The abundances of the OTUs within the Planctomyces genus (i.e. OTU0125, OTU0144, OTU0401, OTU0401, OTU0592) does not significantly differ across depth. None of the linear models generated for these OTUs have a p-value that is below 0.05. The p-values for the OTUs 0125, 0144, 0401, 0592 are 0.7243, 0.7197, 0.9914, 0.366 respectively, hence we cannot reject the null hypothesis that there is no significant relationship between abundance and depth.

The abundances of these OTUs also do not change significantly with oxygen concentration either. The linear models generated to compare the relationship between abundance and oxygen concentration also had p-values that were above 0.05. For the OTUs 0125, 0144, 0401, and 0592, the p-values were 0.7744, 0.7718, 0.7639, and 0.6647 respectively.

* What are the implications of potential differences in pipelines for microbial ecology research and discovery?

There are a number of questions that can be further addressed using this dataset. Since the Planctomyces genus is responsible for the anammox reaction (NO2 - + NH4 + ->N2 + 2H2O), one might ask how their abundance would change at different concentrations of NH4+and NO2-.

Differences in the methodological pipelines can slow microbiology ecology research and discovery. It is more difficult to compare the results of studies if their pipeline differed in terms of the sequencing, bioinformatics tools, and data analysis methods used. Although our study was primarily concerned with the analysis of 16S amplicon data, shogun sequencing is another method commonly employed in modern metagenomic environmental studies. In a study that compared amplicon and shotgun sequencing, only 50% of the phyla identified through amplicon sequencing were recovered from shogun sequencing (1). Amplicon sequencing also identified ~ 27% more families(1), suggesting that studies using shogun sequencing data combined with clade-based taxonomic algorithms delivers should be re-examined. Researchers should be cautious when comparing the results of shotgun sequencing data with amplicon sequencing.

In terms of the bioinformatics pipelines commonly used in metagenomics, some differences are observed, as demonstrated by our study. Between QIIME, mothur, and MG-RAST—differences were mostly observed at the genus level because of mothur’s tendency to have unclassified reads (2). There are also discrepancies between the results we have obtained from QIIME2 compared to mothur at the genus level that support the findings of these studies. Our taxon of interest has a higher relative abundance across all depths our analysis done through mothur, possibly due to the stricter filtering criteria enforced by QIIME2. The Planctomyces genus abundance is also higher than that of mothur’s. There is an additional genus in mothur not eliminated in mothur. Finally, we observe both higher Shannon?? in QIIME2 for O2 and for oxic vs anoxic. These inconsistencies highlight the limitations of bioinformatics pipelines and their ability to distinguish between some 16S rRNA sequences at a genus and species level because of their near identical 16S rRNA sequences. NO2- NH4+

1. Tessler M, Neumann JS, Afshinnekoo E, Pineda M, Hersch R, Velho LFM, Segovia BT, Lansac-Toha FA, Lemke M, Desalle R, Mason CE, Brugler MR. 2017. Large-scale differences in microbial biodiversity discovery between 16S amplicon and shotgun sequencing. Sci Rep 7.

2. Plummer E, Twin J. 2015. A Comparison of Three Bioinformatics Pipelines for the Analysis of Preterm Gut Microbiota using 16S rRNA Gene Sequencing Data. J Proteomics Bioinform 8.

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The general equation for a linear model is:

- Higher Shannon in QIIME2 vs. Mothur for O2

Reason: QIIME2 eliminates more data than Mothur (???)

- Higher Shannon in QIIME2 vs. Mothur for O2 Oxic vs. Anoxic

Reason: QIIME2 eliminates more data than Mothur (???)

- Extra genus in Mothur (Pla3\_lineage\_ge)

Reason: Not eliminated in Mothur, but eliminated in QIIME2 (???)

- Genus distribution in QIIME2 has more Planctomyces than Mothur in terms of abundance

Reason: ???

- Higher abundance in Mothur vs. QIIME2 (2X Values)

Reason: QIIME2 has stricter filtering vs. Mothur (???)

O2