Into the woodwork - An investigation into the composition and structure of the termite gut microbiome

Termites are known to cause millions of dollars worth of damage to houses annually[1] which results in them being perceived as pests that must be destroyed. Sadly, this reputation overshadows the various useful abilities these fascinating critters have. Termites are master-adapters, having colonised most landmasses except Antarctica[2]. Their quickly regenerating exterior allows metabolic processes to take place within the shell to enable survival in various terrains and environmental conditions[3]. Nonetheless, this is not at the hands of termites alone. It has long been known that the microbiome of the termite gut plays a significant role in their survival and adaptation successes[4]. However, this ability to adapt to environments may not only have beneficial effects on our ecosystem but is suspected to also contribute to its degradation. This is understood to be a response to human's destruction of natural resources, resulting in the adaptation of the termites metabolic systems[5].

Necessary to the maintenance of overall ecosystem health are interactions between all biotic communities. In the ecosystem, termites were thought to have taken on the essential role of cellular breakdown to degrade organic matter[6]. As mentioned above, this role is actually shared between the microorganisms within the termites as well as the termites themselves. Current investigations into this topic continue to discover new details and will hopefully eventually allow us to reveal the true extend of the internal life of the termite microbiome. Most recently it has been found that protists within termites host further endosymbiotic bacteria. Often the metabolic processes of the termite are conducted in a "chain" of processes where one material is passed down from one organisms to the next to create or degrade compounds.
There are various examples of these chains, for instance acetogenesis[7], nitrogen fixation[7] or digestion of organic matter[7, 8], i.e. wood. The latter is of particular interest to ecological research that attempts to find alternatives for breaking down organic matter produced by humans (e.g. in landfills). So far, various forms of this research[8] have shown that termites ingest cellular material which protists break down into xylan. The Xylan is then broken down further by bacteria and excreted as greenhouse gas by the termite. Whilst this process might be useful in the efficient degradation of human-generated waste, it would also add greenhouse gases and hence, to the deterioration of our planet.

Striking about research on termites is an obvious lack of consideration for termite colonies. These are known to have social hierarchies[9], much like ants, where some termites even have different morphologies (i.e. no wings for worker termites). This could potentially mean that their microbiome differs based on the role they hold within the colony, and the diverse interactions with the environment. It seems that none of the studies conducted on termites have contrasted the microbiome diversity *within* individual colonies. The question stands whether a large part of community diversity reported could be a confounding factor of the sampling, i.e. having sampled from termites of different casts. Additional confounds could be created by selecting for termites with a favourable microbiome to degrade plastic. There, it would be possible that colonies will not be sustainable in the long-term as only particular casts would have been selected for.
Whilst termite research in New Zealand is rather slim, the overall results based on only the native termite *Stolotermes ruficeps*, show that the symbiotic relationships are similar in nature[10, 11]. Other natives to New Zealand are *Kalotermes brouni* and *Stolotermes inopinus*[11], some of which this research intended on sampling. However, due to a COVID-19 lockdown, the sampling method was changed. Instead, data from two locations in the North-Eastern USA (Massachusetts and Connecticut) was analysed to find differences in alpha- and beta-diversity and identify changes in taxa abundance across the samples. No information about the sampling method, termite species and environmental conditions is known. However, both US states are known to have mild winters and warm summers, which provides favourable conditions for termite colony formation[12, 13, 14].

Results

Alpha diversity analysis shows that both locations are displaying an even, diverse community with most taxa being relatively abundant and few taxa being less abundant (Fig. 1). Massachusetts is displaying a significantly larger abundance of the most represented taxa, with the first OTU rank showing 1000 samples. In comparison, the Connecticut samples show more pooling of ~200 OTU's with the highest rank having a representation of 650 OTU's.

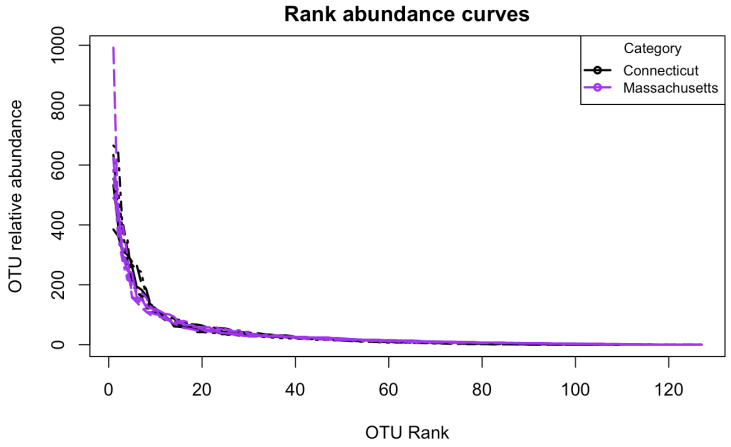

Figure 1. Rank abundance curves of the gut microbiome of termites collected in Connecticut (black) and Massachusetts (purple). *The relative abundances of taxa (represented as 97% identical OTU) are plotted against the respective OTU rank within the community.*

Moving from alpha diversity into beta diversity, the NMDS ordination based on Bray-Curtis dissimilarity scores (Fig. 2) allows closer examination of the microbial community composition within the two collection sites. Two completely separate clusters indicate that the collection sites vary greatly in their microbial community composition. Massachusetts samples are pooling together slightly more than Connecticut samples. This shows that the microbial communities within

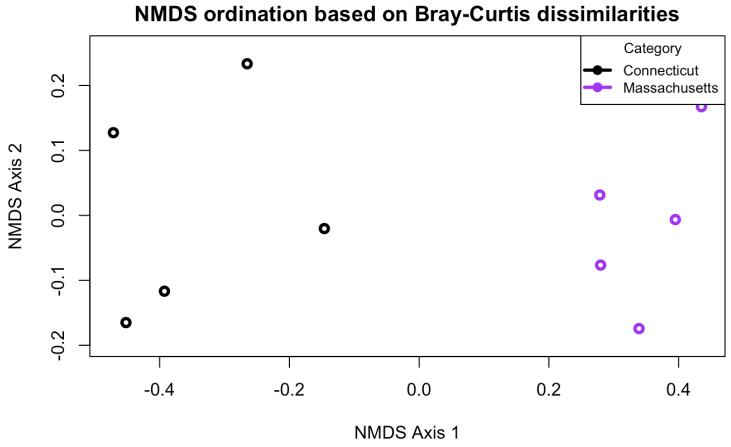

Figure 2. Non-metric Multidimensional Scaling (NMDS) ordination based on Bray-Curtis dissimilarity scores of samples collected in Connecticut (black) and Massachusetts (purple). *Each circle represents a termite sample, i.e. microbial community. K=2 was selected to account for large differences between the groups.*

Massachusetts differ less than the microbial communities within Connecticut. Notably, the samples in Connecticut show quite a larger beta diversity as illustrated by the broader spread of the samples.

Assessing the phylum diversity within and between the two sample sites using a heatmap (Fig. 3) it becomes abundantly clear that *Spirochaetes* are dominating the microbial communities in both locations. Additionally, both, Connecticut and Massachusetts samples, are relatively similar in the patterns of phylum abundance. Massachusetts shows individual variation between the samples, with *Spirochaetes* as the most and *Euryarchaeota* as the least abundant. In contrast, Connecticut exhibits a more regular abundance representation between the different phyla, with high abundances of both *Spirochaetes* and *Elusimicrobia* and low abundances of *Synergistetes* and *Euryarchaeota*. Also represented in the heatmap are *Proteobacteria*, *Tenericutes* and *Firmicutes*. It should be noted that the differences shown in Figure 1 and 2 are mirrored in the heat map. Massachusetts showed a large abundance of one particular OTU/taxa, which the heatmap indicates these to be Spirochaetes.

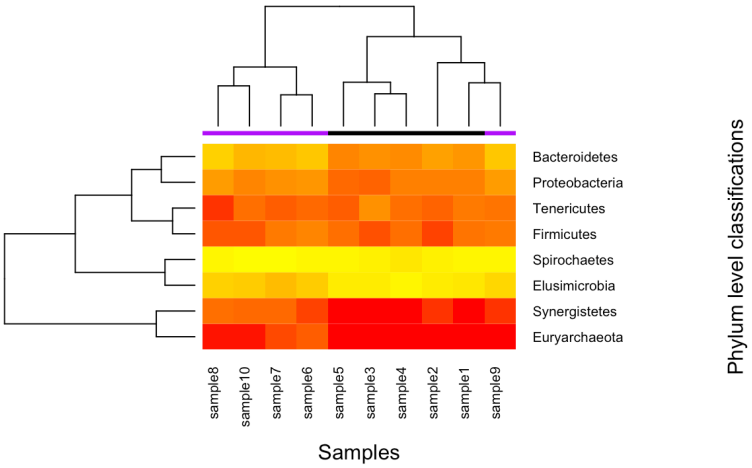

Figure 3. Heatmap illustrating taxa trends at phylum level of microbial community samples in Connecticut (black) and Massachusetts (purple). *Log-normalised relative abundance of OTU's present are illustrated on a gradient from red (low abundance) to yellow (high abundance). Phylogenetic trees have been allocated taxa (rows) and samples (columns) t be clustered with dendrograms.*

Looking at the relative abundances of classes (Fig. 4) by examining the presence and distribution of the bacterial and archaeal classes across the samples, the previously reported data from Figures 1-3 is reiterated. The abundances of classes in samples from both locations show similarities in the abundance of Spirochaetia. In the termite gut microbial community across all samples of the Connecticut location there is a distinct representation of Endomicrobia, with sample 4 showing that over half of microbiome is comprised of this class. Overall, the microbial community in the Connecticut samples is distributed evenly and similarly. This comes in contrast with the samples in Massachusetts. These show a larger diversity in relative abundance of classes between the samples as well as a larger class diversity when compared to Connecticut. The samples from Massachusetts each exhibit a representation of Bacteroidia which is only found to a small degree in the Connecticut samples.

Breaking down the relative abundances of classes even further to identify individual genera (Fig. 5), it becomes clear that the classes are often represented by a single one or two specific genera rather than a diversity within the class. However, the differences between the samples from Connecticut and Massachusetts are now revealed. The largest abundance in Massachusetts is *Treponema*, making up at least half of all sample's genera and really clarifying the genus representing the overwhelming abundance of the *Spirochaetia* class. The Bacteroidia is represented by *Porphyromonadaceae*, making up nearly one fifth of all samples. The Endomicrobia is largely represented by *Candidatus Endomicrobium*, yet a representation of other,

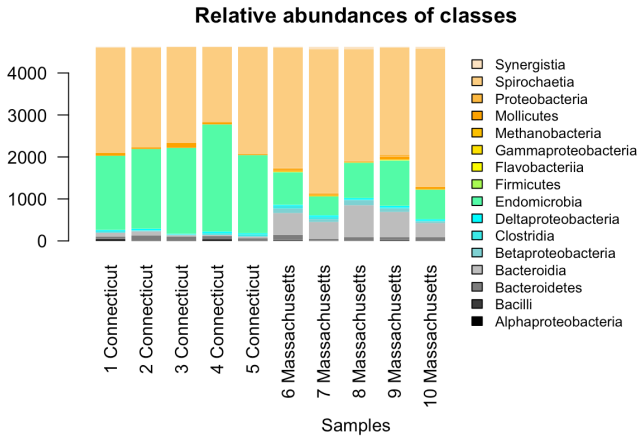

Figure 4. Stacked bar chart plotting relative abundances of microbial classes in Connecticut and Massachusetts. *Each bar is illustrating the presence and distribution of microbial classes across the samples.*

unidentified Endomicrobia can be found in all samples. When examining the genera abundance of the Connecticut samples it becomes evident that there is overall a smaller diversity of genera in the microbiome of the sampled termites when compared to the Massachusetts samples. *Treponema* and *Candidatus Endomicrobium*, representing Spirochaetia and Endomicrobia respectively, dominate the genera by covering between 90-95% of the total genera abundance. Differences between Massachusetts and Connecticut samples, aside the abundance of *Treponema*, become clear upon closer examination of the different genera. The absence of *Porphyromonadaceae* in Connecticut samples is noted, as opposed to this genus holding a relatively large representation in Massachusetts. Both locations display small and slightly differing representations of *Clostridiales*, *Mycoplasma*, *Methanobrevibacter,* and *Proteobacteria.*

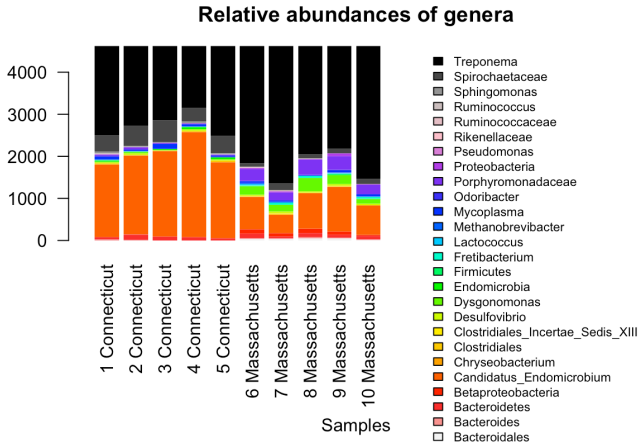

Figure 5. Stacked bar chart plotting relative abundances of microbial genera in Connecticut and Massachusetts. *Each bar is illustrating the presence and distribution of microbial genera across the samples.*

Discussion

In this study, microbiome data from termite guts in Massachusetts and Connecticut (North-Eastern USA) was analysed to gain insight into its alpha- and beta diversity and identify key changes in taxa abundance across the samples. Visual comparison of graphs created for said taxa allows the contrasting of these two locations as well as placing them within the context of the overall literature available on the microbial life within termite guts.

When comparing the alpha diversity (Fig. 1) between the Massachusetts and Connecticut samples it is noted that at first look both are even, diverse communities. Striking, however, is that Massachusetts displayed a more extreme diversity distribution. This trend is reiterated when examining the beta diversity (Fig. 2) which showed great variances between the two sample sites and great variances within the Connecticut sample site. This could simply point to the general idea that termite gut microbiomes in Massachusetts are more alike compared to Connecticut samples that show a larger diversity. However, as mentioned previously, there should be concerns around microbial diversity *within* the termite colonies. A lack of information on where the termites were found make it difficult to determine what the underlying reason for this diversity is. No current research has been found that investigates why there could be such extreme differences between termite guts from one location to another. The author would strongly encourage research into this possibility and contrasting the microbiome between the locations to specifically taking samples from differently casted termites to compare the microbiome within colonies.

Further investigation into the beta diversity allows insight into the metabolical processes guided by microbes. Established in the phyla heatmap (Fig. 3) and both bar-charts representing genera and class abundances (Figs. 4 & 5) was that *Spirochaetia* dominated all termite guts. *Spirochaetia*, particularly the genus *Treponema* found in the samples, is known to be responsible for xylan degradation. Finding this genus therefore indicates that the termites sampled are likely wood feeders. Notable, however, is that the samples from Massachusetts exhibited a larger relative abundance of *Treponema* compared to Connecticut. The fact that all samples show this distinct pattern indicates that the termite microbiome in Massachusetts could generally have a higher abundance of this genus. Alternatively, as nothing is known about the sampling method, it is possible that this could be related to the state of the termite, i.e. whether there was a large amount of nutritional wood available at time of collection. This could be a feasible option as other research has also shown that the termite gut microbiome is dynamic, depending on the nutritional availability at particular times[15].

When examining the less abundant genera and classes it can be seen that in both locations microbes occupying smaller niches still undertake other metabolic processes, such as *Proteobacteria* for Nitrogen fixation or *Methanobrevibacter* for methanogenesis. The presence of *Porphyromonadaceae* only in the samples from Connecticut indicates that there might be dietary differences between the termites in the two locations. *Porphyromonadaceae* are carbohydrate consumers, there might be the possibility that the termites were taken from an area that is closer to urban environments. This difference in diet would be reiterated by the previously mentioned difference in *Trepomena* abundance, as well as the *Proteobacteria* and *Bacteroidetes* abundance, with the latter contributing to lignin abundance.

Overall it can be said that the microbial alpha-diversity of the termite guts is similar between the samples from Massachusetts and Connecticut. It is also suspected that the termites are wood feeders. The microbiome of the tested termites shows variety in its beta-diversity, suggesting that they share slightly differing diets. The microbial diversity represented in the samples reiterates what current research has established. Therein, termite gut microbiome enables termites to adapt to environments as well as degrade organic matter efficiently through a job-sharing of metabolic activities.

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