildlife species were used during this study; red-tailed hawk (*Buteo jamaicensis*), mute swan (*Cygnus olor*), North American river otter (*Lontra canadensis*), and bobcat (*Lynx rufus*). The animals were obtained from officers in the Indiana Department of Natural Resources. After the animals were neutralized, they were placed in a - 20°C freezer and were left frozen until they were placed onto the study site. Each species had 3 replicates, except bobcat where n=1. They were placed in the temperate deciduous forest area Cox-Haggerty, property of Purdue's Forestry

and Natural Resources Department, at least 50m apart with the same body orientation (head pointed to cardinal North direction). Each animal was placed in a wire-mesh cage to prevent possible predation. They were sampled twice; first when they were place out in the study site and when skeletonization stage occurred (which in this study is defined as the time when no more insect activity is present on the body for two consecutive days). The bodies were monitored daily. During both sampling periods, sterile swabbing was done with one wet and one dry cotton swabs by a back and forth motion on the entire body for 30 seconds per swab, making sure that the swabs have touched the inside of the mouth/beak, upper and lower area of the torso and the limbs of the animals. Furthermore, any visible fungal growth was sampled by a dry and a wet sterile swab. These swab samples were immediately transferred onto potato dextrose agar (PDA; 39 g/L, Livonia, Michigan) plates with chloramphenicol in the field.

4.3.2 Culturing

Samples streak plated on potato dextrose agar (PDA; 39 g/L, Livonia, Michigan) with chloramphenicol were grown for minimum of 3 days and were re-evaluated for new growth at 5, 10, 15 and 30 days after field collection. Each morphologically different fungal species was isolated and grown in pure culture on PDA at room temperature. Pure cultures were maintained on slants at 4 °C for short-term storage and in 40% glycerol at -80 °C for long-term storage.

4.3.3 Molecular protocol

DNA was extracted from pure cultures grown on PDA. Colony PCR was done on yeasts; for filamentous fungi, Wizard® Genomic DNA Purification kit (Promega Corp., Madison, Wisconsin) was used to extract DNA. To amplify fungal internal transcribed spacer (ITS) region primers ITS1F (Gardes and Bruns 1993) and ITS4 (White et al. 1990) were used. The protocol for the thermocycler was as follows: denaturation step at 95 °C for 5 min, followed by 35 cycles at

94 °C for 30 s, annealing T at 45 °C for 45 s, 72 °C for 45 s, and a final extension step at 72 °C for 7 min. PCR products were electrophoresed in 1% agarose gel and all successful PCR products were sent to Beckman Coulter, Inc. (Indianapolis, IN., USA) for Sanger sequencing.

4.3.4 Sequence analysis

Sequences were edited manually using Sequencher 5.2.3 (Gene Codes Corporation, Ann Arbor, Michigan, USA), then subjected to blast on GenBank to determine their closest relatives.

4.4 Results

A total of eighteen swabs were taken. Six swabs at the time the animals were placed out, six at skeletonization stage and six extra swabs of visible fungal growth on animals at skeletonization stage during the study. A total of eighty-five pure cultures were obtained in total. From these seventy-two cultures were identified to genus or species level using ITS barcodes and using cutoffs of 97% of similarity to an existing NCBI entry for species level and 94% of similarity for genus identification.

Table 4.2 shows that from the 72 cultures identified, the majority were in the phylum of Ascomycota followed by Basidiomycota and lastly Zygomycota with 52.78%, 30.56% and 16.67% respectively.

Table 4.1 Species found through culturing based on ITS region and at least 94% similarity to an NCBI record

Closest Match	Number of Cultures isolated	Hypothetical role in decomposition	NCBI Accession Number	ITS % Identity	Author of sequence
Apiotrichum laibachii	2	Soil associated yeast	KY101676.1	100	(Vu,D. et al., 2016)
Aureobasidium pullulans	3	"black yeast" has a wide range of habitats, can be found in soil and as a pathogen of plants and humans as well	KT693733.1	99.81-100	(van Nieuwenhuijzen,E.J. et al., 2016)
Cladosporium herbarum	4	Common mold found on various decomposing matters	MH863124.1	100	(Vu,D. et al., 2019)
Cladosporium phaenocomae	2	Found in soil and is helpful with decomposing organic matters	MH865096.1	100	(Vu,D. et al., 2019)
Cystofilobasidium infirmominiatum	3	Yeast which can be found in aquatic environment	NR_073232.1	100	(Schoch,C.L. et al., 2014)

Table 4.1 continued

Didymella keratinophila	4	Was previously recovered from a human superficial tissue specimen in the USA	NR_158275.1	99.80	(Valenzuela-Lopez,N.M.A. et al., 2018)
Filobasidium magnum	7	Common decomposer	KY103433.1	96.33-96.49	(Vu,D. et al., 2016)
***Fusarium* sp.**	2	Plant pathogenic fungi	MH668840.1 MH862691.1	95.94 94.59	(Santos,A.C. et al., 2019) (Vu,D. et al., unpublished)
Gnomoniopsis paraclavulata	1	Plant pathogen	EU254834.1	100	(Sogonov,M.V. et al., unpublished)
Lachancea kluyveri	3	Yeast associated found in soil, and flies mostly	NR_138159	99.66	(Oda,Y. & Fujisawa,T., 2001)
Mortierella rishikesha	1	Soil fungi	NR_111564.1	97.64	(Schoch,C.L. et al., 2014)
Mucor circinelloides	1	It can be found in soil and dung, some ff. ssp. can be pathogenic	NR_126116.1	99.67	(Schwarz,P. et al., 2006)
***Mucor* sp.**	5	Soil and dung fungi	NR_152948	94.68	(Hoffmann,K. et al., 2009)

Table 4.1 continued

Rhodotorula dairenensis	5	Yeasts which are transient colonizers of wet skin, important for decomposition	AF444501.1	99.87-100	(Scorzetti,G., Fell,J.W. & Fonseca,A., 2002)
Rhodotorula mucilaginosa	5	Yeasts which are transient colonizers of wet skin, important for decomposition	KY104874.1	99.66-100	(Vu,D. et al., 2016)
Trichoderma atroviride	8	Filamentous cosmopolitan fungus commonly found in soil	AF456917.1	99.83-100	(Dodd,S.L., Lieckfeldt,E. & Samuels,G.J., 2003)
Trichoderma simmonsii	8	Filamentous cosmopolitan fungus commonly found in soil	NR_137297.1	99.83-100	(Robbertse,B. et al., 2017)
***Umbelopsis* sp.**	5	Prefers lipid rich environment	MH859921.1 KC489501.1	94.82 95.42	(Vu,D. et al., unpublished) (Wang,Y.-N., Liu,X.-Y. & Zheng,R.-Y., 2014)

Table 4.1 continued

Yarrowia lipolytica	3	This species of fungi can use hydrocarbons live off of hydrocarbons, which can be found in a decomposing remains	AF218983	97.44	(Chen,Y.C. et al., 2000)

Table 4.2 Higher level taxonomy for cultures isolated

Phylum	**Family**	**Genus**	**Number of Cultures Isolated**
Ascomycota	Cladosporiaceae	*Cladosporium*	6
	Didymellaceae	*Didymella*	4
	Dipodascaceae	*Yarrowia*	3
	Gnomoniaceae	*Gnomoniopsis*	1
	Hypocreaceae	*Trichoderma*	16
	Nectriaceae	*Fusarium*	2
	Saccharomycetaceae	*Lachancea*	3
	Saccotheciaceae	*Aureobasidium*	3

Table 4.2 continued

Basidiomycota	Cystofilobasidiaceae	*Cystofilobasidium*	3
	Filobasidiaceae	*Filobasidium*	7
	Sporidiobolaceae	*Rhodotorula*	10
	Trichosporonaceae	*Apiotrichum*	2
Zygomycota	Mortierellaceae	*Mortierella*	1
	Mucoraceae	*Mucor*	6
	Umbelopsidaceae	*Umbelopsis*	5

Table 4.3 Species list of cultures collected during mute swan decomposition

Fresh Stage	Skeleton Stage
Filobasidium magnum	*Alternaria alstroemeriae*
Marasmius sp.*	*Apiotrichum laibachii*
Mortierella rishikesha	*Aureobasidium pullulans*
	Cladosporium herbarum
	Cladosporium phaenocomae
	Didymella keratinophila
	Fusarium sp.
	Lachancea kluyveri
	Marasmius sp.*
	Mucor circinelloides
	Rhodotorula dairenensis
	Rhodotorula mucilaginosa
	Russula sp.
	Trichoderma atroviride
	Trichoderma simmonsii
	Umbelopsis sp.

Fresh stage reflects the time the carcasses (n=3) were placed on the research site and skeleton stage was determined when no more insect activity was present on the carcasses for 2 consecutive days. Species labeled with * are present during both stages.

Table 4.4 Species list of cultures collected during red-tailed hawk decomposition

Fresh Stage	Skeleton Stage
Aureobasidium pullulans	*Cladosporium herbarum*
Filobasidium magnum	*Didymella keratinophila*
Pseudopithomyces palmicola	*Fusarium* sp.
	Gnomoniopsis paraclavulata
	Rhodotorula dairenensis
	Rhodotorula mucilaginosa
	Trichoderma atroviride
	Trichoderma simmonsii
	Umbelopsis sp.

Fresh stage reflects the time the carcasses (n=3) were placed on the research site and skeleton stage was determined when no more insect activity was present on the carcasses for 2 consecutive days.

Table 4.5 Species list of cultures collected during fresh stage of North American river otter and bobcat decomposition

Otter	Bobcat
Cystofilobasidium infirmominiatum	*Mucor* sp.
*Trichoderma atroviride**	*Trichoderma atroviride**
*Trichoderma simmonsii**	*Trichoderma simmonsii**
Yarrowia lipolytica	

Carcasses (n=3, n=1 for otter and bobcat respectively) were sampled during the initial collection they were placed onto the research site. The species with * indicates fungal species which are present on both animal species.

4.5 Discussion

When we look at Table 4.2, where the species found are listed with their corresponding percent identity to a NCBI database sequence, there are several species which stand out as cultures found more often on a wildlife species than others. Two frequently found species were *Trichoderma atroviride* and *T. simmonsii* (Table 4.1). Both are reported decomposers, found in the soil and prefer lipid rich environments (Serrano-Carreon, 1992), which is present during decomposition as animal bodies are an abundant source of lipids. They have also appeared on the other two species with only initial swab data (Table 4.5). *Filobasidium* sp. which is another common decomposer found in soil (Botha, 2006), and was found during the initial swabbing of the carcasses (Table 4.3 and 4.4). Moreover, *Rhodotorula dairenensis* and *R. mucilaginosa* were both present on the animals and are yeasts which were observed on skin and showed to be important decomposers (Percival, 2011) and were previously observed in other animal carcasses (Dillon, 1991). These two species of *Rhodotorula* were present during skeleton stages on both mute swan and red-tailed hawk carcasses (Table 4.3 and 4.4). This indicates that *Rhodotorula* has the possibility to become important for determining PMI at a later stage of decomposition due to its absence in the beginning of decomposition, and its presence during the skeletonization stage. Also, *Rhodotorula* ssp.were found on the avian bodies but not on the mammals which indicates that there are fungal species which prefer certain wildlife species over another. Another genus

which was shown to be present later during decomposition, on both wildlife species, was *Umbelopsis*, a group of soil fungi which prefers the lipid rich environment as well (Meeuwse, 2011). The genus *Cladosporium* was also found on multiple samples, and is yet another soil fungus (Bensch, 2012), which is present during the late stages of decomposition and could be used as an indicator species in the future by forensic scientists. This is a cosmopolitan genus and can have a pathogenic effect on animals (Bensch, 2012).

Furthermore, a few species unique to specific environment were found on the carcasses. From those *Lachancea kluyveri* was found on one of the mute swan carcasses. This species of fungi is associated with flies, which are present on bodies during decomposition (Lachance, 1995). This shows that Locard's exchange principle is in motion when one is looking at fungi as well, as a fly interacts with the decomposing body, it leaves its trace, in this case a fungus, which is found on the insect's body. Knowing that flies can transfer fungal traces and other fungal material to carcasses, the specific location of the carcass could be determined after further studies. *Cystofilobasidium infirmominiatum*, a fungus from aquatic environments (Libkind, 2003; Libkind, 2009), was also present on the North American river otter bodies. This shows that there is a possibility for forensic investigators to determine a carcass' living habitat based on the fungal flora present. *Yarrowia lipolytica*, a fungus capable of using lipids and protein containing substances as food source (Barth, 1996) was found as well and has the potential to be used an indicator for decomposition.

Comparing the stages of decomposition for mute swans and red-tailed hawks (Tables 4.3 and 4.4) show that more fungal species can be found in a skeleton stage in comparison to the fresh stage. Moreover, by looking at the fungal communities found on the fresh and skeleton stages, one

can see that the communities drastically change over time, leaving only one genus (*Mucor*) which was present during both fresh and skeleton stages on the mute swan remains (Table 4.3).

This preliminary study resulted in some important information for future forensic mycologists. It provides a checklist of fungi which could potentially be found on the carcasses. Also, this study lists some genera (*Filobasidium*, *Rhodotorula*, *Trichoderma* and *Umbelopsis*,) which can be important decomposers of wildlife. It also highlights some of the interesting findings based on living habitats and colonizers which could lead to using fungi to determine the original location of a wildlife species in poaching cases in the future.

CHAPTER 5. CONCLUSIONS

This research focused on examining the potential use of fungi in forensic science. To expand our horizon a human study, a swine study and a wildlife study was done. From these three studies several conclusions were reached.

During the human study, we have found the least amount of similarity between the first and the last PMI time. This shows that the human mycoflora does change drastically over decomposition which indicates that fungi can be used to determine PMI. Moreover, fifty-one unique OTUs were found in the <24 hrs PMI group. These OTUs can be indicators for <24 hrs PMI. Other OTUs were also found which were only present in the early or late part of decomposition. These OTUs can also be used to determine early versus late decomposition. Additionally, 35 OTUs were found at every PMI, which makes up the pan mycoflora of the decomposing human body.

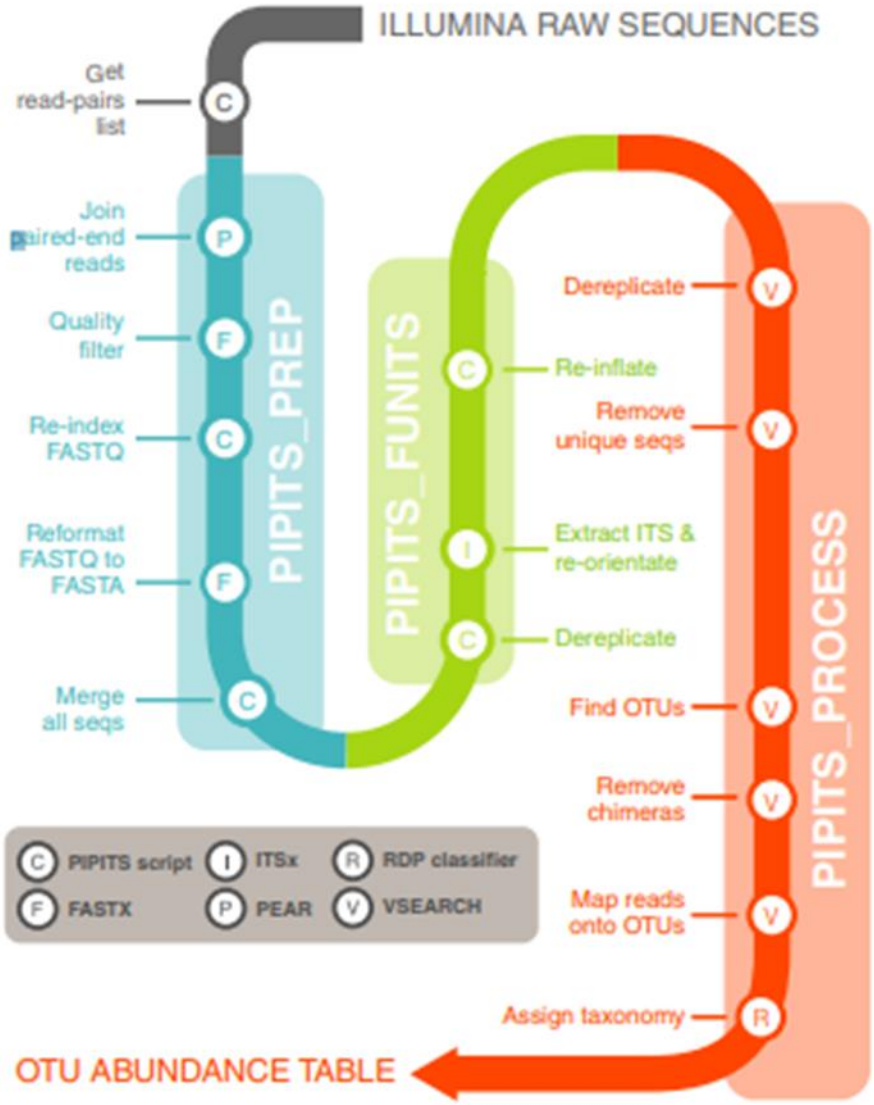
The human study gave an insight on short term decomposition, but to look at long term decomposition the swine study was used. From this study, baseline data was created to look at culturable fungal species that researchers might find in the future on swine carcasses in the West Lafayette, IN. region. Also, we looked at how similar the human and swine mycofora is, and we have seen that over 55% of the fungal species found on the swine were also found on humans. This is a good indicator that swine can be a model animal for fungal studies as well in forensics.

As forensics investigates crimes against animals too, a wildlife study was done as well. Here, baseline data was created for culturable fungal species that could be found on different wildlife species with different diets and living habitats. Furthermore, mycoflora changes were observed between fresh and skeleton stages. This showed that the mycoflora goes through major changes

during decomposition in case of animal species as well. Additionally, looking at the fungal flora of different animals, we can see differences in fungal communities between species as well.

These studies were great in showing that fungi do play a role in decomposition and they could be used for PMI determination. However, more studies are needed to increase sample sizes to refine this data. Also, due to the important role abiotic factors play, these studies must be repeated to get a clear picture on how fungal flora changes between seasons, states and elevations to get a more precise dataset which can be used to determine PMI and location throughout the entire USA.

APPENDIX A WORKFLOW OF PIPITS PIPELINE FOR ILLUMINA ITS SEQUENCES ACCORDING TO GWEON, 2015, FIGURE 1.



APPENDIX B SIGNIFICANCE TABLE BASED OF PMI ON AN OTU LEVEL WITH THE ORIGINAL SAMPLE AREAS AND PMIS FROM TABLE 2.1

```
Terms added sequentially (first to last)

             Df SumsOfSqs MeanSqs F.Model      R2 Pr(>F)
EstimatedPMI  4    1.8067 0.45167 0.97895 0.13089  0.536
Residuals    26   11.9958 0.46138         0.86911
Total        30   13.8025                 1.00000

Permutation test for homogeneity of multivariate dispersions
Permutation: free
Number of permutations: 999

Response: Distances
          Df  Sum Sq   Mean Sq     F N.Perm Pr(>F)
Groups     4 0.03835 0.0095875 0.926    999  0.455
Residuals 26 0.26918 0.0103532
```

APPENDIX C SIGNIFICANCE TABLE BASED OF PMI ON AN OTU LEVEL WITH THE RE-GROUPED SAMPLE AREAS AND PMIS FROM TABLE 2.2

```
Terms added sequentially (first to last)

          Df SumsOfSqs MeanSqs F.Model      R2 Pr(>F)
PMI        2     0.753 0.37650 0.80129 0.04915  0.946
Residuals 31    14.566 0.46987         0.95085
Total     33    15.319                 1.00000

Permutation test for homogeneity of multivariate dispersions
Permutation: free
Number of permutations: 999

Response: Distances
          Df   Sum Sq   Mean Sq      F N.Perm Pr(>F)
Groups     2 0.003511 0.0017557 1.2078    999  0.302
Residuals 31 0.045064 0.0014537
```

APPENDIX D SIGNIFICANCE TABLE BASED OF PMI ON AN OTU LEVEL WITH THE ORIGINAL SAMPLE AREAS AND PMIS FROM TABLE 2.1

```
Terms added sequentially (first to last)

             Df SumsOfSqs MeanSqs F.Model      R2 Pr(>F)
EstimatedPMI  4    1.9022 0.47556  1.2414 0.18415  0.123
Residuals    22    8.4275 0.38307         0.81585
Total        26   10.3297                 1.00000

Permutation test for homogeneity of multivariate dispersions
Permutation: free
Number of permutations: 999

Response: Distances
          Df  Sum Sq  Mean Sq      F N.Perm Pr(>F)
Groups     4 0.16187 0.040466 0.6422    999  0.629
Residuals 22 1.38627 0.063012
```

APPENDIX E SIGNIFICANCE TABLE BASED OF PMI ON A GENUS LEVEL WITH THE RE-GROUPED SAMPLE AREAS AND PMIS FROM TABLE 2.2

```
Terms added sequentially (first to last)

          Df SumsOfSqs MeanSqs F.Model      R2 Pr(>F)
PMI        2    0.7344 0.36721 0.89903 0.05482  0.587
Residuals 31   12.6620 0.40845         0.94518
Total     33   13.3964                 1.00000

Permutation test for homogeneity of multivariate dispersions
Permutation: free
Number of permutations: 999

Response: Distances
          Df  Sum Sq  Mean Sq      F N.Perm Pr(>F)
Groups     2 0.06597 0.032984 1.0621    999  0.356
Residuals 31 0.96276 0.031057
```

APPENDIX F LIST OF OTUS PRESENTED IN EACH SECTION OF THE VENN DIAGRAM PRESENTED IN FIGURE 2.4-21

PMI = 12: None

PMI < 24: OTU121, OTU106, OTU736, OTU515, OTU485, OTU73, OTU275, OTU451, OTU192, OTU282, OTU329, OTU219, OTU173, OTU46, OTU467, OTU541, OTU187, OTU286, OTU151, OTU293, OTU571, OTU679, OTU693, OTU360, OTU703, OTU347, OTU241, OTU268, OTU161, OTU528, OTU324, OTU325, OTU512, OTU337, OTU323, OTU314, OTU92, OTU510, OTU566, OTU591, OTU499, OTU694, OTU559, OTU637, OTU501, OTU744, OTU575, OTU327, OTU399, OTU722, OTU723

PMI > 24: None

PMI > 48: None

PMI > 72: None

PMI = 12 and PMI <24: OTU361, OTU190, OTU483, OTU450, OTU166, OTU171, OTU280, OTU376, OTU104, OTU394, OTU587, OTU518, OTU749

PMI = 12 and PMI >24: None

PMI = 12 and PMI >48: None

PMI = 12 and PMI >72: None

PMI <24 and PMI >24: OTU810, OTU579, OTU108, OTU146, OTU299, OTU200, OTU35, OTU343, OTU194, OTU457, OTU589, OTU196, OTU183, OTU260, OTU464, OTU56, OTU560, OTU690, OTU696, OTU602, OTU702, OTU658, OTU599, OTU620, OTU729, OTU421, OTU629, OTU521, OTU553, OTU817, OTU820, OTU726, OTU834, OTU826, OTU839, OTU819, OTU823, OTU822, OTU827, OTU798, OTU829, OTU841, OTU835, OTU824, OTU825, OTU832, OTU831, OTU828, OTU840, OTU837, OTU836, OTU838, OTU830, OTU842, OTU738, OTU598, OTU833

PMI <24 and PMI >48: OTU695, OTU164, OTU524, OTU733, OTU508, OTU137, OTU365, OTU504, OTU735, OTU563, OTU101, OTU316, OTU601, OTU505, OTU301, OTU304, OTU568, OTU472, OTU495, OTU152, OTU403, OTU116, OTU162, OTU139, OTU3, OTU142, OTU178, OTU531, OTU793, OTU321, OTU315, OTU176, OTU389, OTU370, OTU266, OTU247, OTU359, OTU357, OTU358, OTU737, OTU277, OTU565, OTU597, OTU233, OTU215, OTU97, OTU278, OTU261, OTU229, OTU309, OTU263, OTU156, OTU274, OTU96,

OTU217, OTU50, OTU117, OTU526, OTU402, OTU228, OTU155, OTU95, OTU124, OTU203, OTU539, OTU446, OTU159, OTU574, OTU105, OTU163, OTU167, OTU110, OTU144, OTU44, OTU45, OTU557, OTU100, OTU197, OTU118, OTU165, OTU345, OTU102, OTU72, OTU199, OTU460, OTU383, OTU513, OTU319, OTU454, OTU709, OTU712

PMI <24 and PMI >72: OTU302, OTU570, OTU174, OTU81, OTU582, OTU414, OTU758, OTU543, OTU306, OTU322, OTU815, OTU188, OTU331, OTU395, OTU387, OTU182, OTU208, OTU291, OTU225, OTU292, OTU362, OTU379, OTU7, OTU350, OTU223, OTU561, OTU572, OTU623, OTU608, OTU353, OTU523, OTU218, OTU477, OTU704, OTU478, OTU476, OTU660, OTU40, OTU474, OTU375, OTU710, OTU795

PMI >24 and PMI >48: None

PMI >24 and PMI >72: None

PMI >48 and PMI >72: None

PMI = 12, PMI <24 and PMI >24: OTU186, OTU728, OTU624, OTU550, OTU684, OTU666, OTU671, OTU682, OTU626, OTU668, OTU628, OTU634, OTU675, OTU613, OTU685, OTU655, OTU648, OTU638, OTU607, OTU844

PMI = 12, PMI <24 and PMI >48: OTU298, OTU371, OTU257, OTU349, OTU288, OTU99, OTU279, OTU581, OTU246, OTU432, OTU748

PMI = 12, PMI <24 and PMI >72: OTU848, OTU730, OTU564, OTU772

PMI = 12, PMI >24 and PMI >48: None

PMI = 12, PMI >24 and PMI >72: None

PMI = 12, PMI >48 and PMI >72: None

PMI <24, PMI >24 and PMI >48: OTU417, OTU689, OTU75, OTU160, OTU303, OTU366, OTU533, OTU126, OTU281, OTU153, OTU181, OTU189, OTU437, OTU16, OTU11, OTU364, OTU595, OTU548, OTU431

PMI <24, PMI >24 and PMI >72: OTU79, OTU452, OTU469, OTU489, OTU475, OTU473, OTU502, OTU486, OTU529, OTU739, OTU470, OTU721

PMI <24, PMI >48 and PMI >72: OTU447, OTU170, OTU318, OTU438, OTU425, OTU15, OTU482, OTU662, OTU659, OTU663

PMI >24, PMI >48 and PMI >72: None

PMI = 12, PMI <24, PMI >24 and PMI >48: OTU511, OTU411, OTU368, OTU351, OTU428

PMI = 12, PMI <24, PMI >24 and PMI >72: OTU129, OTU590, OTU583, OTU600, OTU650, OTU621, OTU642, OTU580, OTU606, OTU672, OTU697, OTU641, OTU617, OTU604, OTU677, OTU610, OTU707, OTU657, OTU625, OTU622, OTU669, OTU516, OTU643, OTU692, OTU681, OTU665, OTU649, OTU636, OTU706, OTU700, OTU731, OTU680, OTU683, OTU719, OTU664, OTU603, OTU673, OTU619, OTU651, OTU632, OTU644, OTU640, OTU611, OTU705, OTU618, OTU653, OTU670, OTU674, OTU687, OTU654, OTU588, OTU678, OTU614, OTU646, OTU652, OTU631, OTU647, OTU656, OTU686, OTU718, OTU448

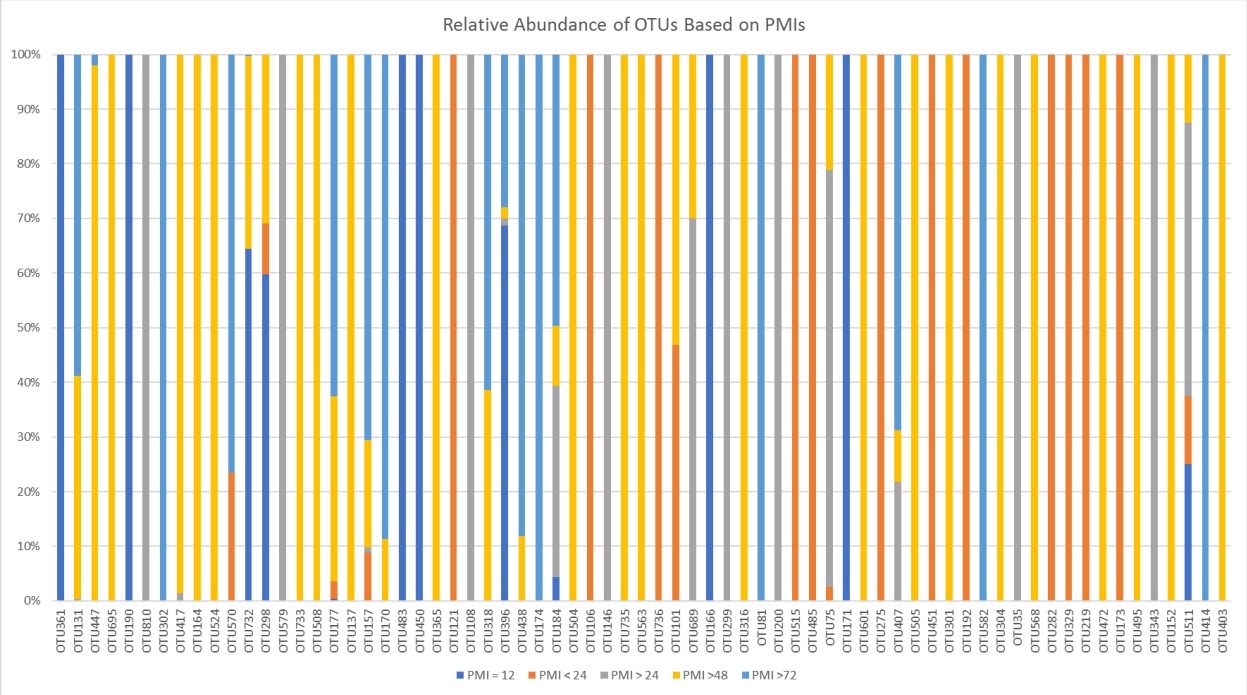
PMI = 12, PMI <24, PMI >48 and PMI >72: OTU732, OTU177, OTU264, OTU491, OTU661, OTU114

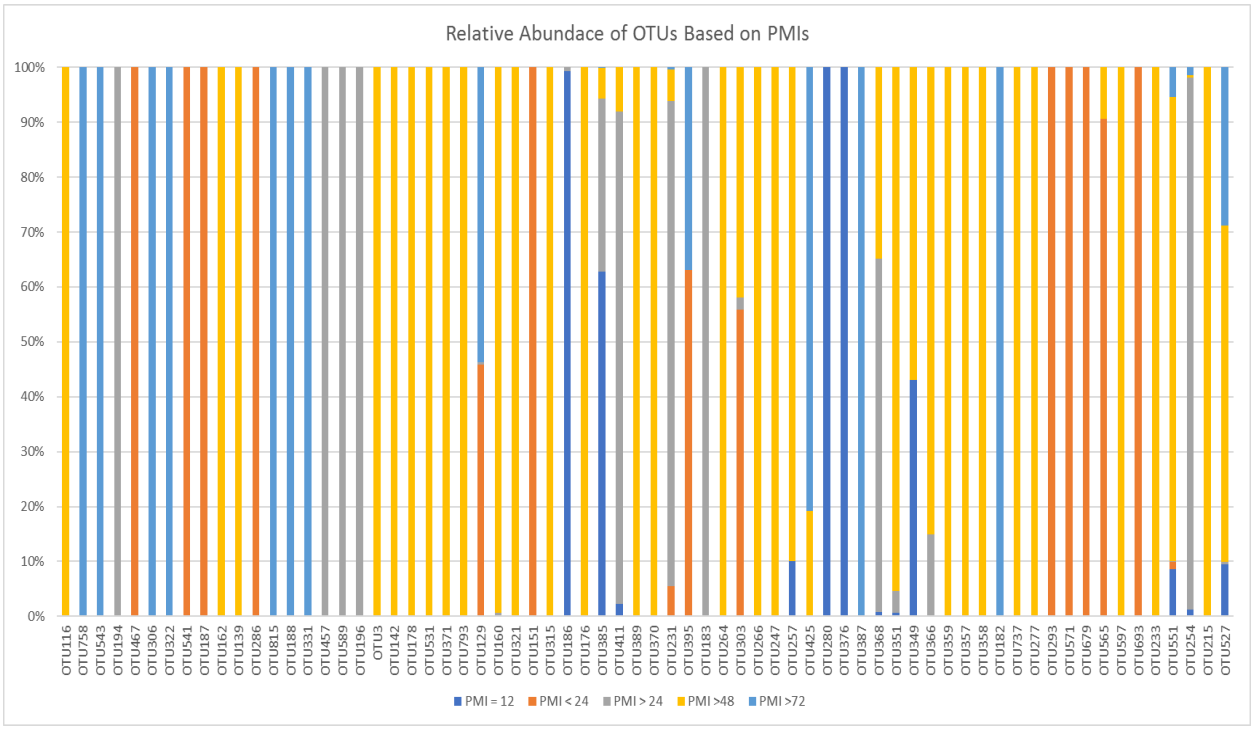
PMI = 12, PMI >24, PMI >48 and PMI >72: None

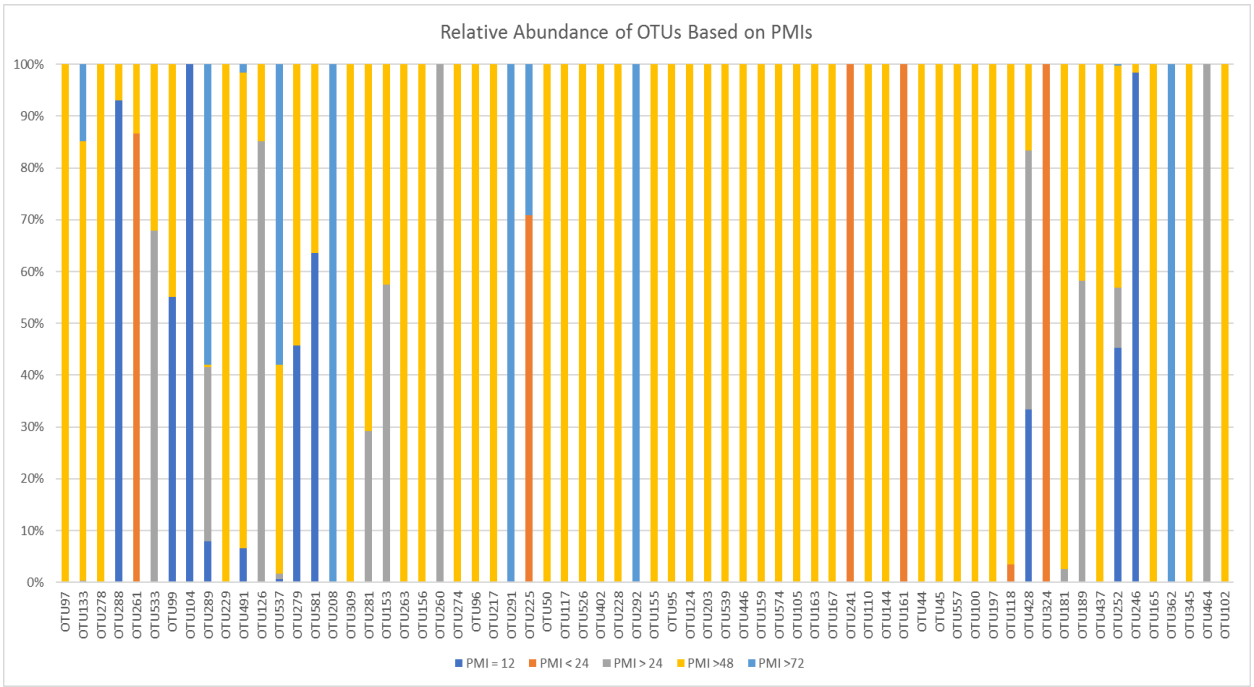
PMI <24, PMI >24, PMI >48 and PMI >72: OTU157, OTU407, OTU231, OTU133, OTU74, OTU549, OTU453, OTU519, OTU609, OTU480, OTU500, OTU809

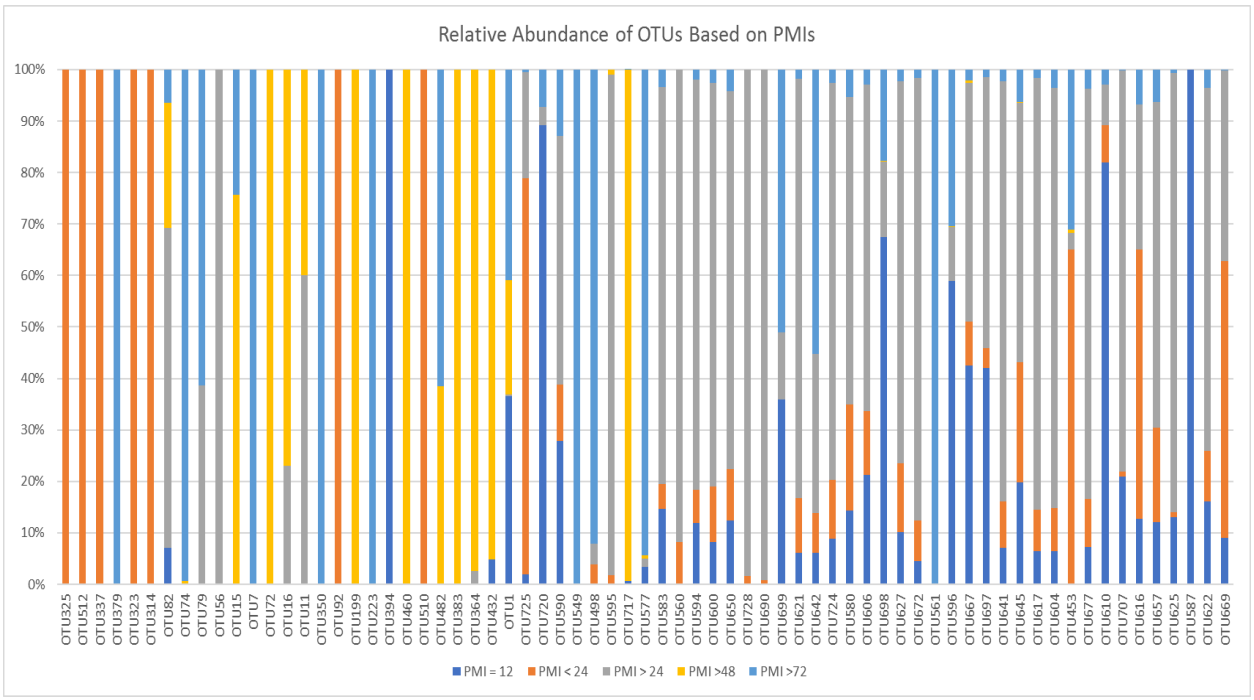
PMI = 12, PMI <24, PMI >24, PMI >48 and PMI >72: OTU131, OTU396, OTU184, OTU385, OTU551, OTU254, OTU527, OTU289, OTU537, OTU252, OTU82, OTU1, OTU725, OTU720, OTU498, OTU717, OTU577, OTU594, OTU699, OTU724, OTU698, OTU627, OTU596, OTU667, OTU645, OTU616, OTU605, OTU639, OTU630, OTU635, OTU615, OTU676, OTU456, OTU743, OTU745

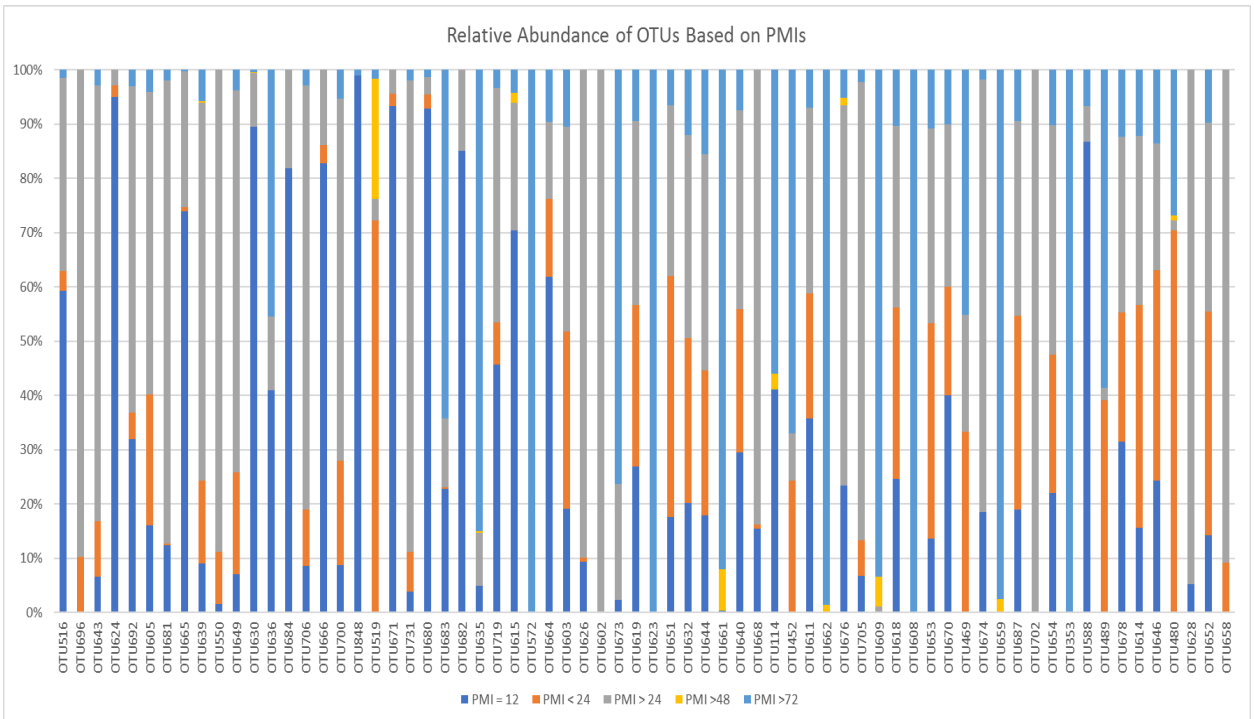
APPENDIX G RELATIVE ABUNDANCE OF OTUS BASED ONPMIS

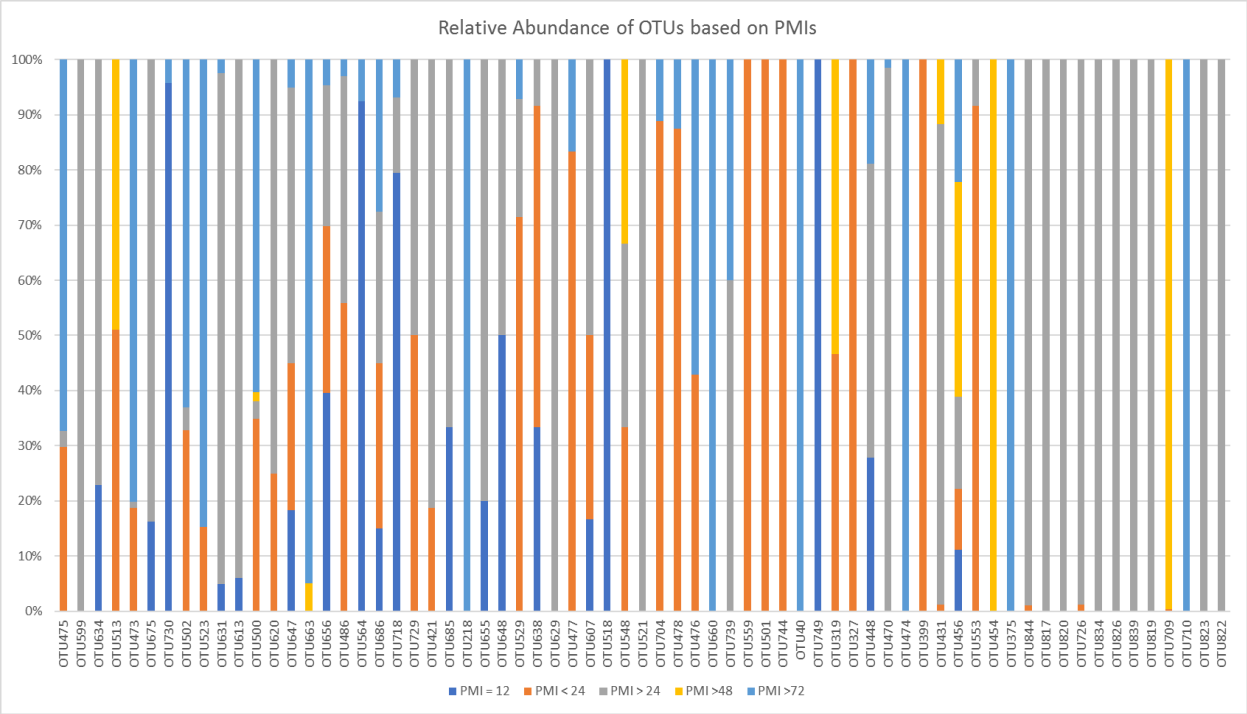


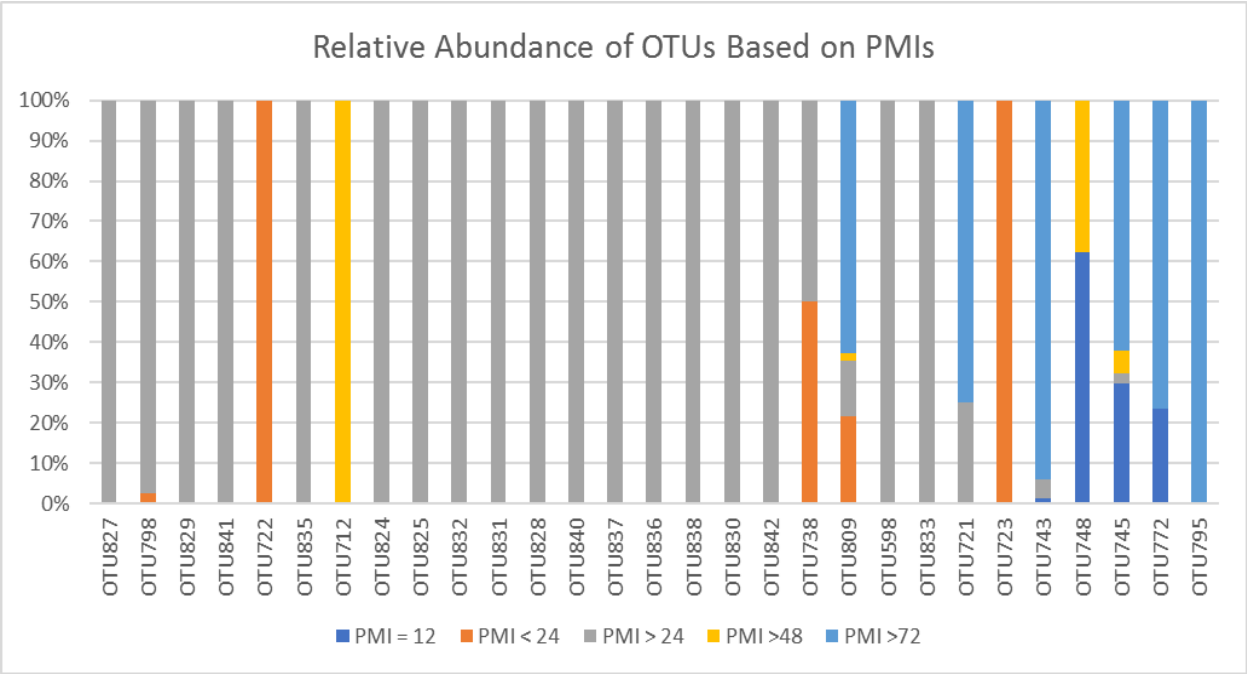

Relative Abundace of OTUs Based on PMIs
100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
OTU116 OTU758 OTU543 OTU194 OTU467 OTU306 OTU322 OTU541 OTU187 OTU162 OTU139 OTU286 OTU815 OTU188 OTU331 OTU457 OTU589 OTU196 OTU3 OTU142 OTU178 OTU531 OTU371 OTU793 OTU129 OTU160 OTU321 OTU151 OTU315 OTU186 OTU176 OTU385 OTU411 OTU389 OTU370 OTU231 OTU395 OTU183 OTU264 OTU303 OTU266 OTU247 OTU257 OTU425 OTU280 OTU376 OTU387 OTU368 OTU351 OTU349 OTU366 OTU359 OTU357 OTU358 OTU182 OTU737 OTU277 OTU293 OTU571 OTU679 OTU565 OTU597 OTU693 OTU233 OTU551 OTU254 OTU215 OTU527
PMI = 12
PMI < 24
PMI > 24
PMI >48
PMI >72


Relative Abundance of OTUs Based on PMIs
100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
OTU97 OTU133 OTU278 OTU288 OTU261 OTU533 OTU99 OTU104 OTU289 OTU229 OTU491 OTU126 OTU537 OTU279 OTU581 OTU208 OTU309 OTU281 OTU153 OTU263 OTU156 OTU260 OTU274 OTU96 OTU217 OTU291 OTU225 OTU50 OTU117 OTU526 OTU402 OTU228 OTU292 OTU155 OTU95 OTU124 OTU203 OTU539 OTU446 OTU159 OTU574 OTU105 OTU163 OTU167 OTU241 OTU110 OTU144 OTU161 OTU44 OTU45 OTU557 OTU100 OTU197 OTU118 OTU428 OTU324 OTU181 OTU189 OTU437 OTU252 OTU246 OTU165 OTU362 OTU345 OTU464 OTU102
PMI = 12
PMI < 24
PMI > 24
PMI >48
PMI >72


Relative Abundance of OTUs Based on PMIs
100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
OTU325 OTU512 OTU337 OTU379 OTU323 OTU314 OTU82 OTU74 OTU79 OTU56 OTU15 OTU7 OTU72 OTU16 OTU11 OTU350 OTU92 OTU199 OTU223 OTU394 OTU460 OTU510 OTU482 OTU383 OTU364 OTU432 OTU1 OTU725 OTU720 OTU590 OTU549 OTU498 OTU595 OTU717 OTU577 OTU583 OTU560 OTU594 OTU600 OTU650 OTU728 OTU690 OTU699 OTU621 OTU642 OTU724 OTU580 OTU606 OTU698 OTU627 OTU672 OTU561 OTU596 OTU667 OTU697 OTU641 OTU645 OTU617 OTU604 OTU453 OTU677 OTU610 OTU707 OTU616 OTU657 OTU625 OTU587 OTU622 OTU669
PMI = 12
PMI < 24
PMI > 24
PMI >48
PMI >72


Relative Abundance of OTUs Based on PMIs
100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
OTU516 OTU696 OTU643 OTU624 OTU692 OTU605 OTU681 OTU665 OTU639 OTU550 OTU649 OTU630 OTU636 OTU684 OTU706 OTU666 OTU700 OTU848 OTU519 OTU671 OTU731 OTU680 OTU683 OTU682 OTU635 OTU719 OTU615 OTU572 OTU664 OTU603 OTU626 OTU602 OTU673 OTU619 OTU623 OTU651 OTU632 OTU644 OTU661 OTU640 OTU668 OTU114 OTU452 OTU611 OTU662 OTU676 OTU705 OTU609 OTU618 OTU608 OTU653 OTU670 OTU469 OTU674 OTU659 OTU687 OTU702 OTU654 OTU353 OTU588 OTU489 OTU678 OTU614 OTU646 OTU480 OTU628 OTU652 OTU658
PMI = 12
PMI < 24
PMI > 24
PMI >48
PMI >72


Relative Abundance of OTUs based on PMIs
100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
OTU475 OTU599 OTU634 OTU513 OTU473 OTU675 OTU730 OTU502 OTU523 OTU631 OTU613 OTU500 OTU620 OTU647 OTU663 OTU656 OTU486 OTU564 OTU686 OTU718 OTU729 OTU421 OTU685 OTU218 OTU655 OTU648 OTU529 OTU638 OTU629 OTU477 OTU607 OTU518 OTU548 OTU521 OTU704 OTU478 OTU476 OTU660 OTU739 OTU559 OTU501 OTU744 OTU40 OTU749 OTU319 OTU327 OTU448 OTU470 OTU474 OTU399 OTU431 OTU456 OTU553 OTU454 OTU375 OTU844 OTU817 OTU820 OTU726 OTU834 OTU826 OTU839 OTU819 OTU709 OTU710 OTU823 OTU822
PMI = 12
PMI < 24
PMI > 24
PMI >48
PMI >72


Relative Abundance of OTUs Based on PMIs
100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
OTU827 OTU798 OTU829 OTU841 OTU722 OTU835 OTU712 OTU824 OTU825 OTU832 OTU831 OTU828 OTU840 OTU837 OTU836 OTU838 OTU830 OTU842 OTU738 OTU809 OTU598 OTU833 OTU721 OTU723 OTU743 OTU748 OTU745 OTU772 OTU795
PMI = 12
PMI < 24
PMI > 24
PMI >48
PMI >72

REFERENCES

Alacs, E. A., Georges, A., FitzSimmons, N. N., & Robertson, J. (2010). DNA detective: a review of molecular approaches to wildlife forensics. *Forensic Science, Medicine, and Pathology*, *6*(3), 180-194.

Anaissie, E. J., McGinnis, M. R., & Pfaller, M. A. (2009). *Clinical Mycology E-Book*. Elsevier Health Sciences.

Anstead, G. M., Sutton, D. A., & Graybill, J. R. (2012). Adiaspiromycosis causing respiratory failure and a review of human infections due to *Emmonsia* and *Chrysosporium* spp. *Journal of Clinical Microbiology*, *50*(4), 1346-1354.

Bååth, E., & Anderson, T. H. (2003). Comparison of soil fungal/bacterial ratios in a pH gradient using physiological and PLFA-based techniques. *Soil Biology and Biochemistry*, *35*(7), 955-963.

Baradkar, V. P., & Kumar, S. (2008). Meningitis caused by *Rhodotorula mucilaginosa* in human immunodeficiency virus seropositive patient. *Annals of Indian Academy of Neurology*, *11*(4), 245.

Barth, G., & Gaillardin, C. (1996). *Yarrowia lipolytica*. In *Nonconventional Yeasts in Biotechnology* (pp. 313-388). Springer, Berlin, Heidelberg.

Benbow, M. E., Lewis, A. J., Tomberlin, J. K., & Pechal, J. L. (2013). Seasonal necrophagous insect community assembly during vertebrate carrion decomposition. *Journal of Medical Entomology*, *50*(2), 440-450.

Bensch, K., Braun, U., Groenewald, J. Z., & Crous, P. W. (2012). The genus *cladosporium*. *Studies in Mycology*, *72*, 1-401.

Bergman, A. G., & Kauffman, C. A. (1984). Dermatitis due to *Sporobolomyces* infection. *Archives of Dermatology*, *120*(8), 1059-1060.

Botha, A. (2006). Yeasts in soil. In *Biodiversity and Ecophysiology of Yeasts* (pp. 221-240). Springer, Berlin, Heidelberg.

Botha, A. (2006). Yeasts in soil. In *Biodiversity and Ecophysiology of Yeasts* (pp. 221-240). Springer, Berlin, Heidelberg.

Bowen, G. J., Wassenaar, L. I., & Hobson, K. A. (2005). Global application of stable hydrogen and oxygen isotopes to wildlife forensics. *Oecologia*, *143*(3), 337-348.

Brack, D. (2004). The growth and control of international environmental crime. *Environmental Health Perspectives*, *112*(2), A80-A81.

Buzina, W., Lang-Loidolt, D., Braun, H., Freudenschuss, K., & Stammberger, H. (2001). Development of molecular methods for identification of *Schizophyllum* commune from clinical samples. *Journal of Clinical Microbiology*, *39*(7), 2391-2396

Callahan, B. J., McMurdie, P. J., & Holmes, S. P. (2017). Exact sequence variants should replace operational taxonomic units in marker-gene data analysis. *The ISME Journal*, *11*(12), 2639.

Caporaso, J. G., Kuczynski, J., Stombaugh, J., Bittinger, K., Bushman, F. D., Costello, E. K., ... & Huttley, G. A. (2010). QIIME allows analysis of high-throughput community sequencing data. *Nature Methods*, *7*(5), 335.

Carter, D. O., & Tibbett, M. (2003). Taphonomic mycota: fungi with forensic potential. *Journal of Forensic Science*, *48*(1), 1-4.

Carter, D. O., Yellowlees, D., & Tibbett, M. (2007). Cadaver decomposition in terrestrial ecosystems. *Naturwissenschaften*, *94*(1), 12-24.

Carvalho, L. M. L. D., Thyssen, P. J., Linhares, A. X., & Palhares, F. A. B. (2000). A checklist of arthropods associated with pig carrion and human corpses in Southeastern Brazil. *Memórias do Instituto Oswaldo Cruz*, *95*(1), 135-138.

Chen, Q., Hou, L. W., Duan, W. J., Crous, P. W., & Cai, L. (2017). Didymellaceae revisited. *Studies in Mycology*, *87*, 105-159.

Chen, Y. C., Eisner, J. D., Kattar, M. M., Rassoulian-Barrett, S. L., LaFe, K., Yarfitz, S. L., ... & Cookson, B. T. (2000). Identification of medically important yeasts using PCR-based detection of DNA sequence polymorphisms in the internal transcribed spacer 2 region of the rRNA genes. *Journal of Clinical Microbiology*, *38*(6), 2302-2310.

Chimutsa, M., Olakanye, A. O., Thompson, T. J., & Ralebitso-Senior, T. K. (2015). Soil fungal community shift evaluation as a potential cadaver decomposition indicator. *Forensic Science International*, *257*, 155-159.

Chowdhary, A., Perfect, J., & de Hoog, G. S. (2015). Black molds and melanized yeasts pathogenic to humans. *Cold Spring Harbor Perspectives in Medicine*, *5*(8), a019570.

Cooper, R. A., & Percival, S. L. (2010). 2 Human Skin and Microbial Flora. *Microbiology of Wounds*, 59.

Crous, P. W., Gams, W., Wingfield, M. J., & Van Wyk, P. S. (1996). *Phaeoacremonium* gen. nov. associated with wilt and decline diseases of woody hosts and human infections. *Mycologia*, *88*(5), 786-796.

Crous, P. W., Schoch, C. L., Hyde, K. D., Wood, A. R., Gueidan, C., De Hoog, G. S., & Groenewald, J. Z. (2009). Phylogenetic lineages in the *Capnodiales*. *Studies in Mycology*, *64*, 17-47.

Crous, P. W., Wingfield, M. J., Guarro, J., Cheewangkoon, R., Van der Bank, M., Swart, W. J., ... & Damm, U. (2013). Fungal Planet description sheets: 154–213. *Persoonia: Molecular Phylogeny and Evolution of Fungi*, *31*, 188.

Davoli, P., & Weber, R. W. (2002). Carotenoid pigments from the red mirror yeast, *Sporobolomyces roseus*. *Mycologist*, *16*(3), 102-108.

Dawson, L. A., & Hillier, S. (2010). Measurement of soil characteristics for forensic applications. *Surface and Interface Analysis: An International Journal Devoted to the Development and Application of Techniques for the Analysis of Surfaces, Interfaces and Thin Films*, *42*(5), 363-377.

de Almeida, J. N., Gimenes, V. M. F., Francisco, E. C., Siqueira, L. P. M., de Almeida, R. K. G., Guitard, J., ... & Rossi, F. (2017). Evaluating and improving Vitek MS for identification of clinically relevant species of *Trichosporon* and the closely related genera *Cutaneotrichosporon* and *Apiotrichum*. *Journal of Clinical Microbiology*, 55(8), 2439-2444.

De Jong, G. D. (1994). An annotated checklist of the *Calliphoridae* (Diptera) of Colorado, with notes on carrion associations and forensic importance. *Journal of the Kansas Entomological Society*, 378-385.

Deak, T. (2007). *Handbook of Food Spoilage Yeasts*. CRC press.

Dillon, V. M., Davenport, R. R., & Board, R. G. (1991). Yeasts associated with lamb. *Mycological Research*, *95*(1), 57-63.

Dodd, S. L., Lieckfeldt, E., & Samuels, G. J. (2003). *Hypocrea atroviridis* sp. nov., the teleomorph of *Trichoderma atroviride*. *Mycologia*, *95*(1), 27-40.

Dosa, A. (1955). Mold findings on exhumated cadavers and their medicolegal importance. *Deutsche Zeitschrift fur die Gesamte Gerichtliche Medizin*, *43*(5-6), 506-516.

Druzhinina, I., & Kubicek, C. P. (2005). Species concepts and biodiversity in *Trichoderma* and *Hypocrea*: from aggregate species to species clusters. *Journal of Zhejiang University. Science. B*, *6*(2), 100.

Dupuy, A. K., David, M. S., Li, L., Heider, T. N., Peterson, J. D., Montano, E. A., ... & Strausbaugh, L. D. (2014). Redefining the human oral mycobiome with improved practices in amplicon-based taxonomy: discovery of *Malassezia* as a prominent commensal. *PLoS One*, *9*(3), e90899.

Feng, P. Y., de Hoog, G. S., Najafzadeh, M. J., van den Ende, A. G., Stielow, J. B., Badali, H., ... & Vicente, V. A. (2014). *Cladophialophora abundans*, a novel species of *Chaetothyriales* isolated from the natural environment. *Mycological Progress*, *13*(2), 381-391.

Fincher, R. M., Fisher, J. F., Lovell, R. D., Newman, C. L., Espinel-Ingroff, A., & Shadomy, H. J. (1991). Infection due to the fungus Acremonium(cephalosporium). *Medicine*, *70*(6), 398-409.

Forensic Sciences. (n.d). *National Institute of Justice*, Retrieved from www.nij.gov/topics/forensics/Pages/welcome.aspx

Gajjar, D. U., Pal, A. K., Santos, J. M., Ghodadra, B. K., & Vasavada, A. R. (2011). Severe pigmented keratitis caused by *Cladorrhinum bulbillosum*. *Indian Journal of Medical Microbiology*, *29*(4), 434.

Gardes, M., & Bruns, T. D. (1993). ITS primers with enhanced specificity for basidiomycetes-application to the identification of mycorrhizae and rusts. *Molecular Ecology*, *2*(2), 113-118.

Giraldo, A., Gené, J., Sutton, D. A., Madrid, H., De Hoog, G. S., Cano, J., ... & Guarro, J. (2015). Phylogeny of *Sarocladium* (Hypocreales). *Persoonia: Molecular Phylogeny and Evolution of Fungi*, *34*, 10.

Godoy-Vitorino, F., Romaguera, J., Zhao, C., Vargas-Robles, D., Ortiz-Morales, G., Vazquez Sanchez, F., ... & Bittinger, K. (2018). Cervicovaginal fungi and bacteria associated with cervical intraepithelial neoplasia and high-risk Human Papillomavirus infections in a Hispanic population. *Frontiers in Microbiology*, *9*, 2533.

Greer, D. L., & Bolanos, B. (1974). Pathogenic potential of *Schizophyllum* commune isolated from a human case. *Sabouraudia: Journal of Medical and Veterinary Mycology*, *12*(2), 233-244.

Gweon, H. S., Oliver, A., Taylor, J., Booth, T., Gibbs, M., Read, D. S., ... & Schonrogge, K. (2015). PIPITS: an automated pipeline for analyses of fungal internal transcribed spacer sequences from the Illumina sequencing platform. *Methods in Ecology and Evolution*, *6*(8), 973-980.

Hagler, A. N., Mendonça-Hagler, L. C., & Pagnocca, F. C. (2017). Yeasts in aquatic ecotone habitats. In *Yeasts in Natural Ecosystems: Diversity* (pp. 63-85). Springer, Cham.

Hansford, C. G. (1937). Annotated Host List of Uganda Parasitic Fungi and Plant Diseases—Part IV. *The East African Agricultural Journal 3.3*, 235-240.

Hawksworth, D. L., & Luecking, R. (2017). Fungal Diversity Revisited: 2.2 to 3.8 Million Species. *Microbiology Spectrum*, *5*(4).

Hawksworth, D. L., & Wiltshire, P. E. (2011). Forensic mycology: the use of fungi in criminal investigations. *Forensic Science International*, *206*(1-3), 1-11.

Hawksworth, D. L., & Wiltshire, P. E. J. (2015). Forensic mycology: current perspectives. *Res Rep Forensic Med Sci*.

Hitosugi, M., Ishii, K., Yaguchi, T., Chigusa, Y., Kurosu, A., Kido, M., ... & Tokudome, S. (2006). Fungi can be a useful forensic tool. *Legal Medicine*, *8*(4), 240-242.

Hoffmann, K., Telle, S., Walther, G., Eckart, M., Kirchmair, M., Prillinger, H., ... & Vágvölgyi, C. (2009). Diversity, genotypic identification, ultrastructural and phylogenetic characterization of zygomycetes from different ecological habitats and climatic regions: limitations and utility of nuclear ribosomal DNA barcode markers. *Current Advances in Molecular Mycology. Nova Science Publishers, Inc, New York*, 263-312.

Horswell, J., Cordiner, S. J., Maas, E. W., Martin, T. M., Sutherland, K. B. W., Speir, T. W., ... & Osborn, A. M. (2002). Forensic comparison of soils by bacterial community DNA profiling. *Journal of Forensic Science*, *47*(2), 350-353.

Inácio, J., & Daniel, H. M. (2017). Commensalism: the case of the human zymobiome. In *Yeasts in Natural Ecosystems: Ecology* (pp. 211-228). Springer, Cham.

Ishii, K., Hitosugi, M., Kido, M., Yaguchi, T., Nishimura, K., Hosoya, T., & Tokudome, S. (2006). Analysis of fungi detected in human cadavers. *Legal Medicine*, *8*(3), 188-190.

James, Stuart H., and Jon J. Nordby. *Forensic Science: An Introduction to Scientific and Investigative Techniques*. CRC Press, 2002.

Khan, Z., Ahmad, S., Kapila, K., Ramaswamy, N. V., Alath, P., Joseph, L., & Chandy, R. (2011). *Pyrenochaeta romeroi*: a causative agent of phaeohyphomycotic cyst. *Journal of Medical Microbiology*, *60*(6), 842-846.

Konuray, G., & Erginkaya, Z. (2015). Antimicrobial and antioxidant properties of pigments synthesized from microorganisms. *The Battle Against Microbial Pathogens: Basic Science, Technological Advances and Educational Programs; Méndez-Vilas, A., Ed*, 27-32.

Kuehn, H. H., Orr, G. F., & Ghosh, G. R. (1964). Pathological implications of Gymnoascaceae, *Mycopathologia* et *Mycologia Applicata*, 24(1), 35-46.

Lachance, M. A., Gilbert, D. G., & Starmer, W. T. (1995). Yeast communities associated with *Drosophila* species and related flies in an eastern oak-pine forest: A comparison with western communities. *Journal of Industrial Microbiology*, *14*(6), 484-494.

Li, J., Chen, D., Yu, B., He, J., Zheng, P., Mao, X., ... & Luo, Y. (2018). Fungi in gastrointestinal tracts of human and mice: from community to functions. *Microbial Ecology*, 1-9.

Libkind, D., Brizzio, S., Ruffini, A., Gadanho, M., van Broock, M., & Sampaio, J. P. (2003). Molecular characterization of carotenogenic yeasts from aquatic environments in Patagonia, Argentina. *Antonie van Leeuwenhoek*, *84*(4), 313-322.

Libkind, D., Gadanho, M., van Broock, M., & Sampaio, J. P. (2009). *Cystofilobasidium lacus-mascardii* sp. nov., a basidiomycetous yeast species isolated from aquatic environments of the Patagonian Andes, and *Cystofilobasidium macerans* sp. nov., the sexual stage of *Cryptococcus macerans*. *International Journal of Systematic and Evolutionary Microbiology*, *59*(3), 622-630.

Lombard,L. (2018) Fungal Planet. Unpublished manuscript.

López-Martínez, R., Hernández-Hernández, F., Millán-Chiu, B. E., Manzano-Gayosso, P., & Méndez-Tovar, L. J. (2007). Effectiveness of imazalil to control the effect of fungal deterioration on mummies at the Mexico City Museum" El Carmen". *Revista Iberoamericana de Micologia*, *24*(4), 283-288.

Macdonald, L. M., Singh, B. K., Thomas, N., Brewer, M. J., Campbell, C. D., & Dawson, L. A. (2008). Microbial DNA profiling by multiplex terminal restriction fragment length polymorphism for forensic comparison of soil and the influence of sample condition. *Journal of Applied Microbiology*, *105*(3), 813-821.

Marples, M. J. (1965). The ecology of the human skin. *The Ecology of the Human Skin.*

Mašínová, T., Bahnmann, B. D., Větrovský, T., Tomšovský, M., Merunková, K., & Baldrian, P. (2017). Drivers of yeast community composition in the litter and soil of a temperate forest. *FEMS Microbiology Ecology*, *93*(2).

Maudet, C., Miller, C., Bassano, B., Breitenmoser-Würsten, C., Gauthier, D., Obexer-Ruff, G., ... & Luikart, G. (2002). Microsatellite DNA and recent statistical methods in wildlife conservation management: applications in Alpine ibex [Capra ibex (ibex)]. *Molecular Ecology*, *11*(3), 421-436.

Meeuwse, P., Tramper, J., & Rinzema, A. (2011). Modeling lipid accumulation in oleaginous fungi in chemostat cultures: I. Development and validation of a chemostat model for *Umbelopsis isabellina*. *Bioprocess and Biosystems Engineering*, *34*(8), 939-949.

Mirhendi, H., Diba, K., Rezaei, A., Jalalizand, N., Hosseinpur, L., & Khodadadi, H. (2007). Colony PCR is a rapid and sensitive method for DNA amplification in yeasts. *Iranian Journal of Public Health*, 40-44.

Nascimento, M. M., Selbmann, L., Sharifynia, S., Al-Hatmi, A. M., Voglmayr, H., Vicente, V. A., ... & Almaghrabi, O. A. (2016). *Arthrocladium*, an unexpected human opportunist in *Trichomeriaceae* (Chaetothyriales). *Fungal Biology*, *120*(2), 207-218.

Oda, Y., & Fujisawa, T. (2001). Intraspecific divergence of *Saccharomyces kluyveri* as revealed by the nucleotide sequences of 18S-28S rRNA spacer regions and α-galactosidase MEL genes. *Bioscience, Biotechnology, and Biochemistry*, *65*(1), 164-166.

Ohm, R. A., Feau, N., Henrissat, B., Schoch, C. L., Horwitz, B. A., Barry, K. W., ... & Hesse, C. N. (2012). Diverse lifestyles and strategies of plant pathogenesis encoded in the genomes of eighteen *Dothideomycetes* fungi. *PLoS Pathogens*, *8*(12), e1003037.

Parkinson, R. A., Dias, K. R., Horswell, J., Greenwood, P., Banning, N., Tibbett, M., & Vass, A. A. (2009). Microbial community analysis of human decomposition on soil. In *Criminal and Environmental Soil Forensics* (pp. 379-394). Springer, Dordrecht.

Pechal, J. L., Crippen, T. L., Benbow, M. E., Tarone, A. M., Dowd, S., & Tomberlin, J. K. (2014). The potential use of bacterial community succession in forensics as described by high throughput metagenomic sequencing. *International Journal of Legal Medicine*, *128*(1), 193-205.

Pechal, J. L., Crippen, T. L., Tarone, A. M., Lewis, A. J., Tomberlin, J. K., & Benbow, M. E. (2013). Microbial community functional change during vertebrate carrion decomposition. *PloS One*, *8*(11), e79035.

Percival, S. L., Emanuel, C., Cutting, K. F., & Williams, D. W. (2012). Microbiology of the skin and the role of biofilms in infection. *International Wound Journal*, *9*(1), 14-32.

Robbertse, B., Strope, P. K., Chaverri, P., Gazis, R., Ciufo, S., Domrachev, M., & Schoch, C. L. (2017). Improving taxonomic accuracy for fungi in public sequence databases: applying 'one name one species' in well-defined genera with *Trichoderma/Hypocrea* as a test case. *Database*, *2017*.

Robert,V., Epping,W. & Boekhout,T. (2016) *Cryptococcus foliicola* sp. nov. and *Cryptococcus taibaiensis* sp. nov., novel basidiomycetous yeast species from plant leaves. Unpublished manuscript.

Rolo, E. A., Oliveira, A. R., Dourado, C. G., Farinha, A., Rebelo, M. T., & Dias, D. (2013). Identification of *sarcosaprophagous Diptera* species through DNA barcoding in wildlife forensics. *Forensic Science International*, *228*(1-3), 160-164.

Ruibal, C., Millanes, A. M., & Hawksworth, D. L. (2011). Molecular phylogenetic studies on the lichenicolous *Xanthoriicola physciae* reveal Antarctic rock-inhabiting fungi and *Piedraia* species among closest relatives in the Teratosphaeriaceae. *IMA Fungus*, *2*(1), 97-103.

Rush,R.E., Lemons,A.R. & Green,B.J. (2018) Unpublished manuscript.

Sampaio, A., Sampaio, J. P., & Leão, C. (2007). Dynamics of yeast populations recovered from decaying leaves in a nonpolluted stream: a 2-year study on the effects of leaf litter type and decomposition time. *FEMS Yeast Research*, *7*(4), 595-603.

Sampaio, J. P. (2004). Diversity, phylogeny and classification of basidiomycetous yeasts. *Frontiers in Basidiomycete Mycology*, 49-80.

Santos, A. C. D. S., Trindade, J. V. C., Lima, C. S., Barbosa, R. D. N., da Costa, A. F., Tiago, P. V., & Oliveira, N. (2019). Morphology, phylogeny, and sexual stage of *Fusarium caatingaense* and *Fusarium pernambucanum*, new species of the *Fusarium incarnatum-equiseti* species complex associated with insects in Brazil. *Mycologia*,1-16.

Schinabeck, M. K., & Ghannoum, M. A. (2003). Human hyalohyphomycoses: a review of human infections due to *Acremonium* spp., *Paecilomyces* spp., *Penicillium* spp., and *Scopulariopsis* spp. *Journal of Chemotherapy*, *15*(sup2), 5-15.

Schoch, C. L., Robbertse, B., Robert, V., Vu, D., Cardinali, G., Irinyi, L., ... & Kirk, P. M. (2014). Finding needles in haystacks: linking scientific names, reference specimens and molecular data for Fungi. *Database*, *2014*.

Schwarz, P., Bretagne, S., Gantier, J. C., Garcia-Hermoso, D., Lortholary, O., Dromer, F., & Dannaoui, E. (2006). Molecular identification of zygomycetes from culture and experimentally infected tissues. *Journal of Clinical Microbiology*, *44*(2), 340-349.

Scorzetti, G., Fell, J. W., Fonseca, A., & Statzell-Tallman, A. (2002). Systematics of basidiomycetous yeasts: a comparison of large subunit D1/D2 and internal transcribed spacer rDNA regions. *FEMS Yeast Research*, *2*(4), 495-517.

Scorzetti, G., Fell, J. W., Fonseca, A., & Statzell-Tallman, A. (2002). Systematics of basidiomycetous yeasts: a comparison of large subunit D1/D2 and internal transcribed spacer rDNA regions. *FEMS Yeast Research*, *2*(4), 495-517.

Serrano-Carreon, L., Hathout, Y., Bensoussan, M., & Belin, J. M. (1992). Lipid accumulation in *Trichoderma* species. *FEMS Microbiology Letters*, *93*(2), 181-187.

Sert, H. B., & Sterflinger, K. (2010). A new *Coniosporium* species from historical marble monuments. *Mycological Progress*, *9*(3), 353-359.

Shirley, J. A., Revill, S., Cohen, B. J., & Buckley, M. M. (1987). Serological study of rubella-like illnesses. *Journal of Medical Virology*, *21*(4), 369-379

Sidrim, J. J. C., Moreira Filho, R. E., Cordeiro, R. A., Rocha, M. F. G., Caetano, E. P., Monteiro, A. J., & Brilhante, R. S. N. (2010). Fungal microbiota dynamics as a postmortem investigation tool: focus on *Aspergillus, Penicillium and Candida* species. *Journal of Applied Microbiology*, *108*(5), 1751-1756.

Stratford, M. (2006). Food and beverage spoilage yeasts. In *Yeasts in Food and Beverages* (pp. 335-379). Springer, Berlin, Heidelberg.

Sudduth, E. J., Crumbley III, A. J., & Farrarn, W. E. (1992). Phaeohyphomycosis due to *Exophiala* species: clinical spectrum of disease in humans. *Clinical Infectious Diseases*, *15*(4), 639-644.

Tibbett, M., & Carter, D. O. (Eds.). (2008). *Soil Analysis in Forensic Taphonomy: Chemical and Biological Effects of Buried Human Remains*. CRC Press.

Torsvik, V., Daae, F. L., Sandaa, R. A., & Øvreås, L. (1998). Novel techniques for analysing microbial diversity in natural and perturbed environments. *Journal of Biotechnology*, *64*(1), 53-62.

Tournas, V. H., Heeres, J., & Burgess, L. (2006). Moulds and yeasts in fruit salads and fruit juices. *Food Microbiology*, *23*(7), 684-688.

Tranchida, M. C., Centeno, N. D., & Cabello, M. N. (2014). Soil fungi: Their potential use as a forensic tool. *Journal of Forensic Sciences*, *59*(3), 785-789.

Tsuneda, A., Tsuneda, I., & Currah, R. S. (2004). Endoconidiogenesis in *Endoconidioma populi* and *Phaeotheca fissurella*. *Mycologia*, *96*(5), 1136-1142.

Valenzuela-Lopez, N., Cano-Lira, J. F., Guarro, J., Sutton, D. A., Wiederhold, N., Crous, P. W., & Stchigel, A. M. (2018). *Coelomycetous Dothideomycetes* with emphasis on the families Cucurbitariaceae and Didymellaceae. *Studies in Mycology*, *90*, 1-69.

Van de Voorde, H., & Van Dijck, P. J. (1982). Determination of the time of death by fungal growth. *Zeitschrift für Rechtsmedizin*, *89*(2), 75-80.

van Nieuwenhuijzen, E. J., Houbraken, J. A., Meijer, M., Adan, O. C., & Samson, R. A. (2016). *Aureobasidium melanogenum*: a native of dark biofinishes on oil treated wood. *Antonie van Leeuwenhoek*, *109*(5), 661-683.

Vu, D., Groenewald, M., De Vries, M., Gehrmann, T., Stielow, B., Eberhardt, U., ... & Boekhout, T. (2019). Large-scale generation and analysis of filamentous fungal DNA barcodes boosts coverage for kingdom fungi and reveals thresholds for fungal species and higher taxon delimitation. *Studies in Mycology*, *92*, 135-154.

Vu, D., Groenewald, M., Szöke, S., Cardinali, G., Eberhardt, U., Stielow, B., ... & Robert, V. (2016). DNA barcoding analysis of more than 9 000 yeast isolates contributes to quantitative thresholds for yeast species and genera delimitation. *Studies in Mycology*, *85*, 91-105.

Wang, Y. N., Liu, X. Y., & Zheng, R. Y. (2014). *Umbelopsis changbaiensis* sp. nov. from China and the typification of *Mortierella vinacea*. *Mycological Progress*, *13*(3), 657-669.

Weatherbee, C. R., Pechal, J. L., & Eric Benbow, M. (2017). The dynamic maggot mass microbiome. *Annals of the Entomological Society of America*, *110*(1), 45-53.

Weiss, M., Bauer, R., Sampaio, J. P., & Oberwinkler, F. (2014). 12 *Tremellomycetes* and Related Groups. In *Systematics and Evolution* (pp. 331-355). Springer, Berlin, Heidelberg.

White, T. J., Bruns, T., Lee, S. J. W. T., & Taylor, J. L. (1990). Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. *PCR protocols: A Guide to Methods and Applications*, *18*(1), 315-322.

Wilson, J. M., Lafon, N. W., Kreitlow, K. L., Brewster, C. C., & Fell, R. D. (2014). Comparing growth of pork-and venison-reared *Phormia regina* (Diptera: Calliphoridae) for the application of forensic entomology to wildlife poaching. *Journal of Medical Entomology*, *51*(5), 1067-1072.

Wilson-Wilde, L. (2010). Wildlife crime: a global problem. *Forensic Science, Medicine, and Pathology*, *6*(3), 221-222.

Wiltshire, P. E., Hawksworth, D. L., Webb, J. A., & Edwards, K. J. (2014). Palynology and mycology provide separate classes of probative evidence from the same forensic samples: a rape case from southern England. *Forensic Science International*, *244*, 186-195.

Woudenberg, J. H. C., Groenewald, J. Z., Binder, M., & Crous, P. W. (2013). *Alternaria* redefined. *Studies in Mycology*, *75*, 171-212.

Zhang, E., Tanaka, T., Tajima, M., Tsuboi, R., Nishikawa, A., & Sugita, T. (2011). Characterization of the skin fungal microbiota in patients with atopic dermatitis and in healthy subjects. *Microbiology and Immunology*, *55*(9), 625-632.

Zhou, L. W. (2015). *Fulvifomes imbricatus* and *F. thailandicus* (Hymenochaetales, Basidiomycota): two new species from Thailand based on morphological and molecular evidence. *Mycological Progress*, *14*(10), 89.