Final Report

Exploring the Potential of Native Microbial Consortium for Biodegradation of Plastic Wastes in Compost

Report to Divert Nova Scotia

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Summary

Increase in global population and advances in technology have necessitated the use of plastic materials for domestic and industrial application. However, plastics are recalcitrant and hence, remain inert to degradation and deterioration leading to their accumulation in the environment. These create serious environmental, health and socioeconomic problems. One of the biggest challenges of the compost industry is contamination from plastic wastes. The ability of microorganisms to use polyethylene (plastics) as a carbon source has been recently established and this can be affected by environmental, chemical and biological factors. Native microbial communities can vary with variations in abiotic and biotic factors and from region to region. The biodegradability of plastics by native microbial consortium in compost in Canadian context was explored.

Key word: Plastics, Micro organisms, biodegradation, degradation, Metagenomics

Introduction

The current rate of global use of polyethylene and plastic product is approximately 12% per annum, and this continues to rise. The high consumer demand drove global production to approximately 140 million tons of synthetic polymers, which has increased by 1.74-fold over the past 15 years to about 243 million tons (Manika *et al.,* 2015; Raziyafathima *et al.,* 2016). The rise in production and use of synthetic polymers has increased the amount of global plastic wastes with numerous adverse effects on the environment, a concern expressed by the public including environmental advocates, growers and researchers. Plastic material and its utilization has found wide application in virtually all aspects of human life in both domestic and commercial settings. Thus, hardly will one do without encountering plastics or its product daily. However, one of the major environmental threats posed by these plastics is their inability to breakdown or their low rate of breakdown, which thereby, lead to environmental pollution, blockage of water ways and causing death of marine fresh water flora and fauna. Another negative impact is that plastic contamination in the soil can affect seed germination, plant establishment, root penetration, and impede nutrient and water uptake. Interestingly, there are few published scientific literatures to establish these facts.

Figure 1. Compost contaminated with plastic taken from Fundy Compost, Brookfield, NS.

Globally, plastic wastes are estimated to comprise at least 16% of the total amount of municipal solid wastes in most landfills (Muenmee *et al.*, 2014). Compost is well-known to be a microcosm of large number and diverse populations of microorganism(s) such as bacteria, fungi and actinomycetes, which help in the decomposition of organic and some inorganic materials (Friend and Smith, 2017). Plastic wastes in compost has been labelled as "devil" (Shah, 2001; Walter Termeer, CEO, Fundy compost, pers. comm. 2014) due to the numerous negative impacts. However, many research in the area of biodegradation of plastics has been carried out on soils, raw plastics and they are done *in vitro* while decomposition of plastics in compost is understudied especially, in North America. Native microbial communities can vary with variations in abiotic (e.g. light, temperature, pressure) and biotic (e.g. microorganisms and enzymes) factors, and from one geographic region to another. Thus, there is the need to investigate the potential of native microbial species in any locality that can decompose plastics in compost. It was therefore, hypothesized that age of compost and pile environmental conditions (i.e. location) and presence of plastic can affect the diversity and richness of native microbial community in compost. Hence the objectives of the study are:

General Objective

To evaluate the potential of microbial communities to degrade plastic in compost.

Specific objectives are to:

- evaluate the diversity and richness of the microbial community in compost.
- evaluate the effect of the presence of plastics on the microbial community in compost.

Materials and Method

Site Description

Samples were collected from four different composting facilities, namely:

(1) Colchester Composting Facility, Kemptown; (45°27'24.6"N 63°06'20.1"W)

(2) Valley – Northridge Farms, Aylesford (45°03'20.9"N 64°50'27.6"W)

(3) Fundy Compost Inc., Brookfield (45°15'01.5"N 63°20'46.9"W)

(4) Guysborough Composting Facility, Boylston (45°29'33.7"N 61°32'15.2"W)

Sample collection

Five (5) different bulk compost associated with partially decomposed plastics/low density polyethene (LDPE) were collected from compost piles per location. In addition, 500 g of compost samples within 10 cm radius around the sampled plastic films was collected at each location using sterile hand auger. The plastic and compost samples were kept in labeled sterile plastic bags and immediately placed in a cooling box with icepacks before transporting to the laboratory. The samples were then processed within 24 hrs.

Preparation of compost samples

Approximately 10 g of the compost samples were sieved using 2-mm sieve and kept at -80^{o}C for further analysis at the Dalhousie Faculty of Agriculture Microbiology lab. DNA was isolated from 0.25 g of fine compost after sieving.

Preparation of plastic samples

Five (5) g of bulk compost associated with partially decomposed plastics was placed into a conical flask and 150 ml of sterile 10% glycerol was added before placing on a shaker for 15 min. The mixture was sonicated for 15 min before removing the plastic which was again placed in another flask, and the process was repeated. The solution from two cleaning steps was combined and centrifuged at 4000 rpm for 30 min and the supernatant was decanted. The plastic pellets formed were transferred into 1.5 ml Eppendorf tubes and centrifuged again at 8,000 rpm for 10 mins. The supernatant was discarded and the sample was stored at -80^{o}C until processing for DNA isolation.

DNA extraction and sequencing

DNA extraction was carried out using the PowerSoil DNA Isolation kit (MO BIO Laboratories, Carlsbad, CA, USA) according to the manufacturer's protocol. DNA quality and concentration will be measured by NanoDrop 1000 spectrophotometer (Thermo Scientific, Waltham, USA). Five microlitres of each isolated DNA sample were sent to the Dalhousie University Centre for Comparative Genomics and Evolutionary Bioinformatics – Integrated Microbiome Resource (CGEB-IMR) (http://cge.b-imr.ca/) for V6-V8 16S rRNA gene and fungal ITS (internal transcribed spacer) gene library preparation and sequencing. Samples were multiplexed using a dual-indexing approach and sequencing using an Illumina MiSeq with paired-end 300+300 bp reads. Polymerase chain reaction (PCR), primers and Illumina sequencing details were as described by Comeau *et al*., (2017).

Sequencing data processing

Microbiome Helper Standard operating procedure as described by Comeau *et al*., 2017 were used to process and analyse the sequencing data. Overlapping paired-end reads were stitched together using PEAR (v0.9.6; (Zhang *et al.,* 2014)). The 16S and ITS reads were successfully stitched with 94.8% and 68.9% respectively, FASTX-Toolkit (v0.0.14; Gordon 2009) was later ran to filter out reads that did not have at least 90% of nucleotides (nt) with a quality score greater than 30. In addition, we filter out reads shorter than 400 bp that did not contain matching 3'and 5' sequences to the appropriate forward and reverse primers with BBMap (v35.85; Bushnell 2014). Lastly, we ran USEARCH (v6.1; Edgar *et al.,* 2011)) to screen out chimeric reads using the options mindiv=1.5 and minh=0.2.

OTU picking and statistical analyses.

Following these filtering steps above, we ran open-reference OTU picking using QIIME wrapper scripts (Caporaso *et al.,* 2010). Specifically, SortMeRNA (v2.0-dev; (Kopylova *et al.,* 2012)) was used for the reference OTU picking steps with sortmerna_coverage=0.8 and sumaclust (v1.0.00; Mercier et al. 2013) for the *de novo* OTU picking steps with 10% of the failures sub-sampled. OTUs that contained fewer than 0.1% of the total sequences were filtered out in order to compensate for MiSeq run-to-run bleed-through (Comeau *et al.,* 2016). Then alpha-diversity (richness and Chao1) and beta diversity i.e. weighted Unifrac distance (Lozupone *et al.,* 2011) metrics using QIIME was generated (Caporaso *et al.,* 2010). The "cca" function from the R package vegan (v2.4-0; Oksanen et al. 2016) was used to run our canonical correspondence analyses (CCAs). Spearman and Tukey's pairwise tests were carried out using Past3 package (Hammer et al., 2001). Adonis tests (999 permutations) were run in QIIME to calculate how sample groupings, Sample types and location are related to microbial community structure. These tests are a measure of how much variation in community structure is explained by the variable of interest. Pairwise Spearman correlations between factors was performed using the "rcorr" function of the Hmisc R package (http://biostat.mc.vanderbilt.edu/wiki/Main/Hmisc). To test for fungi that have differential abundance across metadata categories, the ITSS BIOM table was parsed to containing only fungal OTUs. Analysis of taxonomic profiles was performed using the STAMP

software package (Parks *et al.*, 2014), While analysis of statistical significance ($\alpha < 0.05$) of sample grouping was done using QIIME (Caporaso *et al.*, 2010)

Result and Discussion

Composition of microbial communities across all locations

All the data analysis was conducted as stated in above section using the standard operating procedure of the CGEB-IMR as outlined in the Microbiome Helper package (QIIME). After the first analysis seven reads were very low therefore their DNA were re-isolated and sent for re-amplification. A total of 838,769 16S and 825,446 ITS sequences were obtained. After normalization these reads were distributed among 4,391 bacterial and 653 fungal OTUs and repressed to 593 bacterial and 198 fungal taxa.

The relative abundance of fifteen major microbial taxa (representing 89% and 90% of bacteria and fungi respectively) identified at class level in this study were as follows:

(a) Bacteria16S rRNA: *Alphaproteobacteria, Saprospirae, Deltaproteobacteria, Bacilli, Chloracidobacteria, Gammaproteobacteria, Actinobacteria, Betaproteobacteria, Anaerolineae, Gemmatimonadetes, Cytophagia, Flavobacterii, Sphingobacteriia, Acidobacteria and Gemm-5*

(b) Fungi ITS: *Cystobasidiomycetes, Sordariomycetes, Pezizomycotina_cls_incertae_sedis, Orbiliomycetes, Dothideomycetes, Exobasidiomycetes, Agaricomycetes, Taphrinomycetes, Saccharomycetes, Pezizomycetes, Leotiomycetes, Eurotiomycetes, unclassified and unclassified.*

Also, 16S rRNA and fungal ITS amplicon analysis showed that variations in microbial (bacteria and fungi) populations were significantly ($P<0.01$) influenced by locations and age of the pile not by the presence of plastics or association with plastics (sample type). However, in order to establish the diversity of the microbial communities within the four locations studied, Principal Coordinate Analysis (PCoA) was used. This showed the visual separation in microbial compositions from each location of compost sites studied and age of the compost piles sampled (Figures 2 and 3). This ecological dissimilarity was done using Bray-Curtis ecological distance.

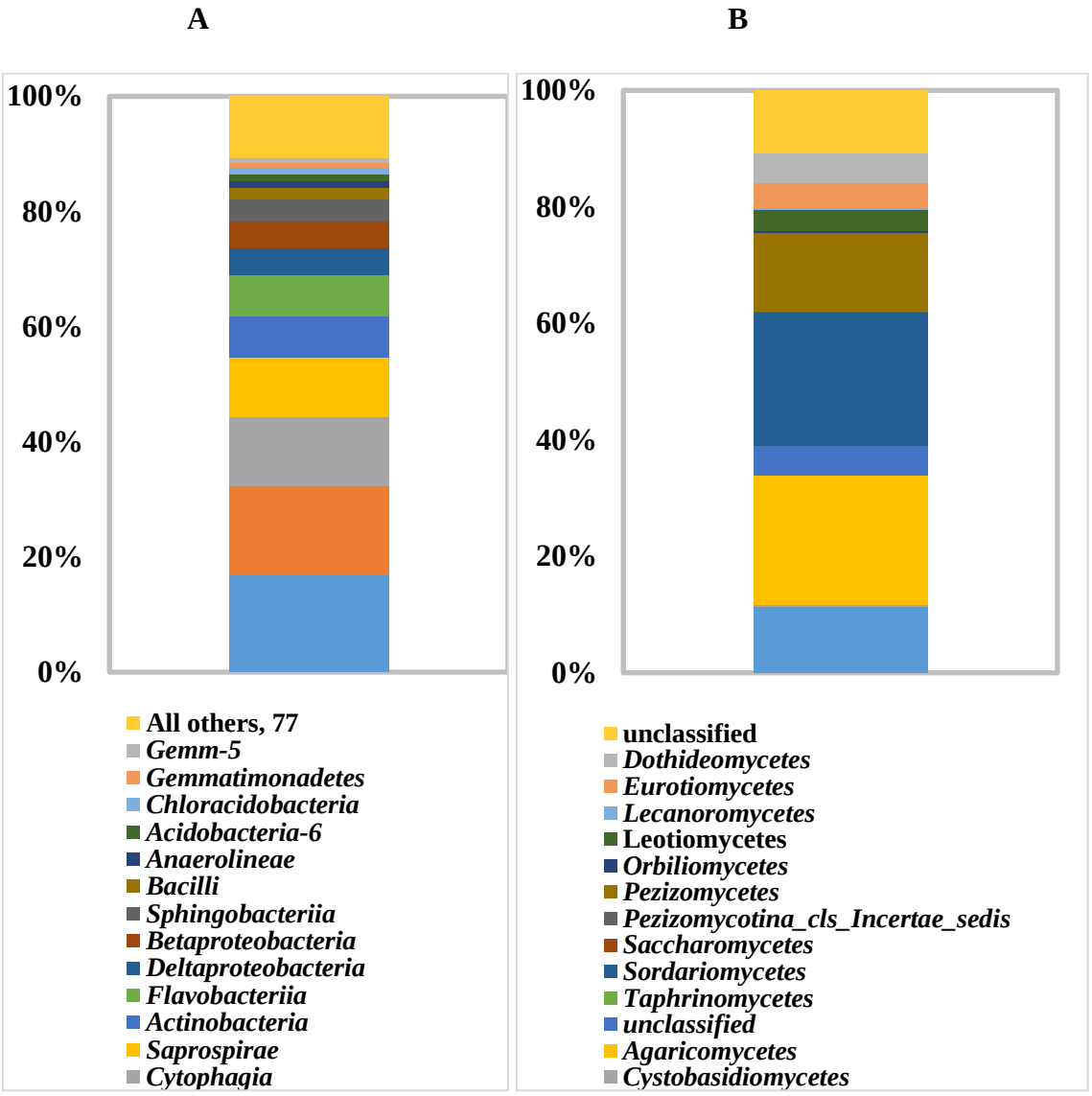


Fig. 1. Relative abundances of major microbial taxa identified in this study bacteria16S rRNA (A) and fungi ITS (B).

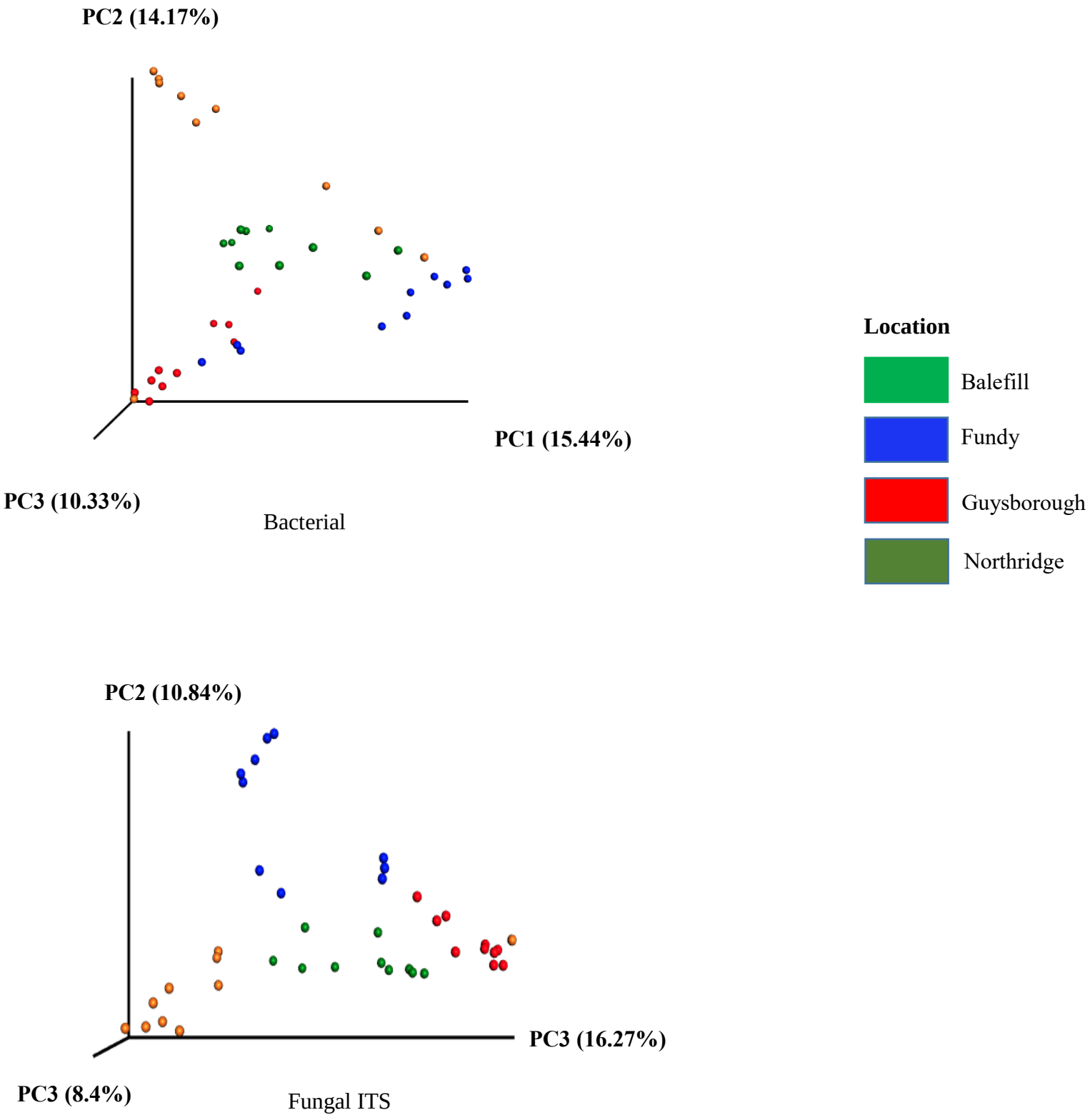


Fig 2. Principal coordinate analysis of microbial communities (upper figure: bacterial and lower figure: fungi) based on Bray-Curtis ecological distances for location.

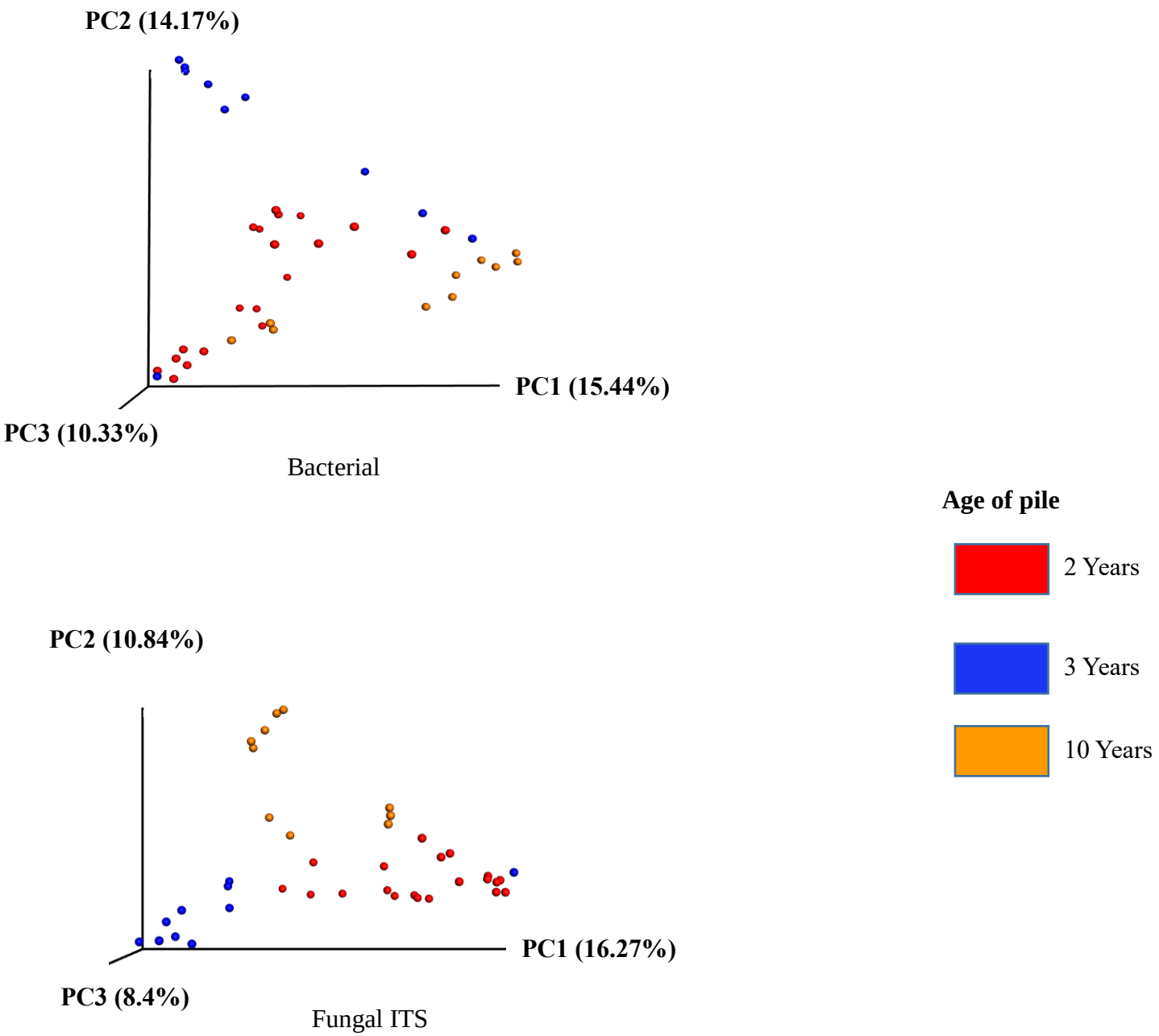


Fig 3. Principal coordinate analysis of microbial communities (upper figure: bacterial and lower figure: fungi) based on Bray-Curtis ecological distances for age of pile.

Analysis of variance using distance matrices for partitioning distance matrices among sources of variation and fitting linear models (Adonis) tests indicated that grouping by location of compost

facility was significant for bacterial (R^2 = 0.26389, P < 0.001) and fungal ITS (R^2 = 0.2774, P < 0.001; Fig. 1 and Table 1). While grouping by age of the piles also showed a strong effect for bacterial (R^2 = 0.1052, P < 0.001) and fungal ITS (R^2 = 0.0938, P < 0.001; Fig. 2 and Table 1).

Table 1: Variation in Time as explained by weighted UniFrac and Bray-Curtis beta-diversity.

	16S		ITS
Grouping (subset)[a]	Weighted Unifrac (R^2)	Bray – Curtis (R^2)	Bray – Curtis (R^2)
Location	0.25734***	0.26389***	0.2774***
Age of pile (Time)	0.08611**	0.1052***	0.09383***
Sample Type	0.03411	0.02601	0.02809

[a] Weighted UniFrac and Bray-Curtis beta-diversity distances were calculated for each subset of samples. Adonis tests were used to assess whether beta-diversity is related to sample groupings, 999 permutations, R^2 and p-values.

Significant differences in relative abundances of several bacterial and fungal taxa were detected between different compost sites locations (Fig. 4). These differences in structures and functions of microbial communities in each location were further established using statistical analysis of metagenomics profiles (STAMP).

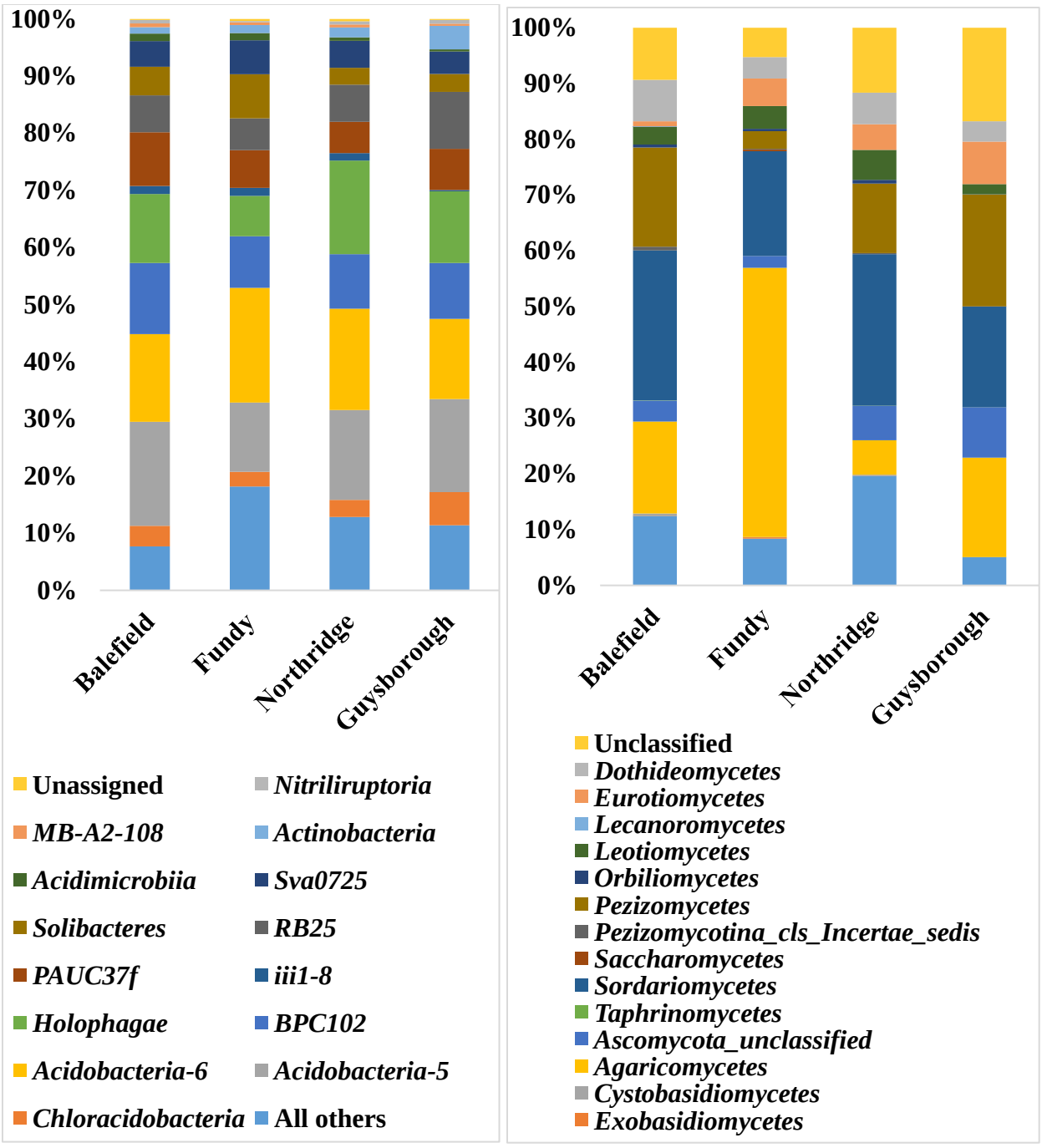


Fig. 4. Microbial taxa identified from different locations.

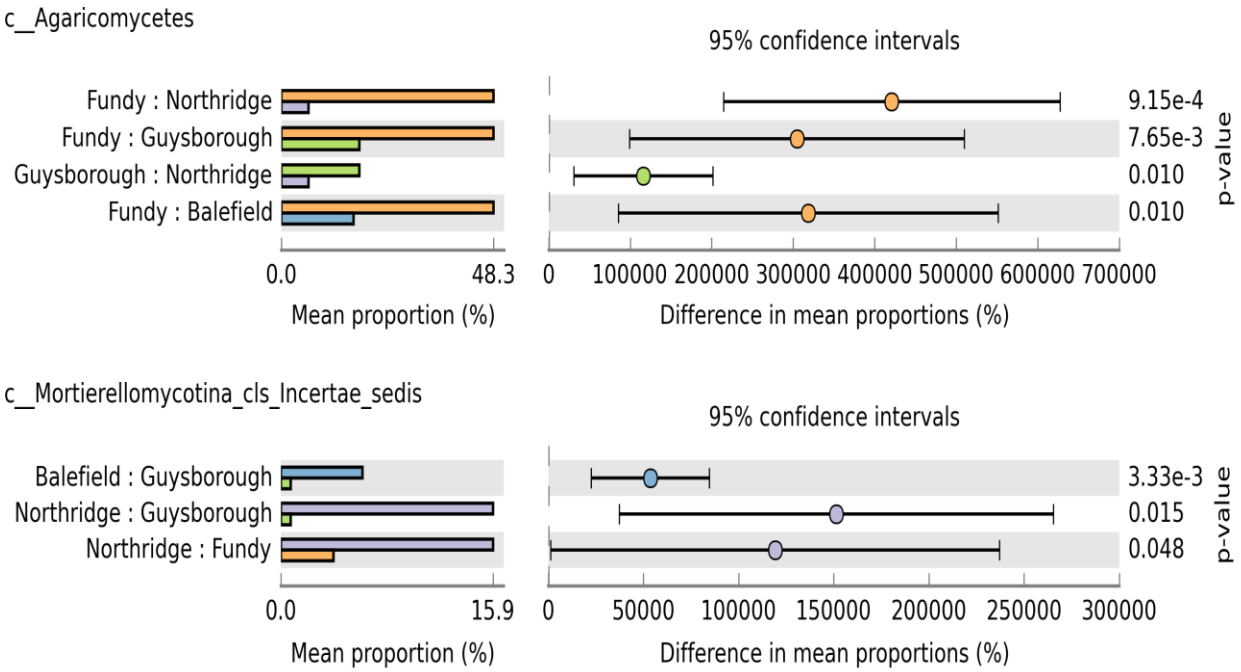
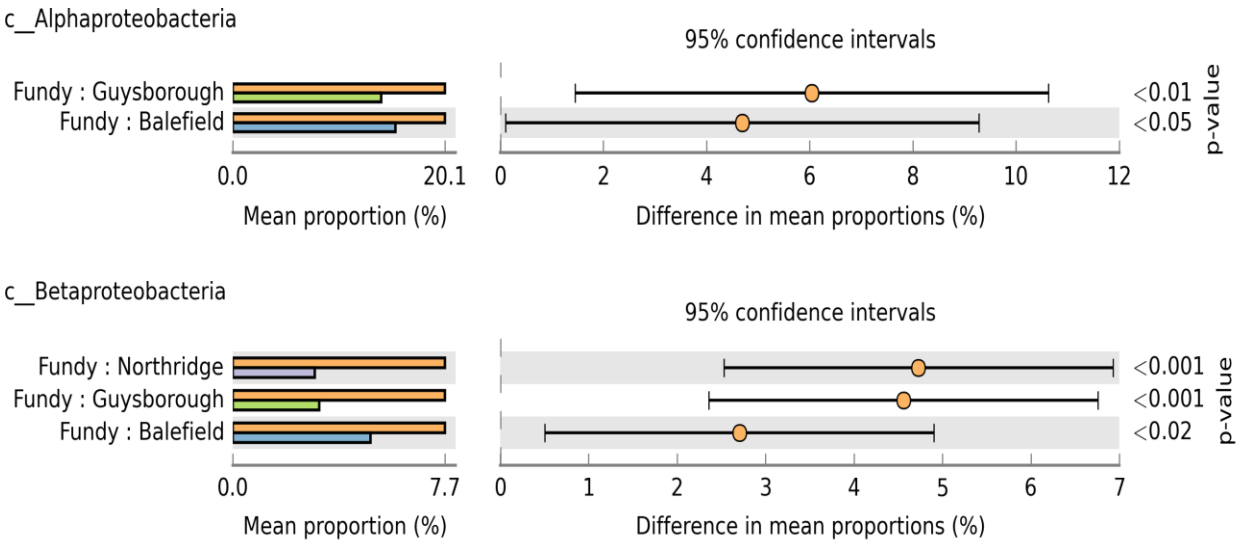


Fig. 5. The relative abundance of fungi at the class level based on ITS sequencing. Corrected p-values were calculated using Benjamini-Hochberg false discovery rate approach (P<0.05).



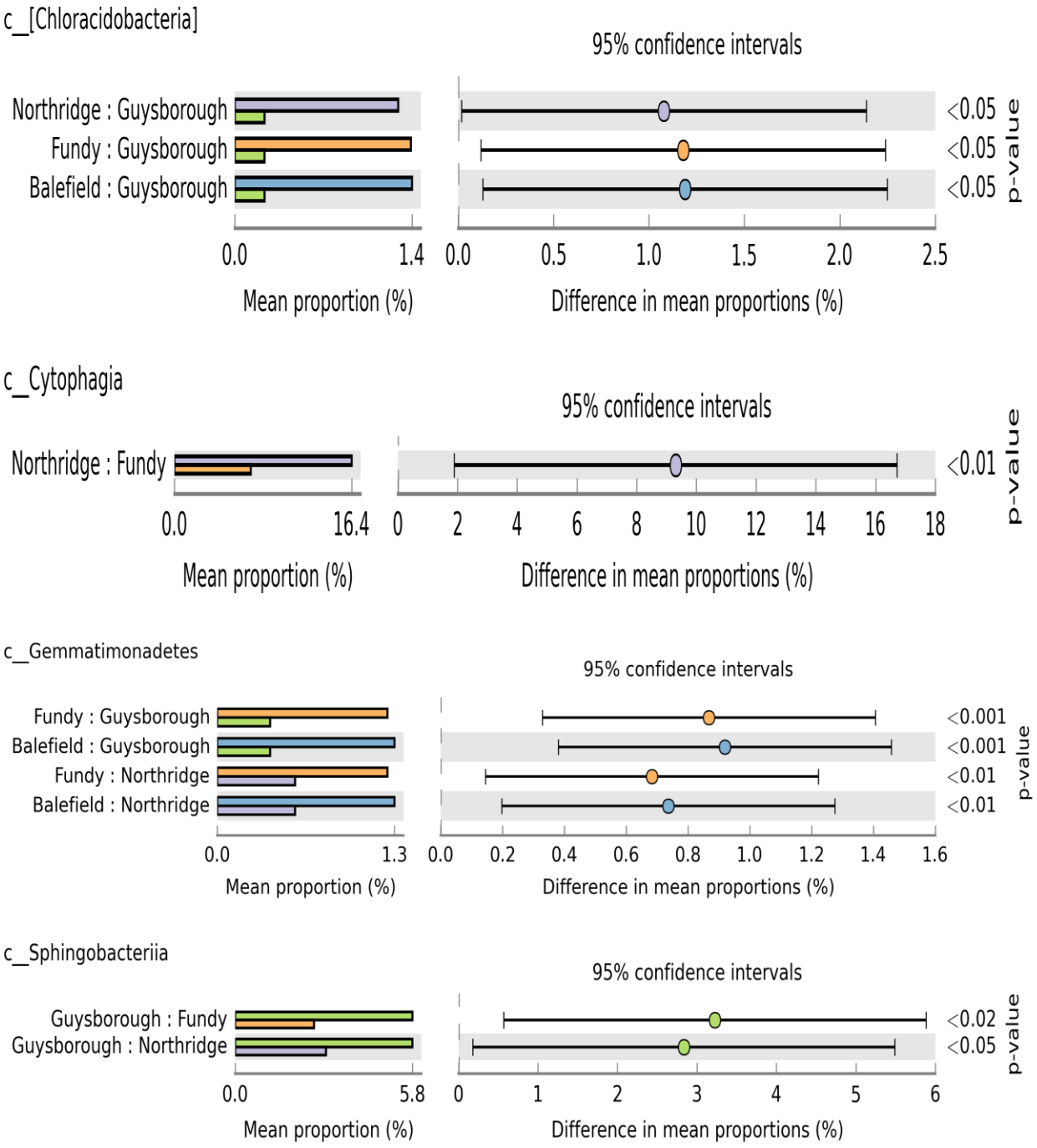


Fig. 6. The relative abundance of bacteria at the class level based on 16SrRNA sequencing. Corrected p-values were calculated using Benjamini-Hochberg false discovery rate approach ($p<0.05$).

Conclusion

All microbial abundances reported in this study are relative and not absolute. This is a well acknowledged limitation for most 16S/ITS microbiome studies. There is limited literature information related to plastics in compost. However, the findings of this study are in agreement with current knowledge of the composition of bacterial communities in soils (Fierer *et al.* 2009; Bulgarelli *et al*. 2013; Shi *et al*. 2015; Foulon *et al*. 2016). Also, some of the identified microorganisms (five classes identified to be relevant) in this work, have been confirmed to have potential for biodegrading plastics. As such, we propose in the next project to assess plastic waste biodegrading efficacy of the five identified native microorganisms classes in compost under laboratory controlled-environment and field conditions. The result from this study also showed that the variations in microbial populations were significantly ($p<0.01$) influenced by locations and time but not association with sample type. The reasons could be that this variation in microbial population may be as a result of source of feedstock, age of pile and processing method. The study also showed that there were significant number of microorganisms in compost that are not yet identified.

Future Direction

We could not do the isolation and inoculation studies due to financial limitation (Divert NS provided SRG funding) and the long duration required for the plastic decay studies.

- To isolate and identify bacteria and fungi (within the microbial
- community) with abilities to degrade plastics
- To conduct experiment to investigate the reason(s) for the variation in the microbial community
- To investigate the effect of carbon and nitrogen content in compost on the diversity and structure of microbial populations

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