

Microbial Ecology of the OSIRIS-REx Assembly Test and Launch Environment

Abstract #1113

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Introduction

- Our goal is to characterize the microbes present during different phases of the OSIRIS-REx ATLO (Assembly Test Launch Operations)
- These data can inform future planetary protection controlled missions help to understand the relationship between organic and biological cleanliness
- We extracted DNA from 17 witness foils and two blanks originally intended for amino acid characterization
- We successfully amplified and sequenced DNA from bacteria, archaea, and fungi.
- We amplified the 16S ribosomal rRNA gene to identify bacteria and archaea
- We amplified the ITS region to identify fungi
- We were not able to sequence DNA from any other Eukaryotes.
- The DNA amplification and sequencing techniques used had a detection limit of ~7 bacterial cells.

Methods

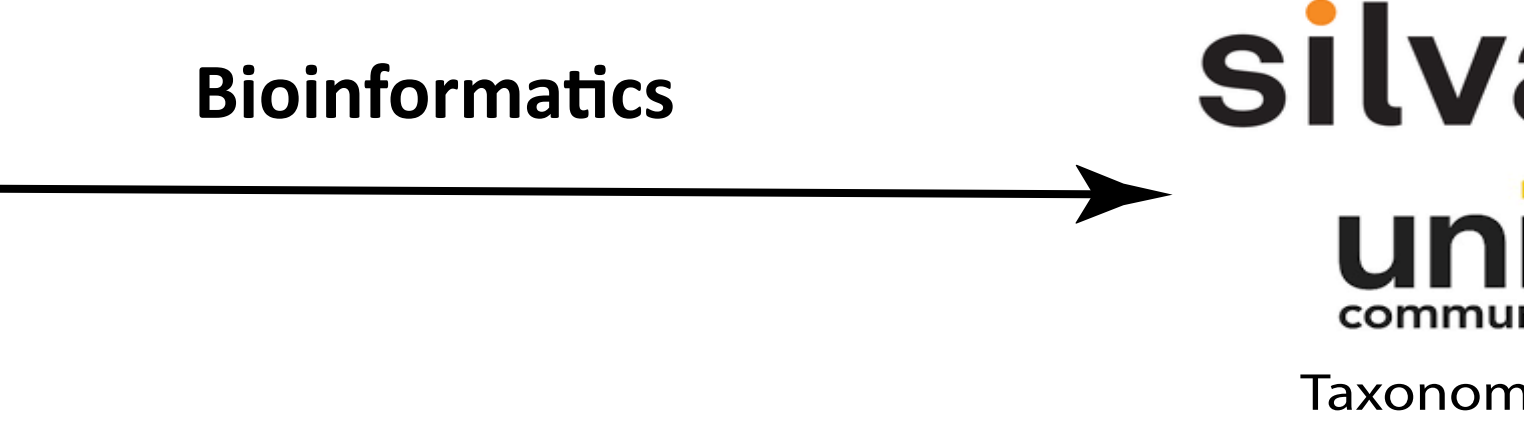
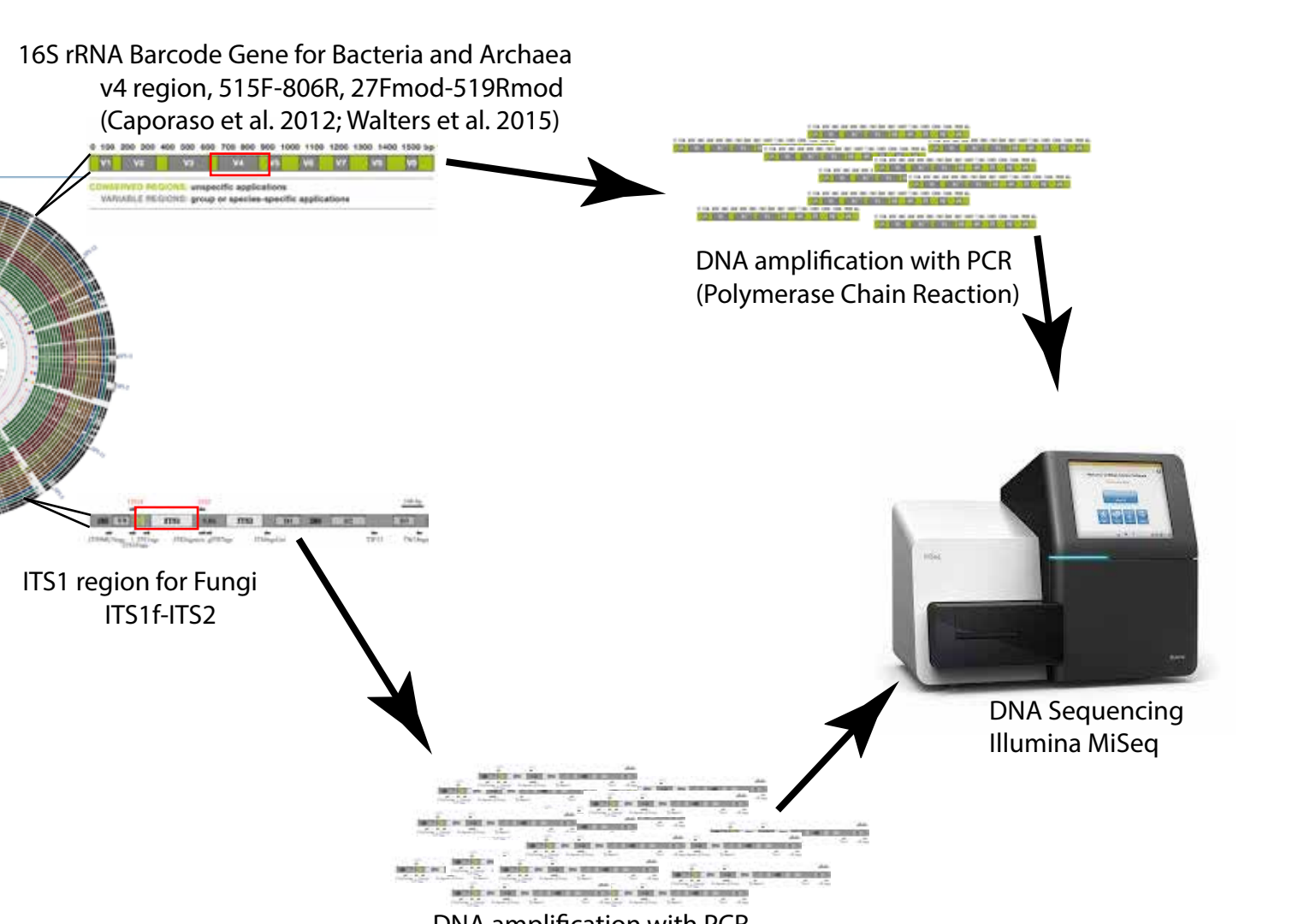
DNA Extraction and Tag Sequencing

Sample Information

Sample	Exposure start	Exposure stop	Location	ATLO Processes
OR-CXP-01-1-A.0	3/11/2015	4/14/2015	LMD/Denver	ISC exam & test, TAGSAM deploy, clean up head
OR-CXP-02-1-A.0	4/14/2015	5/11/2015	LMD/Denver	TAGSAM fuel, Denovo
OR-CXP-03-1-A.0	5/11/2015	6/10/2015	LMD/Denver	airlock bio-ISC tests, test site
OR-CXP-04-1-A.0	6/12/2015	7/14/2015	LMD/Denver	SARA TVAC/lock container/TAGSAM deploy, fuel, Post-ube/DVRS/OTIS
OR-CXP-05-1-A.0	7/14/2015	8/19/2015	LMD/Denver	TAGSAM install, deploy
OR-CXP-06-1-A.0	8/19/2015	9/18/2015	LMD/Denver	OCAS test/ISC deploy
OR-CXP-07-1-A.0	8/18/2015	11/4/2015	LMD/Denver	SARA deploy, /move to HAL
OR-CXP-08-1-A.0	11/4/2015	12/9/2015	LMD/Denver	HAL, move to move to SS
OR-CXP-09-1-A.0	12/9/2015	1/7/2016	LMD/Denver	RESCUA, install/ISS, moving/ISS container
OR-CXP-10-1-A.0	1/8/2016	2/5/2016	LMD/Denver	FT, TAGSAM/ISS, HAL, IAB, IAC/PTAC pre-ent
OR-CXP-11-1-A.0	2/5/2016	3/16/2016	LMD/Denver	TAGSAM/ISS, HAL, IAB, IAC/PTAC pre-ent
OR-CXP-12-1-A.0	3/16/2016	4/26/2016	LMD/Denver	bank, OTC/ISS/ISS
OR-CXP-13-1-A.0	4/26/2016	4/27/2016	LMD/Denver	TAGSAM flight, install
OR-CXP-14-1-A.0	4/27/2016	6/17/2016	KSC	SARA deploy, flight, install
OR-CXP-15-1-A.0	6/17/2016	8/26/2016	KSC	ISC battery install/TAGSAM cleaning
OR-CXP-16-1-A.0	7/14/2016	8/26/2016	KSC	ISC battery install/TAGSAM cleaning
OR-CXP-17-1-A.0	8/26/2016	8/26/2016	KSC	ISC battery install/TAGSAM cleaning
ORX Fairing - FB - 18			KSC	Fairing Blank
ORX Fairing CK FA - 19			KSC	Fairing CK
Swab Blank - SB - 20			KSC	Fairing CK
Kit Blank - KB - 21			KSC	Fairing CK

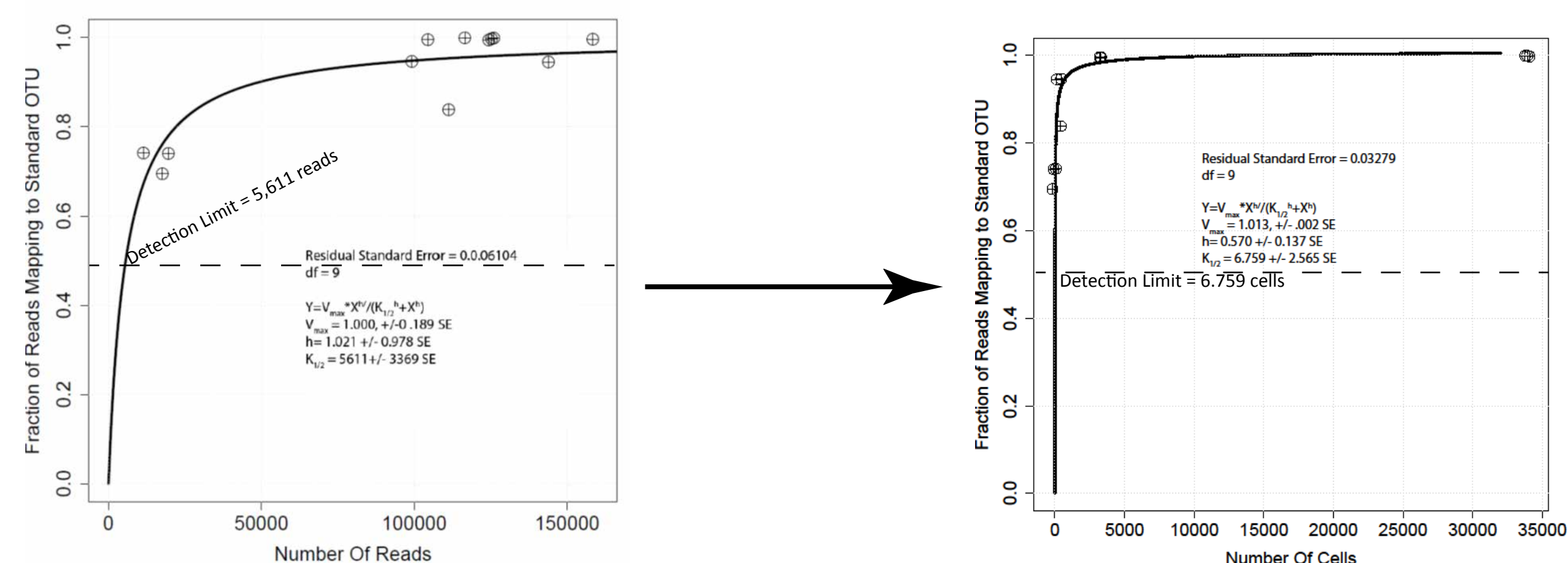


(Dworkin et al., 2018)



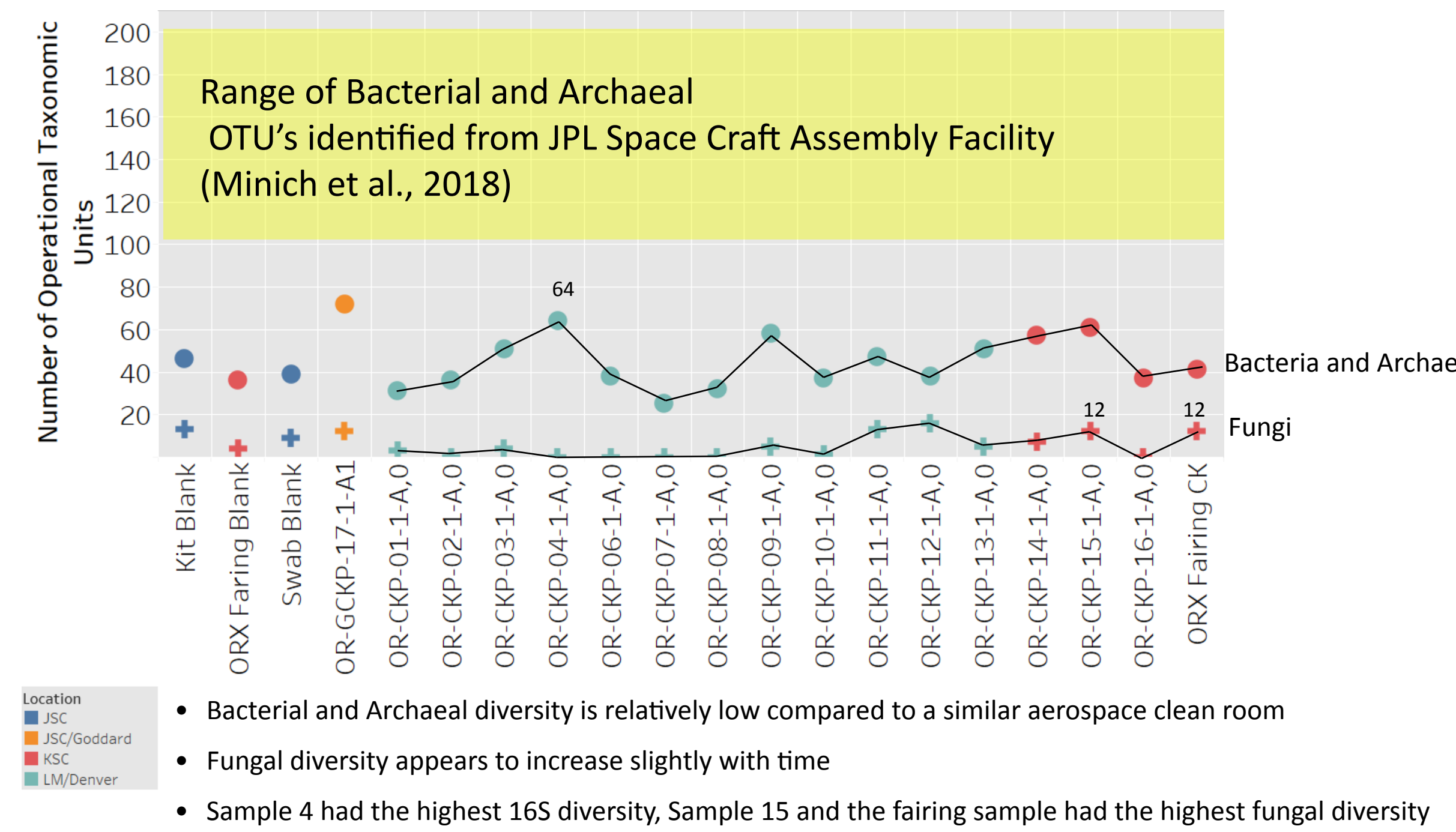
- DNA sequences were quality filtered, trimmed analyzed using the QIIME software package.
- We utilized the Deblur pipeline to identify 100% unique OTU's (Operational Taxonomic Units)
- OTU's were identified using the Silva v132 database for bacteria and archaea and the UNITE database for fungi.

Known concentrations of *Bacillus subtilis* sequenced in order to determine the detection limit for this sequencing run

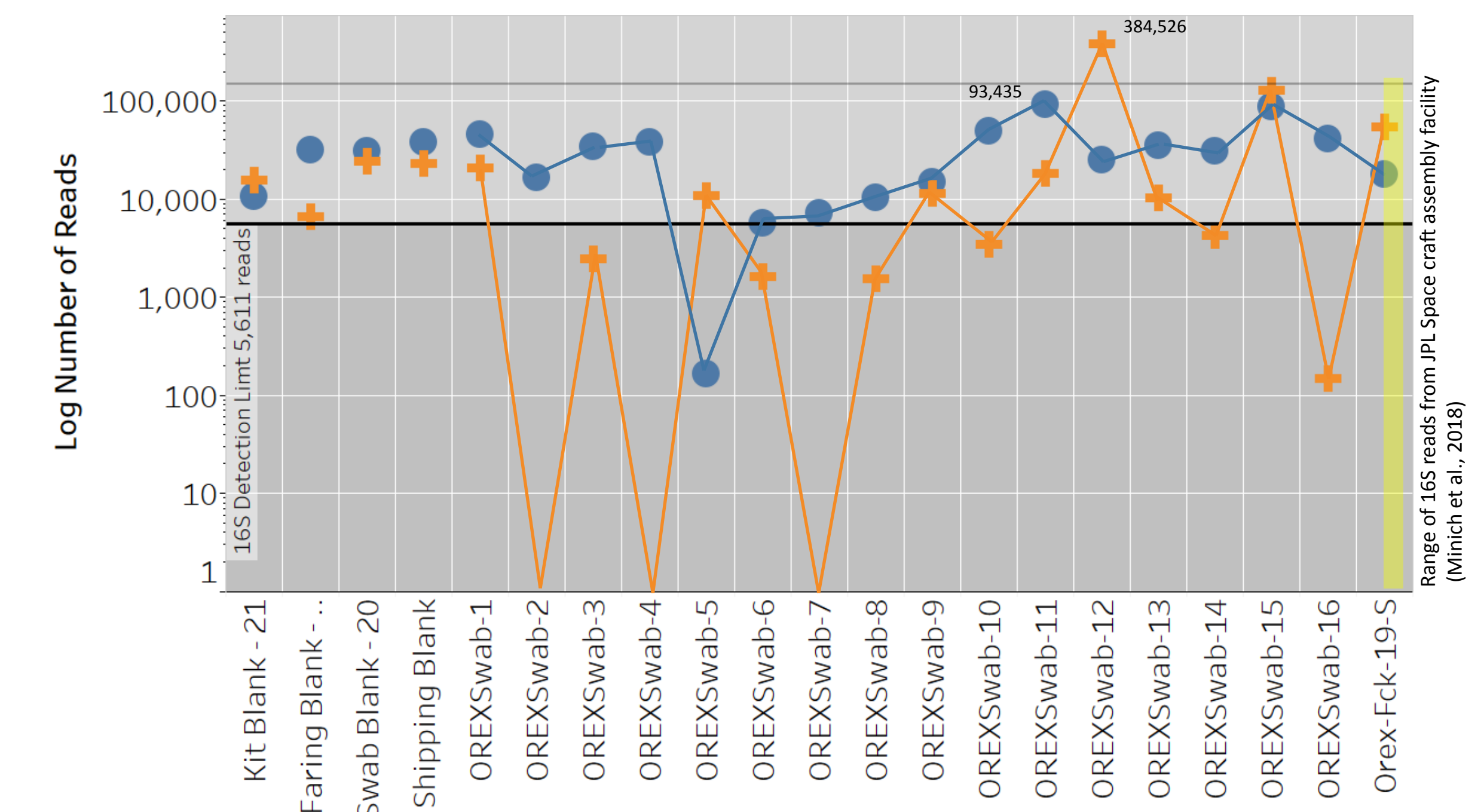


Assuming an average of 5.14 fg of DNA per cell (Blattner et al. 1997), this is a detection limit of approximately 34 fg of DNA

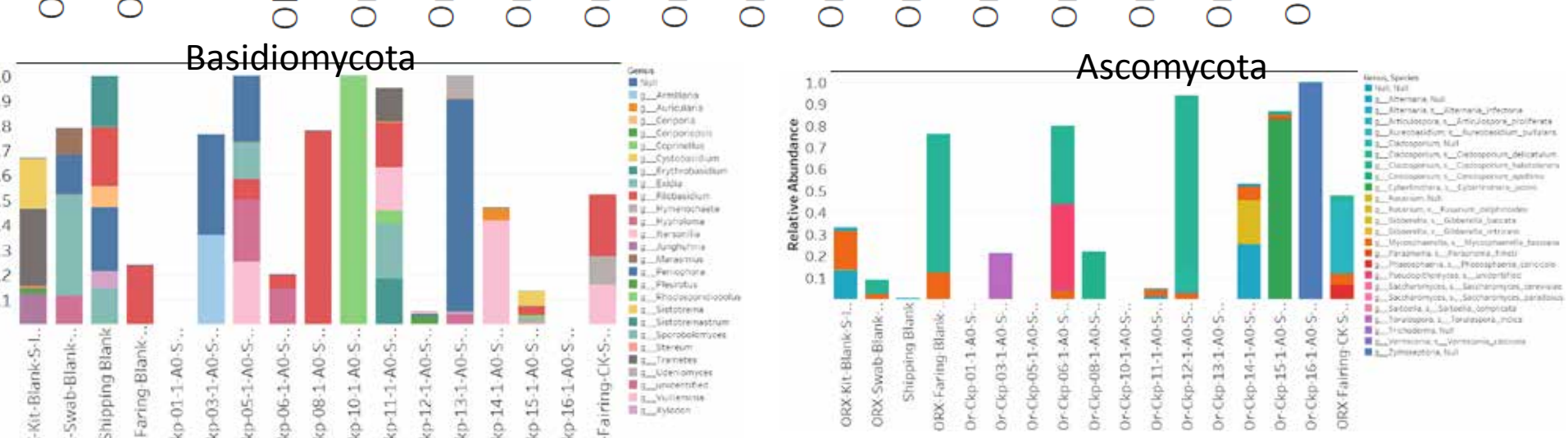
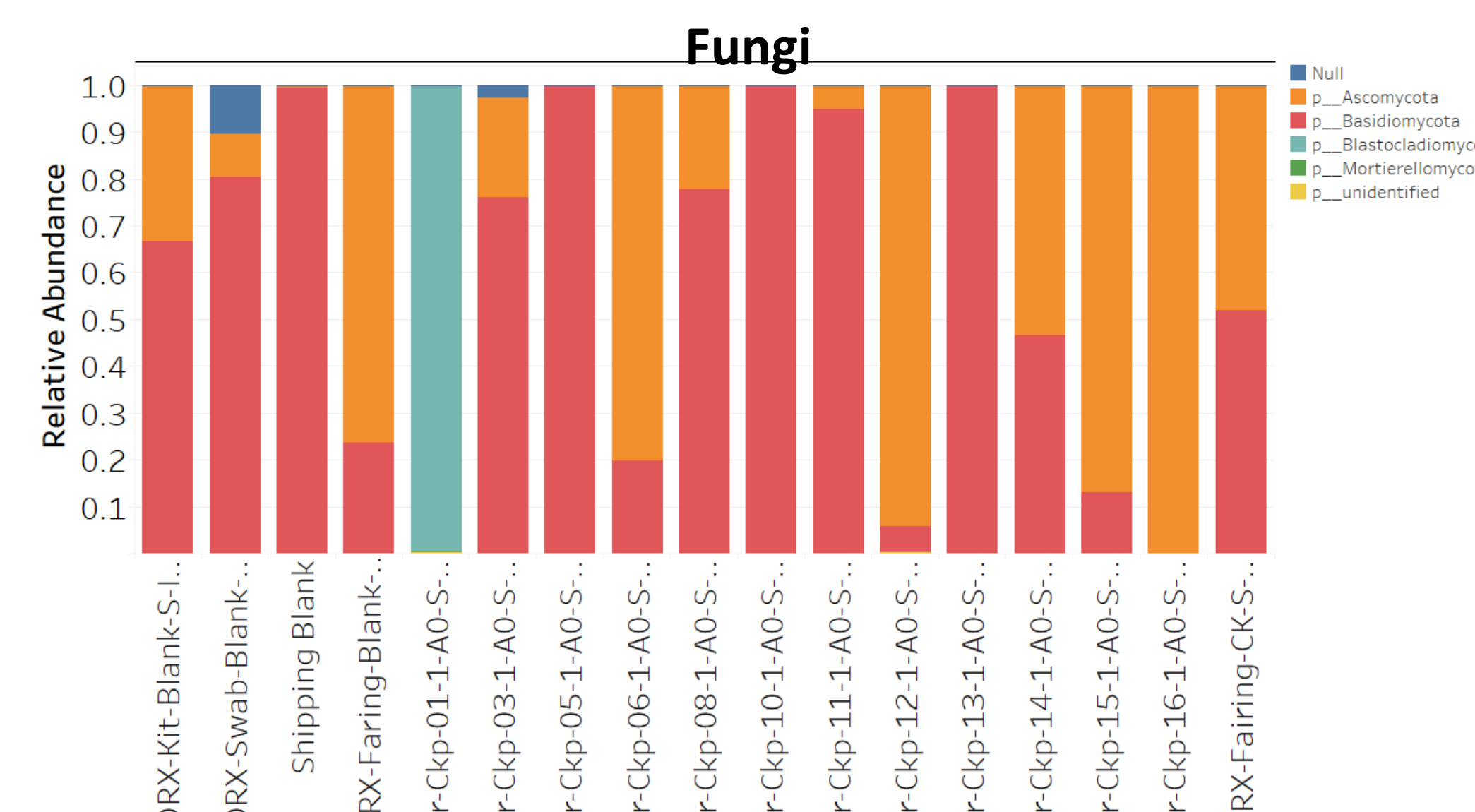
Results



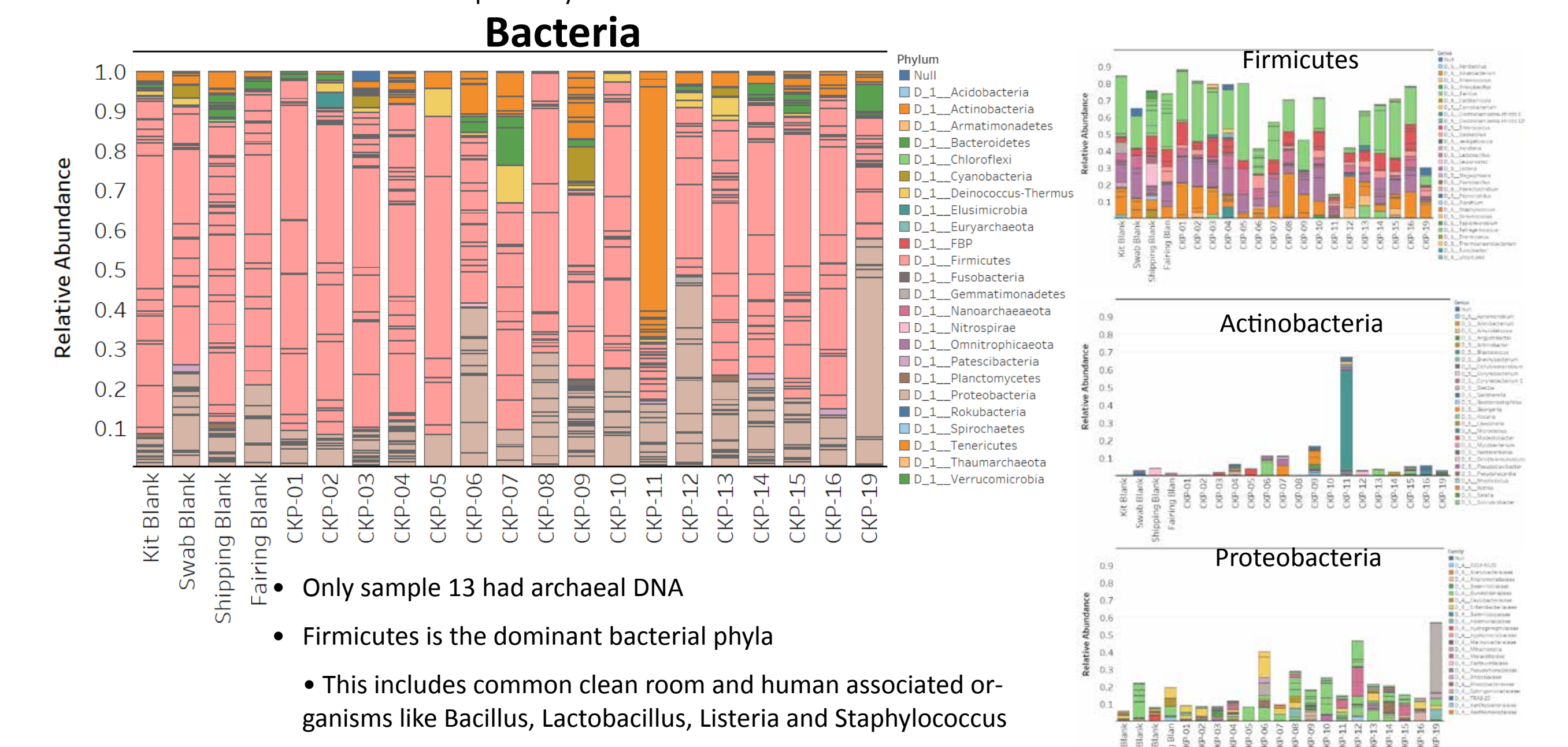
- Bacterial and Archaeal diversity is relatively low compared to a similar aerospace clean room
- Fungal diversity appears to increase slightly with time
- Sample 4 had the highest 16S diversity, Sample 15 and the fairing sample had the highest fungal diversity



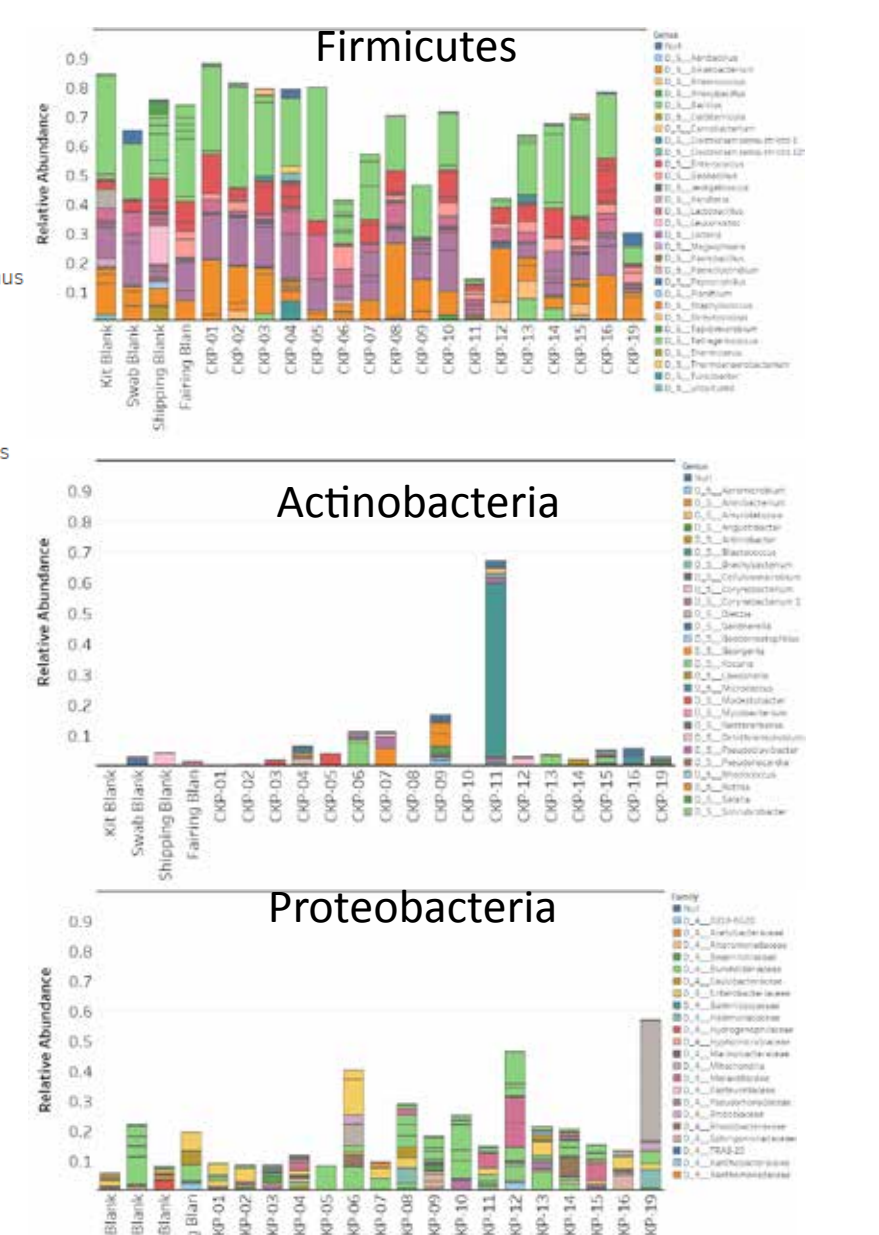
- Only one 16S sample had too few sequences for further analysis
- 3 fungal samples had 0 reads
- It is common for the blanks and control samples to have relatively large numbers of DNA sequences
- The number of bacterial reads appears to increase with time
- The number of fungal reads is more variable
- Sample 11 (TVAC lid opened) and sample 12 (launch container) have highest number of 16S and fungal reads respectively



- Fungal DNA is relatively distinct for each sample
- Sample 12 (launch container) is dominated by *Cladosporium delicatum*, a saprophytic fungi
- Sample 16 is dominated by Zygomycota a genus containing plant pathogens
- Sample 13 (TAGSAM flight install) is dominated by the an unidentified Merulaceae the family responsible for lignin degradation
- Unique organisms from the fairing
- *Udenomyces pyrnicola* (yeast)
- *Itersonilia pannonica* (plant associated fungi)
- *Articulospora proliferata* (aquatic fungi)
- *Phaeosphaeria coricola* (plant fungi)



- Only sample 13 had archaeal DNA
- Firmicutes is the dominant bacterial phyla
- This includes common clean room and human associated organisms like *Bacillus*, *Lactobacillus*, *Listeria* and *Staphylococcus*
- Sample 11 is dominated by *Micrococcus* a type of human associated actinobacteria
- Sample 19 (Fairing) appears to be different, dominated by mitochondrial DNA from an Oomycete (water mold)



Conclusions

- We successfully sequenced bacterial and fungal DNA from clean room witness coupons demonstrating that these types of samples can be useful for biological contamination knowledge
- Witness coupons from the TVAC testing and from the rocket fairing had distinct microbial communities
- The abundance of fungal sequences may correlate to the amount of carbon and iron bearing particles from replicate witness plates.
- Further data analysis is ongoing
- Dedicated biological contamination knowledge samples should be routinely collected for missions where biology could affect mission requirements e.g. Mars2020, Europa Clipper

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