Next Generation Sequencing of Protists as a Measure of Microbial Community in Oil Sands Tailings Ponds: Amplicon Versus Metagenomic Approaches

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Oil Sands Research and Information Network

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REPORT SUMMARY

The Alberta oil sands provide a major benefit to the province as an economic driver. At the same time, their responsible exploitation, particularly in mitigating the environmental impact of oil extraction stands as a significant challenge to be addressed. One of the most contentious aspects is the reclamation of tailings ponds, vast reservoirs of post-processing water and solids mixed with a variety of industrial compounds. Microbiological processes from bacteria and archaea have been previously shown to be at play in the tailings ponds and are factored into plans for their reclamation. However, the impact of microbial eukaryotes, known in all other environments to play a role in the food web, has been relatively poorly addressed. This will be important to know, particularly in light of end pit lake plans for reclamation moving forward.

To better understand the microbial communities in the tailings ponds for improved reclamation planning, we have begun using next generation sequencing (NGS) methods to understand the microbial eukaryotic communities present in tailings. We also compare results from two different NGS strategies, metagenomic versus amplicon based, to assess a productive strategy for analyses going forward.

Metagenomic data sequenced using the Illumina platform from a tailings sample were obtained via the Hydrocarbon Metagenomics project. Amplicon data were generated in the lab from extracted genomic DNA from the same environmental sample that generated the metagenome data and sequenced using the Illumina platform. Informatic analyses of these datasets were run to obtain ecological measures (rank abundances, diversity indices, taxonomic affiliation).

Both the metagenomic and amplicon datasets confirmed the presence of a diverse community of microbial eukaryotes in the tailings. The overall taxonomic affiliations of the sequences were broadly consistent. However, the amplicon-based study gave vastly more data than the metagenomic one, showing a large additional set of low abundance organisms present in the sample.

The community of microbial eukaryotes in the tailings pond is real, non-trivial and diverse. The breadth of the community within different ponds, at different spatial distributions and seasons should be explored to better understand the extent of what is present and how it changes periodically through the year so as to better plan reclamation efforts.

The amplicon-based analysis gave ~1,600x more data and revealed a much more complex picture of eukaryotic diversity. While metagenomic approaches give a broader picture of all genes from all microbes in the environment, for the specific question of assessing eukaryotic diversity an amplicon based approach is recommended at the present time.

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1 INTRODUCTION

1.1 Tailings Ponds and Microbiology for Reclamation

The Alberta oil sands are one of the biggest deposits of bitumen in the world, extending over 77,000 km^2 and distributed in three geographic areas: Athabasca, Cold Lake, and Peace River (Chalaturnyk et al. 2002). They consist of a mixture of highly viscous semi-solid crude oil, quartz, silts, clay, and water (Chalaturnyk et al. 2002). These reserves are one of the most important suppliers of U.S. oil, and contain about 26,798 m^3 (169 billion barrels) of bitumen (Energy Resources Conservation Board 2012), which is of a comparable magnitude to the world's reserves of conventional petroleum. The oil sands are a substantial driver for the Canadian economy. In 2012-13, royalties from the oil sands were $3.56 billion, and in 2012 the energy sector accounted for over 22% of Alberta's GDP (Government of Alberta 2014). The oil sands therefore have global significance as energy reservoirs and economic drivers. However, their exploitation is also a source of environmental concern.

During the process of extraction of mined oil sands, steam, solvents and/or hot air are injected to reduce the viscosity of bitumen and mechanical energy is used to separate the different components (Clark and Pasternack 1944). As result, a very large volume of sludge and wastewater with byproducts from the extraction process such as phenolic compounds, polycyclic aromatic hydrocarbons (PAHs) and naphthenic acids is produced (Nix and Martin 1992), and retained on site in tailings ponds that occupy 176 km^2 (Canadian Association of Petroleum Producers 2014), and contain a total volume of 720 million m^3 (Simieritsch et al. 2009).

Options to reclaim tailings include: (1) placing tailings in mined-out pits and capping them with water to form a lake (Hrynyshyn 2012); and, (2) drying the tailings through a variety of processes and capping with a soil cover to create wetland or upland ecosystems. In both cases the resulting environment is likely to be exposed to process-affected water (OSPW). Therefore understanding the starting composition of organisms within the tailings ponds is an important first step in reclamation planning.

Understanding the role of microbially driven processes is an integral part of reclamation efforts. Extensive work on the prokaryotic communities of the tailings ponds has demonstrated the role of microbes in both the beneficial settling of tailings solids and degradation of hydrocarbon (Siddique et al. 2006) and byproducts, and the detrimental production of greenhouse gasses (e.g., H_2S)(Siddique et al. 2010). Recently, the microbial communities have been examined in a more global way via the use of Next Generation Sequencing (NGS) technologies to describe the entire communities of the tailings ponds (An 2013).

1.2 Microbial Eukaryotes and Their Roles in the Environment

All of the efforts summarized above have focused on the prokaryotic (bacterial and archaeal) components of the communities, leaving aside microbial eukaryotes. However, it is clear that eukaryotes play key roles in microbial ecosystems as primary producers (photosynthesis and incorporation of nutrients), decomposers, or predators that regulate prokaryotic populations

(Walker et al. 2011). They also participate in mutualistic or parasitic relationships with eukaryotes on the higher levels of the food web. In marine environments, all of these complex interactions have been dubbed the *microbial loop* (Azam et al. 1983, Fenchel 2008).

Various environments including freshwater, soil, and marine have been studied for their diversity of microbial eukaryotes (e.g., Bailly et al. 2007, Lovejoy et al. 2006, Richards et al. 2005). However, comparatively less has been established about the microbial eukaryotes in environments that have been anthropogenically influenced.

1.3 Microbial Eukaryotes and Their Roles in Petrochemical Impacted Environments

A few studies have examined the effect of petrochemicals, primarily on phytoplankton, in oceanic environments (e.g., Ozhan et al. 2014). Microcosm work showed that introduction of crude oil was generally harmful to most phytoplankton groups, and the dispersants were highly toxic (Ozhan and Bargu 2013, Pickney 2012). Surprisingly, this effect was non-uniform across taxa and some groups (e.g., the diatom *Pseudonitzia* spp.) actually showed improvement upon addition of the oil (Pickney 2012). Starting conditions of the microcosms, such as seasonality, type of oil added, and nutrient content all affected the results (Ozhan and Bargu 2013). This highlights the fact that addition of hydrocarbons in an environment will affect community structure, but not necessarily in a straight forward or easily predictable manner. It also raises the possibility that petrochemical-enriched environments could allow the growth of previously uncharacterized organisms that, in other environments, would be outcompeted.

Additionally, examination of the organisms showed that the addition of hydrocarbons to the media or microcosm can produce alternations at the genetic and cellular level. It was shown that hydrocarbons can cause modification in organellar function, with plastids[1] being most heavily affected (Wang and Zheng 2008). Early experiments also showed that ciliates[2], when grown in the presence of crude oil, showed modified endomembrane organelles (Wyndham and Costerton 1981). Of particular relevance to the tailings ponds, it was shown that PAHs caused a reduction in expression of genes responsible for photosynthetic or cell division in diatoms (Bopp and Lettieri 2007). This gives rise to the hypothesis that organisms found living in hydrocarbon-enriched environments such as the tailings ponds may well exhibit unusual cell biology either as short-term responses to stimuli (altered gene expression) or due to long-term adaptation (genomic changes).

We have recently reported the first description of microbial eukaryotic communities in the tailings ponds (two ponds, from two different companies) using whole community molecular ecological methods (Aguilar et al. submitted). Therein we showed the communities not only existed, but were diverse, and dominated by taxa normally found in marine environments. This is consistent with the high salinity of the ponds themselves and with the prokaryotic communities characterized from these sites (An et al. 2013). Some of the eukaryotic sequences identified

[1] See http://en.wikipedia.org/wiki/Plastid

[2] See http://en.wikipedia.org/wiki/Ciliate

were highly similar to those found in other environments previously, but others differed and could only be placed as members of larger clades, thus suggesting that novel organisms exist in these environments. This study was greatly facilitated by the recent advances in DNA sequencing technology and their application in microbial ecology.

1.4 Metagenomic vs. Amplicon Based NGS Strategies For Microbial Ecology

The field of microbial ecology is currently undergoing a revolution, stemming from this adoption of new DNA sequencing technology. The traditional approach to study microbial communities was the use of cultures and microscopy, which are laborious and time consuming. Furthermore, these methods are strongly biased as many species cannot be easily grown in culture conditions. Incorporation of molecular methods enabled the use of multiple cloning, PCR and Sanger sequencing to characterize microbial communities, certainly an improvement over visual techniques alone. However, the advent of next generation sequencing (NGS) has reduced the costs and processing time of the samples, enabling a more in-depth knowledge of microbial communities, and detecting even the rarest organisms.

There are two predominant approaches to using NGS to address questions of microbial ecology, metagenome- and amplicon-based. Each holds theoretical advantages and disadvantages. The use of metagenomes has the advantage of being a more unbiased approach. As this strategy is based on random sequencing across all genomes present in the sample, information is obtained about bacteria, archaea and eukaryotes simultaneously. It also provides information about both functional genes and taxonomically useful regions. On the other hand, the amplicon strategy consists of the use of primers to amplify a specific region of the genome using the polymerase chain reaction (PCR). The small subunit ribosomal DNA (ssu rDNA) gene is most commonly used due to its historical use as a taxonomic marker and thus the extensive reference databases available against which to compare the obtained information for identification. This approach has the advantage of allowing for specificity in the question being asked as the primers can effectively target given taxonomic groups (e.g., eukaryotes to the exclusion of bacteria or archaea). However, primers may also have differential affinity for particular groups and so biases can exist that over-represent some organisms and exclude others. Both methods are compatible and together provide a more complete view of the community.

1.5 Research Objectives

In this report we address two related questions:

1. What information can be obtained about the microbial eukaryotic communities of the tailings ponds using NGS?
2. What are the relative advantages of amplicon and metagenomic NGS strategies going forward. Should one be used to the exclusion of the other to describe tailings pond microbial eukaryotic communities?

2 METHODS

2.1 Sample Description

2.1.1 *Site Information*

To make our analysis as comparable as possible, we analyzed samples from the same source, the pipe transferring tailings from Mildred Lake Settling Basin (MLSB) to West In-Pit (WIP)[3], operated by Syncrude Canada Ltd. Access to the metagenome data and the extracted whole genomic DNA from this sample was generously provided by Dr. Julia Foght, University of Alberta, under the auspices of the Hydrocarbon Metagenomes[4] project (Genome Canada).

2.1.2 *Sequencing Information for Metagenome and Amplicon Samples*

DNA was extracted and the metagenome was sequenced as previously described (An et al. 2013). Genomic DNA was amplified using universal primers for the V4 region of the small subunit of the ssu rDNA gene of eukaryotes (Stoeck et al. 2010), and using the following PCR conditions 95 °C for 5 minutes, 10 cycles of touchdown PCR[5]: 95 °C for 30 s, 60 °C for 30 s (decreasing at 0.5 °C/cycle), and 72 °C for 30 s, 30 cycles of regular PCR: 95 °C for 30 s, 55 °C for 30 s, and 72 °C for 30 s, 72 °C for 5 minutes. Amplicon libraries were created according to published protocols (16S Metagenomic Sequencing Library Preparation document[5]) and sequenced along with 20% PhiX control at 9pM on an Illumina MiSeq using a 500 cycle v2 kit for a paired end 250 bp run. Importantly, we analyzed both a metagenome and amplicon study derived by Illumina sequencing, so as not to introduce sequencing platform as a variable into the study.

2.1.3 *Bioinformatic Analyses of the Obtained Sequences*

The metagenome has been assembled using CLC Genomics Workbench and is publically accessible in IMG-ER under the accession number JGI ID Ga0010868. The V4 amplicon sequences were bioinformatically processed following several steps using the software Mothur. Firstly, a quality filtering based on removing any sequences that were too short or contain misreads, ambiguous bases, or long homopolymers was carried out. After that, potential chimaeric sequences formed by two pieces from different organisms, were identified and removed. Finally, sequences were compared to an existing database (SILVA[6]) of classified sequences to assign a taxonomic placement using BLAST[7].

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[3] Longitude 111.55 W and latitude 57.02 N

[4] See http://www.hydrocarbonmetagenomics.com/

[5] See http://support.illumina.com/downloads/16s_metagenomic_sequencing_library_preparation.html

[6] See http://en.wikipedia.org/wiki/Touchdown_polymerase_chain_reaction

[6] See http://www.arb-silva.de/

[7] See http://blast.be-md.ncbi.nlm.nih.gov/Blast.cgi

Once classified, the composition and abundance of the samples were evaluated. Statistical analyses were performed using the package vegan from R. Measures for richness and diversity (i.e., Shannon and Simpson indexes) were calculated and the structure of the communities was studied using plots with the relative and absolute abundances of the taxonomical groups.

2.2 Total ssu rDNA Sequences and Eukaryotic ssu rDNA Sequences per Sample

The small subunit ribosomal DNA gene was used to assess the microbial community in both the metagenomic and amplicon samples. While this is the gene targeted in the amplicon-based studies, in the case of the metagenomic sample, this information was extracted from the IMG database[8]. We then went on to assess the total number of sequences, and relative classifications of the sequences based at the level of taxonomic Domains (Bacteria vs Archaea vs Eukaryotic) and within eukaryotes.

One of the theoretical advantages of an amplicon-based study is the increased depth of coverage of the gene of interest. Consistent with this, the amplicon sample contained 28,271 SSU sequences, while the metagenome contained 248 ssu rDNA sequences. Of those sequences, all 28,271 of the SSU sequences in the amplicon sample were eukaryotic SSU sequences, as compared to only 17 of the sequences derived from the metagenome.

3 RESULTS

3.1 Classification

The ssu rDNA sequences were classified against the SILVA database, at the third level of classification. This gives an intermediate picture – neither too big nor overly specific in the taxonomic assignment.

3.1.1 *Proportion of Bacterial vs. Archael vs. Eukaryotic*

The amplicon-based study was performed using eukaryote specific primers. This approach was successful, and yielded no prokaryotic sequences in our analysis. By contrast the metagenome gave a picture of the overall microbial community, with only 6.85% of the sequences being derived from eukaryotes (Figure 1).

[8] See http://img.jgi.doe.gov/

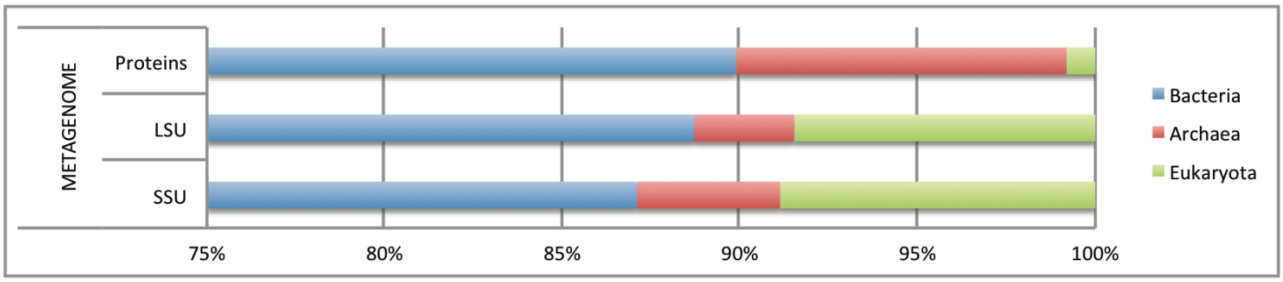

Figure 1. Relative proportion of total ssu rDNA sequences classified by domain. This graph shows the percentages of bacterial, archael and eukaryotic ssu rDNA sequences present in the MLSB metagenome dataset. Note that the number of non-eukaryotic ssu rDNA sequences in the amplicon dataset was 0 and thus the data are not shown.

3.1.2 *Classification of Eukaryotic Sequence at Level 3 of SILVA Database*

Both Shannon and Simpson diversity indexes (Table 1) were calculated for the two samples studied. The metagenome showed a much lower richness value (number of taxonomic groups present in the sample), but higher diversity indexes (measures of how evenly the organism are distributed across the groups present in the sample). This can be explained by the lower capacity of the metagenome to detect rare organisms. Rare groups contain a much lower proportion of the organisms that affect the diversity measurements when put together with the most abundant groups. These results are consistent with the rank abundance plots of the two samples (Figure 2).

Table 1. Diversity indexes for the metagenome and amplicon samples.

	Metagenome	Amplicon
Richness	44	8
Shannon index	1.931598	1.756537
Simpson index	0.8208465	0.7681661

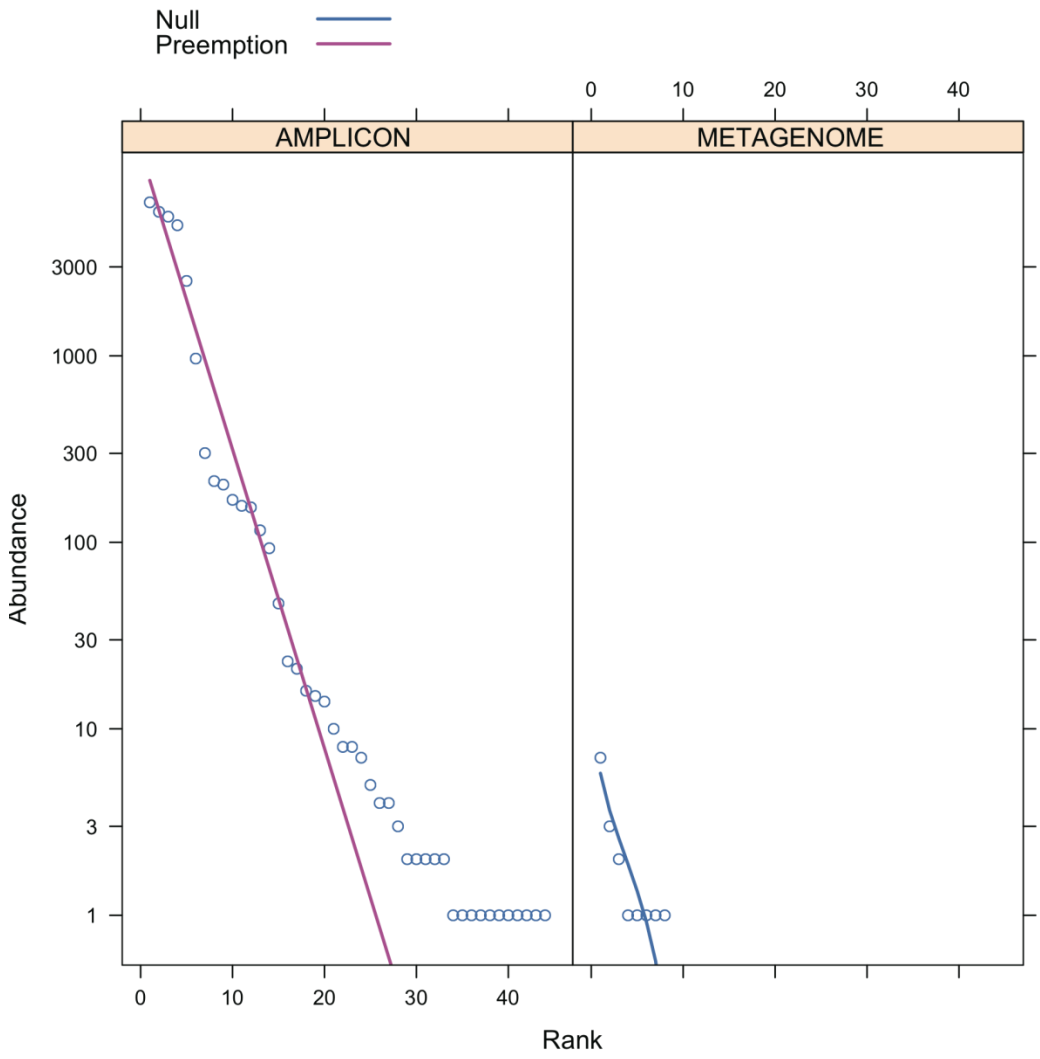

Figure 2. Rank abundance plots of metagenome and the amplicon samples based on SSU classification using SILVA database.
The horizontal axis represents the abundance rank. The most abundant taxon is given rank 1, the second most abundant is 2, etc.
The vertical axis represents relative abundance of the taxa. The metagenome sample showed a curve with a shorter tail than the amplicon sample, suggesting a more even distribution of the groups in the later case.

Accumulation curves (Figure 3) were also calculated for both samples. This allowed us to assess the extent to which the study has been exhaustive and how close are our results to the true numbers of the organisms present in the tailings ponds. The curve for the amplicon dataset was much closer to reaching a horizontal asymptote than was the metagenome dataset. This clearly shows that the amplicon analysis is a more exhaustive method and provides much more detailed information about the eukaryotic community than the metagenome.

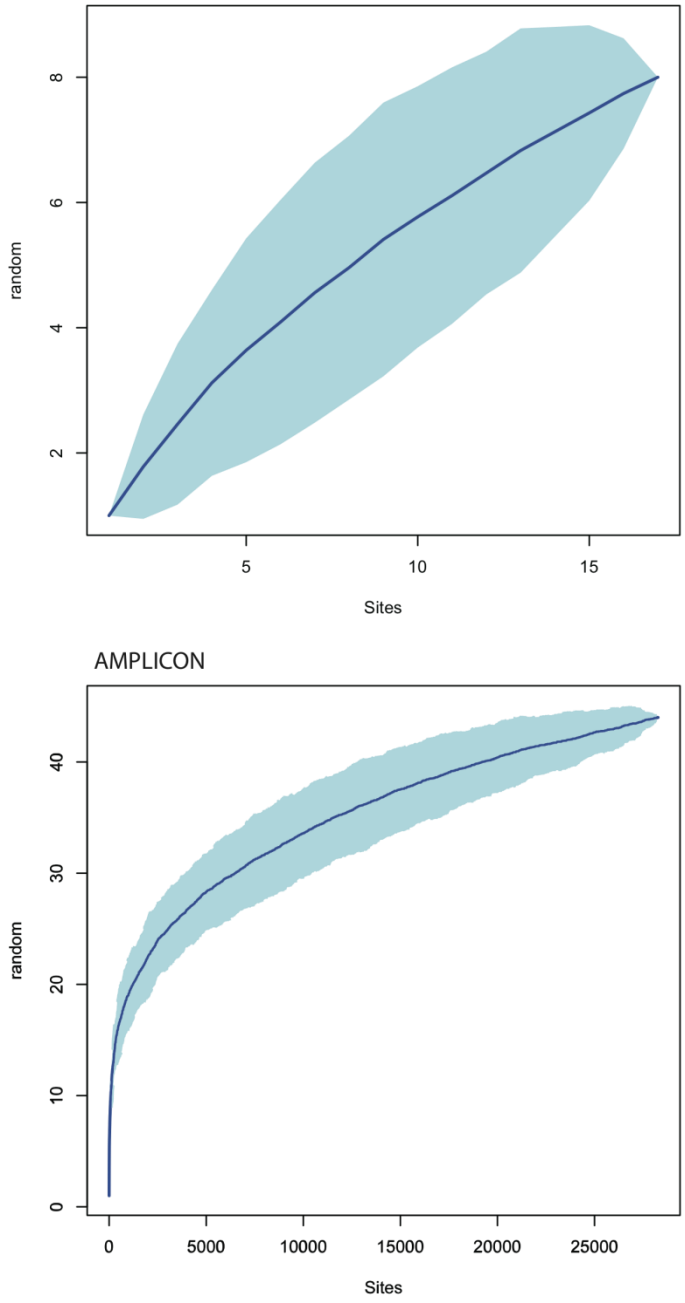

Figure 3. Accumulation curves for the metagenome (top) and the amplicon sample (bottom) based on SSU classification with SILVA.
The horizontal axis represents the number of sequences considered and the vertical axis the number of accumulated taxa. Sequences were randomized 100 times and the accumulated numbers of taxa with an increasing number of sequences were calculated. The solid lines represent the accumulation curves obtained with the "exact" method of the "specaccum" function from the R package vegan, and the textured areas correspond to their standard deviations.

3.2 Comparisons of Community Structure and Hypothesis Generation

The classification of sequences using SILVA database (Figures 4 and 5) showed that in both the metagenome and the amplicon-based analyses Nucletmycea LKM11, Cercozoans, Fungi and Chlorophyta are dominant groups. However, the amplicon analysis also revealed a higher number of low abundance groups of organisms that were not detected in the metagenomic analysis.

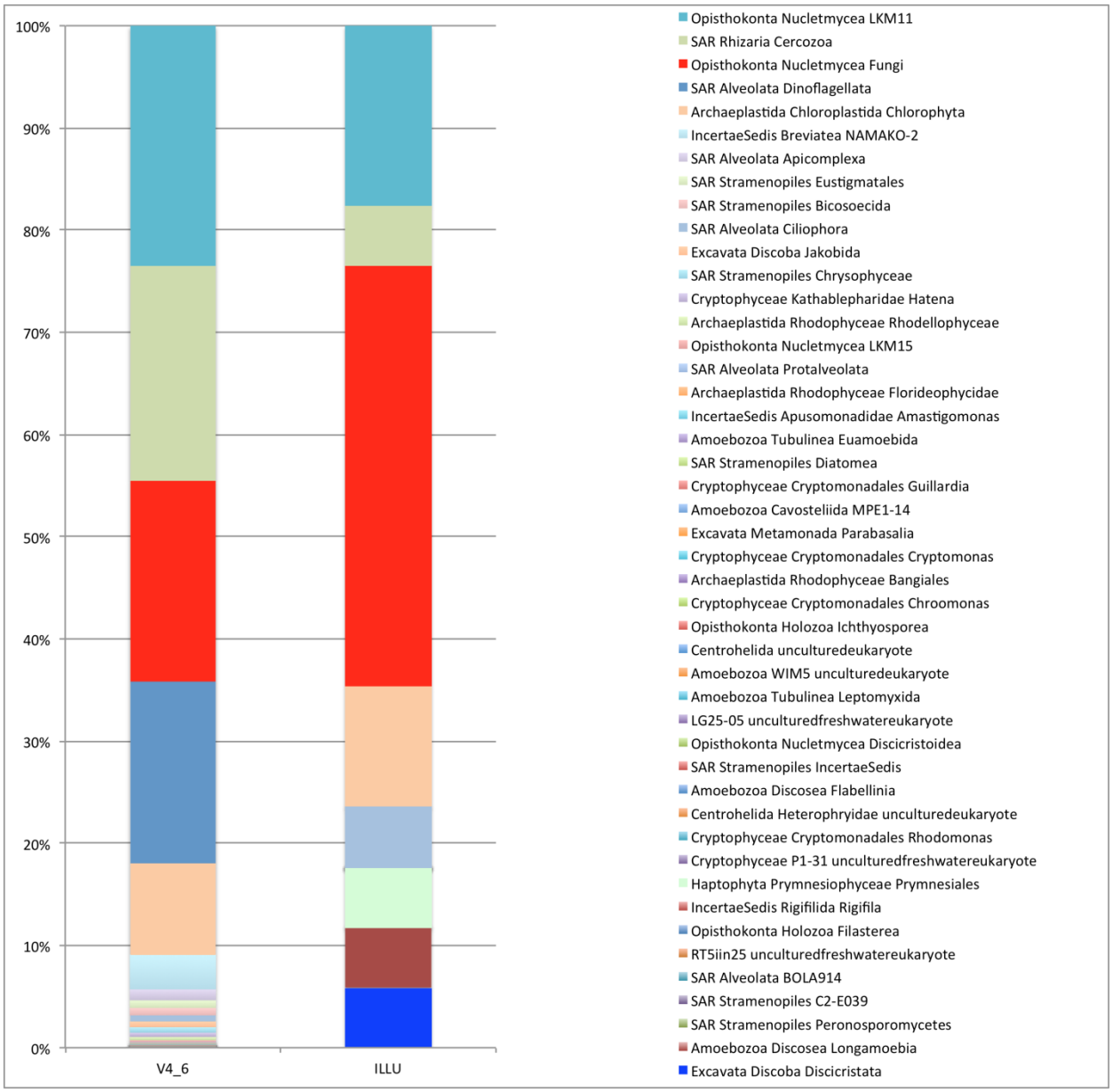

Figure 4. Relative abundance of eukaryote groups found in the tailings ponds amplicon (left) and metagenome (right) sample based on a SILVA classification of the SSU.

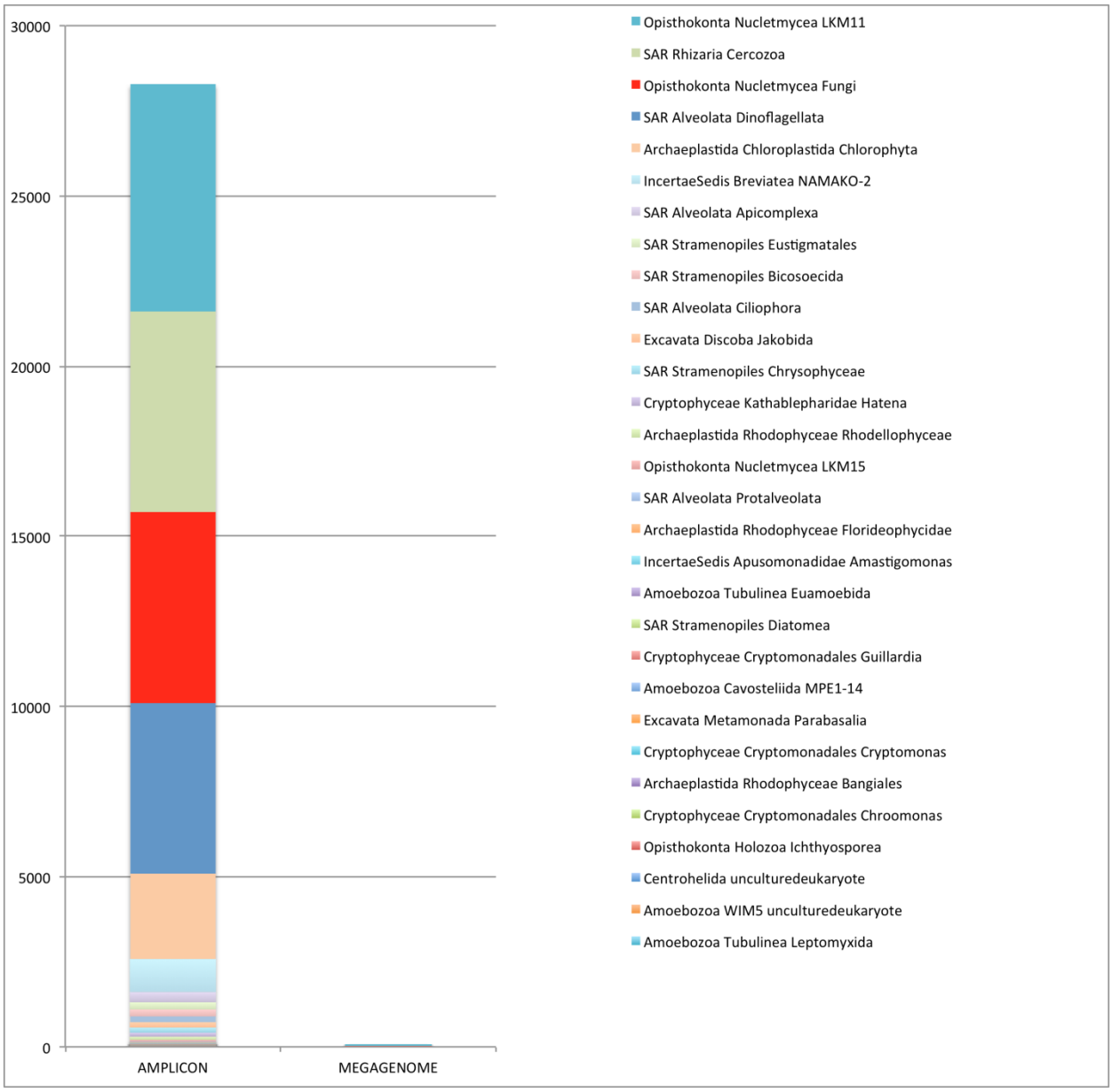

Figure 5. Absolute abundance of eukaryote groups found in the tailings ponds amplicon and metagenome sample based on a SILVA classification of the SSU.

4 DISCUSSION

This report provides the first analysis of metagenomic versus amplicon-based data of microbial eukaryotes from a tailings pond. Importantly for comparability, we used samples from the same tailings pond and as far as possible used the same analytical tools and assessment measures between the samples. Overall, the work reinforces the findings, from metagenomic data alone (Aguilar et al. submitted), that diverse microbial eukaryotes are present within the ponds and thus need to be taken into account when considering the overall microbial community.

It is worth noting that the major groups of organisms identified in both samples were consistent. This was despite the massive difference in the total number of eukaryotic ssu sequences sampled in the two datasets (Figure 5). Overall this trend suggests that the initial glimpse shown by the metagenome dataset is still representative of the overall microbial eukaryotic community. What is missing is the extent to which the groups dominate and the tail of vastly lower abundance organisms also present in the sample. These low abundance groups may nonetheless be important, as we currently have no information about the community dynamics of the tailings ponds due to physical stratification of the ponds, or seasonality.

It is tempting to interpret the identity of the organisms found in the samples in the light of the chemistry of the tailings ponds. However, given these results are only from a single metagenome and amplicon-based sample, care should be taken in doing so. This is even further reinforced by the fact that using a different database, somewhat different taxonomic assignments were made for the same metagenome sample. Further investigation into the effect that the choice of database plays on taxonomic assignment is warranted, as is more robust assignment of the sequences by phylogenetic analysis.

What is clear is that regardless of the precise taxonomic assignments, the microbial eukaryotic communities are complex and warrant much more extensive investigation.

4.1 Advantages of Amplicon Data

The second aim of the project was to assess the relative strengths and weaknesses of metagenomic versus amplicon-based strategies to studying the microbial eukaryotic community of the tailings ponds. The advantages of using amplicon-based approaches to assess microbial eukaryotic communities are manifest in this report. Due to the focus on a single region of a single gene, equivalent sequencing efforts are able to yield far greater depth into the overall community. The 1/12 of the MiSeq run[9] here yielded ~1,600 times more sequences than the metagenome dataset. Thus while the metagenome did show that microbial eukaryotes are present, the amplicon data enabled an assessment far deeper into the abundant and the rare members of the community. It also gave a more complete picture of the community, reaching near saturation in the accumulation curves, while the metagenome clearly just scratched the surface.

As well, since the amplicon data are derived from the homologous region of the ssu rDNA gene, the sequences are directly comparable at the operational taxonomic unit (OTU) level. Unfortunately, due to time considerations and limits on computational resources, the OTU analyses were not possible to include in this report. Theoretically, these would give far more precision in the assessment of abundance, as compared to metagenomic data. This is because, due to the random nature of metagenomic data, the sequence obtained for a given ssu rDNA gene does not necessarily correspond to the same region in every case. Consequently, sequences that

[9] As the library reported here was run along-side 11 others, it corresponds to 1/12th of the total sequence obtained in that sequencing run.

share the same taxonomic classification at a given rank might not be the same actual organisms or could in fact be portions of the same individual sequence. As future analyses move into comparisons of fine-scale spatial distribution (vertical and horizontal) within a given pond (and thus potentially an assessment of succession as tailings are added over time), seasonality within the same pond, or between ponds, it will be even more important to be able to assess whether the same organisms versus the same types of organisms are responsible for differences between samples.

4.2 Advantages of Metagenome Data

Although amplicon-based data have clear advantages, there are other theoretical advantages to taking a metagenomic approach. Firstly, is the issue of bias. Because amplicon-based studies are based on an initial PCR step, this introduces a danger of primer bias. As metagenomic data uses total DNA this risk is eliminated. Furthermore, because of the amplicon study design, eukaryotic sequences alone were targeted. This yielded vastly more depth of enquiry into the sample. However, the whole metagenome provided information about the bacterial, archaeal and eukaryotic components, thus giving a broader view of the microbial community. Finally, although not utilized in this study, the metagenome does give information beyond the one gene of interest. For enzyme discovery, particularly where the taxonomic information is less central to the question being asked, a metagenomic approach obviously yields far more relevant information.

Although the fact that the metagenomes do not always give sequence information for the same region of the ssu rDNA gene can be a disadvantage limiting analyses at the OTU level, in some cases it can yield more sequence data for the gene of interest than an amplicon-based strategy, which is intentionally limited to the V4 region. As more sequence can give more phylogenetic information, this can lead to greater precision to the taxonomic assignment of a given sequence. As an example, in the recent metagenomic study of tailings ponds (Aguilar submitted), it was possible to use all sequences over 1,000 bp in length found in the metagenomes and subject them to phylogenetic analysis. To perform an equivalent study starting from an amplicon-based strategy, this would require follow-on experiments using PCR to obtain the entire sequence before being able to do phylogenetic placement or identification of the cell by fluorescence in-situ hybridization.

5 CONCLUSIONS

We have here demonstrated the feasibility and utility of metagenomic and amplicon-based strategies to understanding microbial eukaryotic communities in tailing ponds. These components, although of relatively low abundance compared with prokaryotes, are diverse and non-trivial and thus should be taken into consideration in any future assessments of microbial-based strategies for management and reclamation.

Metagenomic and amplicon-based analyses have both theoretical and demonstrated strengths in assessing the microbial communities in the tailings ponds. The value of their respective use depends in large part on the question being asked. For assessments of community structure of

microbial eukaryotes only, a targeted amplicon-based strategy is clearly superior. For assessments of the entire community, it is possible that an amplicon-based strategy with universal primers (i.e., those that can amplify both prokaryotic and eukaryotic sequences) is advisable. If information beyond the taxonomic composition of the environment is required, then the metagenomic approach begins to be more attractive. In such a case, far more depth of sequencing of each individual sample would likely be advisable in order to overcome the relatively shallow picture obtained in the single metagenome that was analyzed here.

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7 GLOSSARY

7.1 Terms

Accumulation Curve

The more intensively a community of organisms is studied, the more likely it is to find a higher number of the organisms that are actually present. The accumulation curve is a graphical representation of the number of taxonomical groups found using a given sampling effort. The probability of finding new organisms progressively decreases as our knowledge about the community grows. In other words, the more we know about the system, the more difficult it gets to learn something new. This causes the curve to have a flattened appearance (saturation) when most of the taxonomical groups are found.

Amplicon

Region of DNA produced via an amplification reaction, in this case by a polymerase chain reaction.

Archaea

One of the three fundamental "Domains" of life, as assessed by phylogenetic methods that judge genetic distance and by a suite or shared morphological features that are exclusive to these organisms. These cells lack a nucleus enclosed by a membrane. They possess lipids that are based on an ether linkage of their acyl-glycerides to the phosphate backbone and possess a specialized isoprenyl lipid.

Base Pair

DNA is assembled of polymerized deoxyribonucleic acids (referred to as a base). Each DNA chain is composed of two polymers whereby the corresponding subunits interact or pair-up via hydrogen bonds. The unit of two bases that are bonded, one on each chain, are deemed a base pair.

Bioinformatic

The use of computational methods to analyze biological information, in this case DNA sequence. Often this involves large-scale analyses of data and may involve programming or modification of scripts for use on computational clusters, rather than the use of 'out of the box' software.

Chimaeric Sequence

In the sequencing process, using a "paired end read" approach, the same DNA molecule is read from both ends (5' and 3'). Therefore an important step of the post-processing bioinformatic analysis is pairing up of sequences that the correct reads from both ends of the same DNA molecule. In cases where the program incorrectly assembles reads from two different molecules and treats it as a new or unique sequence, this is deemed a chimaeric sequence.

Clade

All organisms that are the descendents of a common ancestor.

Eukaryote

One of the three fundamental "Domains" of life, as assessed by phylogenetic methods that judge genetic distance and by a suite or shared morphological features that are exclusive to these organisms. Although defined by the presence of genomic DNA enclosed by a double lipid bilayer (the nuclear envelope/endoplasmic reticulum), the presence of other organelles such as the Golgi body, mitochondria-related organelle, endosomes are also often taken as pan-eukaryotic features.

Homopolymer

Any substance that is composed of a single repeating subunit. In this case, this refers to regions of DNA that have the same single DNA base in succession.

Metagenome

The composite assemble of all genomic DNA, from many different organisms, found in an environment.

Next Generation Sequencing

Refers to a set of post-sanger sequencing methodologies that produce large numbers of, often short, reads. Also treated as synonymous with "high through-put" sequencing. In this case we use the Illumina platform.

Operational Taxonomic Unit (OTU)

This term describes a proxy for "species-level" identity in DNA sequences derived from the environment, where the organisms from which the DNA was taken were not identified. An OTU made up of numerous sequence "reads" would be regarded as representing numerous instances of the organism from which the DNA sequence came. However in cases where an organism has more than one copy of the DNA sequence, there may not be a linear 1:1 match between number of reads and number of organisms.

Paired End

Protocol for next generation sequencing where the same molecule is sequenced from both directions and the reads are matched.

Prokaryote

Cells that do not have their genomic DNA enclosed by a membrane. Can be either Bacteria or Archaea.

Richness

The total number of taxonomical groups present in a community.

Sequence

The order of bases in a DNA chain or molecule.

Shannon Index

A measure of diversity that simultaneously takes into account how many taxonomical groups are present and how evenly distributed the organisms are across the groups. The index increases both with an increasing number of groups and with a more uniform distribution of the organisms between the groups. It is a measure of entropy in the system, as the higher the number of groups and the more equally distributed the individuals between the different groups the more difficult is to predict to which group one randomly selected individual belongs to. The formula used for its calculation is:

$$H' = -\sum_{i=1}^{R} p_i \ln p_i$$

Where R is the number of groups and p is the number of individuals that belong to each group.

Simpson Index

A measure of diversity based on similar principles as the Shannon index. It is calculated using the formula below:

$$\lambda = \sum_{i=1}^{R} p_i^2$$

Where R represents the number of groups and p is the number of individuals that belong to each group.

7.2 Acronyms

bp	Base Pairs
DNA	Deoxyribonucleic Acid

NGS	Next Generation Sequencing
OSPW	Oil Sands Process-affected Water
OSRIN	Oil Sands Research and Information Network
MLSB	Mildred Lake Settling Basin
PAH	Polycyclic Aromatic Hydrocarbon
PCR	Polymerase Chain Reaction
SEE	School of Energy and the Environment
WIP	West In-Pit

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OSRIN reports are available on the University of Alberta's Education & Research Archive at http://hdl.handle.net/10402/era.17209. The Technical Report (TR) series documents results of OSRIN funded projects. The Staff Reports (SR) series represent work done by OSRIN staff.

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