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Metagenomic Analysis of Microbial 18s Eukaryotes Communities and Environmental factors in the Western Antarctic Peninsula waters during Austral Summers

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Metagenomic Analysis of Microbial 18s Eukaryotes Communities and Environmental factors in the Western Antarctic Peninsula waters during Austral Summers

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Abstract

Little is known about the environmental factors that impact eukaryotic microbial populations in the Western Antarctic Peninsula. Metagenomic and environmental data have been collected over the course of three consecutive austral summers in the Western Antarctic Peninsula off Palmer Station. More than 13 million 18S rRNA eukaryotic sequences have been taxonomically identified and categorized from the Antarctic water samples collected. Here we will investigate the environmental factors that affect eukaryotic organism populations, as well as possible indicator species that could provide insight as to the status of other eukaryotic species. Due to climate change, understanding these factors and identifying status indicating species is becoming increasingly important in understanding microbial systems, and to inform future research of Antarctic ecosystems and environmental conditions. We identified several groupings of correlated taxonomic operational units. Additionally, we found that Stramenopiles.Diatomea.ME-Euk-FW10, an uncultured diatom with a large population presence, had a particularly strong correlation to temperature.

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Introduction

Microbial eukaryotes exhibit broad lifecycle diversity. Competition, environmental stresses, predation, and viruses are all factors that affect eukaryotic diversity and lifespan (Jungblut et al., 2012). Amoeboid heterotrophic protists have been shown to feed on, and significantly decrease, diatom populations (Thomsen et al., 1991). Algae and Phytoplankton have developed population survival and reproduction strategies. Most notably they have been observed to have two distinct life strategies: boom-and-bust and a persistent presence (Assmy et al., 2013). Species that have a persistent life strategy are generally more resilient to environmental changes and stressors. Boom-and-bust organisms require a narrower range of environmental parameters but tend to have a large increase in population when those parameters are met, as well as a large decrease in population under stress (Mock et al., 2017). Environmental factors, such as seasonal upwelling in surface waters off the coast of Spain, have been shown to cause a shift in microbial eukaryotic populations, and temperature showed a significant correlation with changes of community composition (Hernández-Ruiz et al., 2018).

Palmer Station is located off of the Western Antarctic Peninsula (WAP) on Anvers Island. The Palmer Station Long Term Ecological Research (PAL-LTER) program has been actively studying the WAP marine ecosystem since 1990 (Smith et al., 1995). The WAP undergoes extreme seasonal climate variation. During the austral winters, a layer of sea ice covers the surface and water salinity levels increase as temperature and light levels decrease. The austral summers give way to open waters and warmer weather, causing glacial and sea ice melt. In turn

this creates density shifts and induces ocean upwelling (Ducklow et al., 2006; Seyitmuhammedov et al., 2022). The Antarctic peninsula has experienced an average rise of 6C˚ since 1950, causing it to be amongst the most rapidly warming regions on Earth (Ducklow et al., 2006). This rise in temperatures has altered the ecosystem on both the macro and micro scale, as penguin, krill, and zooplankton populations change to reflect the newly developing environment (Ducklow et al., 2006). Historically, increased winter ice extent, ice duration, and reduced summer winds have been favorable conditions for phytoplankton in WAP. This results in an increased krill biomass which subsequently affects the diets of local fish and penguins. Specifically, colder temperatures and higher ice caps result in narrower depths of water stratification and a decrease in the mixing of water columns. This increases diatom concentrations driving the krill reproduction and feeding habits (Saba et al., 2014).

In addition to the importance of commercial fishing, the Southern Ocean is an important atmospheric CO_2 sink. An estimated third of all atmospheric carbon is captured by the world's oceans, of which the Southern Ocean is responsible for approximately 40% (Kock et al., 2007; Sabine et al., 2004; Frölicher et al., 2015). Due to climate change, understanding what environmental factors affect microbial eukaryotes such as diatoms, and to what extent these factors affect the ecosystem, is critical in our anticipation and ability to combat downstream effects. Additionally, identifying status indicating species is becoming increasingly important in understanding microbial systems, helping to inform future research of Antarctic ecosystems and environmental conditions. The ability to estimate the population of a difficult to identify

species by the population of an easily identifiable, correlated species could free up resources for researchers in the field.

The majority of marine microbial organisms have yet to be successfully cultured (Suzuki et al., 1997). Therefore, metagenomic bioinformatics techniques for analyzing environmental samples are necessary for identifying microorganisms and capturing an accurate representation of the ecosystem. 18s ribosomal RNA has become the most frequently used biomarker in the determination of environmental microbial eukaryotes. This is in large part due to the highly conserved 18s rRNA regions which allows for the use of universal primers (Meyer et al., 2010). This allows for relatively accessible, cost effective, and quantitative sequencing of environmental microbial eukaryotes.

Taxonomic background

Archaeplastida

Archaeplastida is a broad supergroup of eukaryotes composed of Chloroplastida green algae, Rhodophyceae red algae, and land plants. Archaeplastida are typically capable of photosynthesis, however some have lost that ability (Ball et al., 2011). The Archaeplastida species that are capable of photosynthesis have chloroplasts surrounded by two membrane layers; this suggests an endosymbiosis event with a cyanobacterium. Note that, other eukaryotic organisms with chloroplasts have three membrane layers or more (Tikhonenkov,

2020). Taxonomic classification of Archaeplastida is still contested; however molecular analysis supports that Picozoa are a part of the Archaeplastida group (Blaby-Haas & Merchant, 2019).

Stramenopile

Stramenopiles, also referred to as Heterokonts, are a major eukaryotic clade and are part of the supergroup SAR (Stramenopila, Alveolate, Rhizaria) (Keeling & Burki, 2019). The clade consists of photoautotrophic, zoosporic, and phagotrophic organisms causing the clade to have a large degree of diversity (Cho et al., 2022). The Stramenopiles clade includes of the subgroups MAST (Marine Stramenopiles), diatoms, Dictyochophyceae, and others (Keeling & Burki, 2019; Han et al., 2019). Stramenopiles.Diatomea.ME-Euk-FW10, Stramenopiles.MAST-2, and Stramenopiles.MAST-3 are Stramenopiles that have not yet been successfully cultured.

Diatomea

Diatoms are an abundant class of Stramenopile algae which are commonly photoautotrophic and are exhibit silica cell walls. These silica cell walls form arrays of hierarchical pores that are used for gas and nutrient exchange (Lengyel et al., 2020; Zhou et al., 2022). The silica cell walls are thought to protect the diatoms from predation, as well as harmful ultraviolet radiation. It is estimated that diatoms are responsible for 25% of the world's primary production and 40% of marine primary production (Sethi et al., 2020; Lakshmi et al., 2022). As such, diatoms play an important role in carbon fixation and oxygen production. It has been shown that

photosynthesizing organisms, in particular diatoms, are sensitive to environmental temperature and conductivity. Higher temperatures, and higher environmental conductivity, increases the efficiency of ion exchange, and consequently increases the efficiency of photosynthesis (Lengyel et al., 2019; Lengyel et al., 2020).

Dictyochophyceae

Dictyochophyceae are marine Stramenopiles phytoflagellates that have a wide range of cellular morphology (Eckford-Soper & Daugbjerg, 2016). Dictyochophyceae blooms have been correlated with fish killing events and phycotoxin secretion (Eckford-Soper & Daugbjerg, 2016; Skjelbred et al., 2011; Mardones et al., 2022). Phycotoxins have been linked to amnesic shellfish poisoning, paralytic shellfish poisoning, and diarrhetic shellfish poisoning in humans, through human consumption of seafood exposed to harmful algae blooms (Mardones et al., 2022). Like most Stramenopiles, Dictyochophyceae generally contain chloroplasts and perform photosynthesis (Cassar et al., 2015).

Jakobida

Jakobida belong to the supergroup Excavata and are heterotrophic, flagellar-driven eukaryotes consisting of only twenty identified species (O'Kelly, 1993). Some Jakobida have been observed in anoxic marine environments, as well as hypersaline environments (Strassert et al., 2016). Jakobids are not photosynthesis capable and instead feed on bacteria (Christaki et al., 2005).

Picozoa

Picozoa were once thought to be photosynthesizing algae but have since been determined to be heterotrophic and feed on small organic particles (Moreira & López-García, 2014). Environmental seawater sampling has revealed that Picozoa are widely distributed (Seenivasan et al., 2013).

Haptophyte

Haptophytes are a broadly distributed and relatively abundant clade of algae that are closely related to the SAR clade of eukaryotes (Cuvelier et al., 2010; Parfrey et al., 2011). Haptophytes contain large quantities of fatty acids and are a common food source for oysters and shrimps (Renaud et al., 1995). Haptophytes produce large amounts of alkenones which are believed to be used as a form of energy storage. Haptophytes alkenones production varies as a function of salinity and temperature (Randlett et al., 2014).

Cryptophyte

Cryptophytes are mostly photosynthetic, unicellular eukaryotes that are found in fresh, brackish, and marine environments. Their cell walls contain ejectosomes and two flagella, which results in asymmetrical spiral swimming (Magalhães et al., 2021). The Cryptophyte plasmid is surrounded by four membranes as a result of endosymbiosis with red algae (Douglas & Penny, 1999).

Alveolata

The Alveolata clade is part of the SAR supergroup. The clade consists of predatory flagellates, photosynthetic organisms, and parasitic organisms. Alveolata often have tubular cristae and pitted, pore-like intrusions on the cell wall (Queiroz et al., 2020). In a study conducted off the coast of Japan, Alveolata was strongly correlated with depth, nitrate and nitrite, temperature, and time of year (Sogawa et al., 2022). Dinoflagellates are a common Alveolata superclass that have been observed to feed on diatoms. A study conducted in the Southern Ocean near Australia found a negative correlation between Dinoflagellates and diatoms (Cassar et al., 2015).

Rhizaria

Rhizaria are amoeboid heterotrophs known to feed on diatoms. In the Western Antarctic peninsula, Rhizaria were negatively correlated with net community primary production. This suggests that Rhizaria are preying on diatoms and other photosynthesizing microbial organisms (Magalhães et al., 2021). Rhizaria communities have been observed to be drastically affected by time of year and depth (Sogawa et al., 2022).

Methods

Water Collection

Water samples were collected over the course of 3 years from Palmer Station LTER Sampling Site B at a depth of 10 meters by Dr. Shellie Bench. Samples of interest were collected on dates: 27-11-12, 08-02-13, 27-12-13, 23-01-14, 03-02-14, 10-02-14, 28-02-14, 04-03-14, 01-12-14, 11-12-14, 12-01-15, 19-01-15, 09-02-15, 23-02-15, 09-03-15. Temperature, conductivity, pressure, fluorescence, salinity, density, chlorophyll, phaeopigment, phosphate, silicate, nitrite and nitrate, and primary production were all measured from the water samples collected by the Palmer LTER team (Ducklow et al., 2019).

Taxonomic Identification

Water samples were passed through a 3 µm filter in order to collect eukaryotic microbes and remove any prokaryotic microbial organisms. Retained samples were PCR amplified using 18s rRNA primers and sequenced. Using bbmerge, paired end fastq library files were merged then trimmed using bbduk. The quality threshold was set to 38 (ASCII 'F') with a minimum length of 160. The trimmed files were converted to the fasta format from fastq and adapter sequences were removed. 18s reads were identified through the use of mothur, with the Silva and LTP taxonomy database (Schloss et al., 2009; Quast et al., 2012; Yilmaz et al., 2013). In order to reduce the time cost of balstx, nucleotide reads were translated into all 6 reading frames. If a single translation was substantially longer than the rest, it was accepted as correct.

The translated sequences were then submitted as blast (Venables et al., 2013) queries on March 20, 2019. Reads were retained if the best E-value hit was <= 0.01. Taxonomies for the retained reads were obtained from the GenBank's Taxonomy Database. Taxonomically identified reads of the 18s library were mapped to 22 clades. Over 13 million 18S eukaryotic sequences were identified this way.

Operational taxonomic unit

Operational taxonomic unit (OTU)s were derived from identified sequencing data. OTUs were then further consolidated into compounded groupings with biological similarities. Three degrees of consolidation were established: "minimum", "medium", and "maximum" (Table 1. A/B) (Supplementary Table 1. A/B). Time course data of diatom and Dictyochophyceae groupings were produced at no consolidation, minimum, medium and maximum consolidation. Medium consolidation was then chosen for further consolidation. All OTUs counted were normalized to the population percentage of the day sampled.

Stacked Bar plots

Stacked Bar plots of all the taxa were created. All stacked bar plots consisted of the percent count of observed taxa across all the dates that the samples were collected. Five stacked bar plots from the medium consolidation OTUs were created in total. The plots consist of: all classified OTUs, only Stramenopiles, only diatoms, the sum total of the Diatomea percent

counts with the other OTU groupings, and the sum total of the Stramenopiles percent counts with the other OTU groupings.

Donut Plot

Donut plots were created using the cumulative observed population percentage counts of early, mid, and late austral summers in Antarctica. Early summer consists of November, midsummer consists of December and January, and late summer consists of February and March. Donut plots of each year's summer were cumulatively created and denoted as Trip 1, 2, and 3. Finally, a cumulative Donut Plot of all the sample dates, spanning across three austral summers, was created.

Correlation Plot

A correlation plot was produced using the medium consolidation OTU data. Correlations of the taxa and environmental factors were calculated using the Pearson correlation coefficient. OTUs with a correlation coefficient of over an absolute value of 0.65 were grouped together resulting in four groupings. These four groups were established, along with a fifth containing all the OTUs with no correlations to either other taxa or the environment factors. Group 1 consists of: ***Archaeplastida.Chlorophyta.and.otherArchaeplastida***, ***Stramenopiles.Diatomea.ME_Euk_FW10***, and ***Stramenopiles.Dictyochophyceae.Pedinellales***. Group 2 consists of: ***Excavata.Discoba.Jakobida***, ***Haptophyta.non_Phaeocystis***,

Eukaryota_other. Group 3 consists of: ***Picozoa.Picomonadida***. Group 4 consists of: ***Stramenopiles.Dictyochophyceae.Dictyochales.and.Florenciellales.and.otherDictyochophyceae***, ***Stramenopiles.MAST_2.and.MAST_3***, **Stramenopiles.Ochrophyta.other**.

Principal Component Analysis and Non-metric Multidimensional Scaling

A Principal Component Analysis (PCA) was performed on the environmental data and the classified OTUs. This was done to reduce dimensionality and confirm any Pearson correlation hits of correlated OTUs and environmental factors. Non-metric Multidimensional Scaling (NMDS) was also performed. The NMDS used the Bray–Curtis dissimilarity with a number of dimensions set at 2.

Time course and heat map

A time course of each correlated grouping was created. Percent counts were transformed using Log base 10 across all dates to ensure the figures produced would be interpretable. If the group had an established correlation of an environmental factor with a Pearson correlation coefficient absolute value greater than 0.65, then a heat map of the environmental factor was attached to the time course. While Group 2 had an absolute value Pearson correlation of under 0.65, PCA analysis indicated correlation leading to the inclusion of a heat map. Larger environmental values were chosen to be represented as black, while lower environmental values were represented with white.

Machine and R Specifications

R version 4.2.1 was used with the x86_64-w64-mingw32 compiler. The following packages and their corresponding versions were used: corrgram 1.14, ggplot 3.40, vegan 2.6-4, and webr 0.15. The specification of the machine used were: AMD Ryzen 7 3700X 8-Core Processor 3.59 GHz, 32.0 GB RAM, 64-bit operating system, x64-based processor, and a Nvidia Geforce rtx 3080 super. Code is available at: https://github.com/IdanSiman-Tov/Metagenomic-Analysis-of-Microbial-18s-Eukaryotes-Communities-and-Environmental-factors-in-WAP

Table 1. A.

Chlorophyta & other Archaeplastida	Archaeplastida.Chlorophyta
Chlorophyta & other Archaeplastida	Archaeplastida.otherArchaeplastida
Cryptophyte	Cryptophyceae.Cryptomonadales.Geminigera
Cryptophyte	Cryptophyceae.otherCryptophyceae
Jakobida	Excavata.Discoba.Jakobida
Haptophyte	Haptophyta.Phaeocystis
Haptophyte	Haptophyta.non-Phaeocystis
Picozoa	Picozoa.Picomonadida
Dinoflagellate	Alveolata.Dinoflagellata
Other Alveolata	Alveolata.otherAlveolata
Rhizaria	Rhizaria.Cercozoa

MAST (Marine stramenopile)	Stramenopiles.MAST-2
MAST (Marine stramenopile)	Stramenopiles.MAST-3
Diatom	Stramenopiles.Diatomea.Bacillariophytina
Diatom	Stramenopiles.Diatomea.Coscinodiscophytina
Diatom	Stramenopiles.Diatomea.otherDiatomea
Diatom	Stramenopiles.Diatomea.ME-Euk-FW10
Dictyochophyceae	Stramenopiles.Dictyochophyceae.Dictyochales
Dictyochophyceae	Stramenopiles.Dictyochophyceae.otherDictyochophyceae
Dictyochophyceae	Stramenopiles.Dictyochophyceae.Florenciellales
Dictyochophyceae	Stramenopiles.Dictyochophyceae.Pedinellales
Ochrophyta	Stramenopiles.Ochrophyta.other
Phaeophyceae	Stramenopiles.Phaeophyceae
Other Stramenopiles	Stramenopiles.otherStramenopiles
SAR_unclassified	SAR_unclassified
Holozoa	Opisthokonta.Holozoa
Other Opisthokonta	Opisthokonta.otherOpisthokonta
other Eukaryote	Eukaryota;other

Table 1. B

Chlorophyta & other Archaeplastida	Archaeplastida.Chlorophyta.and.otherArchaeplastida
Cryptophyte	Cryptophyceae.Geminigera.and.otherCryptophyceae
Jakobida	Excavata.Discoba.Jakobida
Haptophyte	Haptophyta.Phaeocystis
Haptophyte	Haptophyta.non_Phaeocystis
Picozoa	Picozoa.Picomonadida
Dinoflagellate	Alveolata.Dinoflagellata
Rhizaria	Rhizaria.Cercozoa
MAST (Marine stramenopile)	Stramenopiles.MAST-2.and.MAST-3
Diatom	Stramenopiles.Diatomea.Bacillariophytina.and.Coscinodiscophytina.and.otherDiatomea
Diatom	Stramenopiles.Diatomea.ME-Euk-FW10
Dictyochophyceae	Stramenopiles.Dictyochophyceae.Dictyochales.and.Florenciellales.and.otherDictyochophyceae
Dictyochophyceae	Stramenopiles.Dictyochophyceae.Pedinellales
Ochrophyta	Stramenopiles.Ochrophyta.other
Phaeophyceae	Stramenopiles.Phaeophyceae
SAR_unclassified	SAR_unclassified.and.otherStramenopiles.and.otherAlveolata
other Eukaryote	Eukaryota;other

Table 1. A. classified OTUs from the taxonomic identification methods. B. classified OTUs from the taxonomic identification methods with a “medium” level of biological consolidation.

Results

Microbial eukaryotic populations have a diverse population distribution (Fig. 1). While some OTUs can be seen to be more dominant than others, most populations have a large temporal population percentage variance. The most consistent OTU observed was ***Haptophyta.Phaeocystis***, while the largest variation observed was ***Stramenopiles.Diatomea.ME-Euk-FW10*** group.

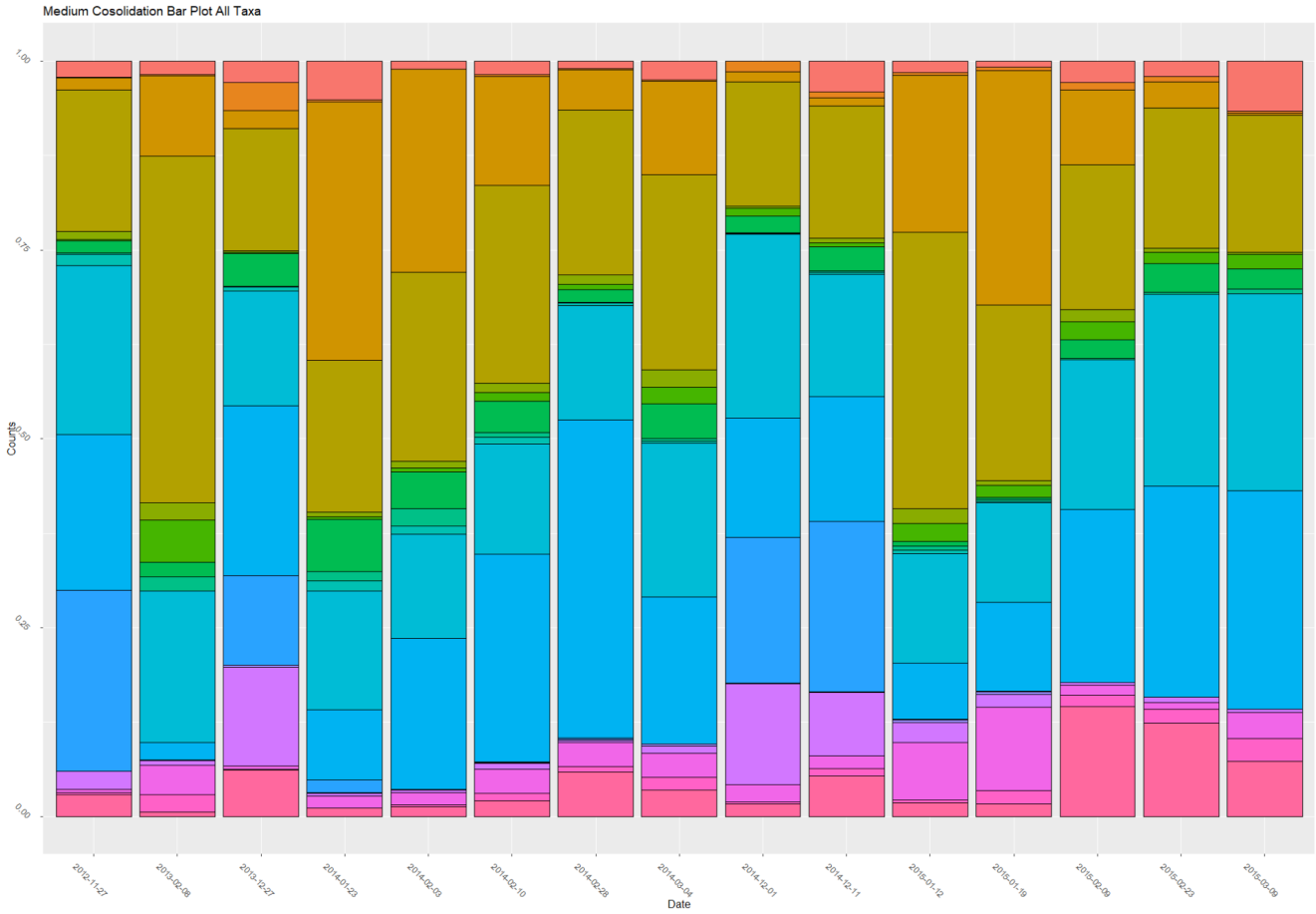
The two most persistent and dominant Stramenopiles OTUs consist of ***SAR_unclassified.and.otherStramenopiles.and.otherAlveolata*** and ***Stramenopiles.Diatomea.Bacillariophytina.and.Coscinodiscophytina.and.otherDiatomea***. Together these OTUs comprise over half the Stramenopile population during all time points except 11/December/2014. Interestingly, ***Stramenopiles.Diatomea.ME-Euk-FW10*** undergo rapid population fluctuations, frequently consisting of less than one percent of the Stramenopiles population or around a fourth of the Stramenopiles population as seen in Fig 1.B.

Within the diatom population, the ***Stramenopiles.Diatomea.Bacillariophytina.&.Coscinodiscophytina.&.otherDiatomea*** dominate the diatom population with over 60% of the observed time points, comprising of almost entirely the ***Stramenopiles.Diatomea.Bacillariophytina.&.Coscinodiscophytina.&.otherDiatomea*** OTU (Fig. 1.C). When examining the minimum consolidation bar plot (Supplemental Fig. 3.A), it should be

noted that ***Stramenopiles.Diatomea.otherDiatomea*** is the most dominant diatom group. ***Stramenopiles.Diatomea.otherDiatomea*** is a grouping which consists of many different diatom species. ***Stramenopiles.Diatomea.ME-Euk-FW10*** is an uncultured diatom species and was either a fraction of a percent of the diatom population or a substantial percent of the population. During three of the five observed spikes of ***Stramenopiles.Diatomea.ME-Euk-FW10***, the OTU was observed to constitute over half of the diatom population.

Diatom populations tend to fluctuate to a large degree and have been observed to consist of anywhere between 2% and 45% of the total microbial eukaryotic population (Fig. 1.D). In contrast the Stramenopiles were consistently a large portion of the population (Fig. 1.E).

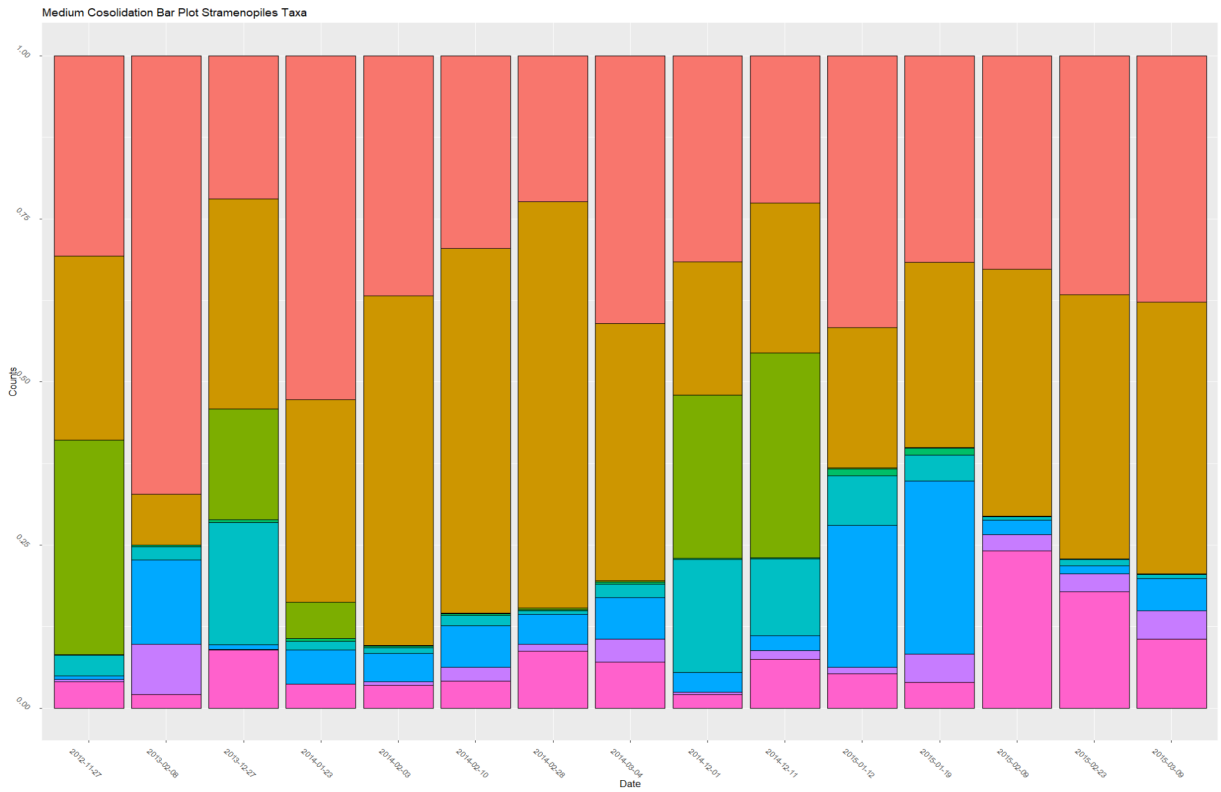
Figure 1. A.



Taxa

- Alveolata.Dinoflagellata
- Archaeplastida.Chlorophyta.and.otherArchaeplastida
- Cryptophyceae.Geminigera.and.otherCryptophyceae
- Eukaryota;other
- Excavata.Discoba.Jakobida
- Haptophyta.non_Phaeocystis
- Haptophyta.Phaeocystis
- Picozoa.Picomonadida
- Rhizaria.Cercozoa
- SAR_unclassified.and.otherStramenopiles.and.otherAlveolata
- Stramenopiles.Diatomea.Bacillariophytina.and.Coscinodiscophytina.and.otherDiatomea
- Stramenopiles.Diatomea.ME-Euk-FW10
- Stramenopiles.Dictyochophyceae.Dictyochales.and.Florenciellales.and.otherDictyochophyceae
- Stramenopiles.Dictyochophyceae.Pedinellales
- Stramenopiles.MAST-2.and.MAST-3
- Stramenopiles.Ochrophyta.other
- Stramenopiles.Phaeophyceae

B.


Medium Cosolidation Bar Plot Stramenopiles Taxa
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0.25
0.00
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2013-12-27
2014-01-23
2014-02-03
2014-02-10
2014-02-28
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2015-01-12
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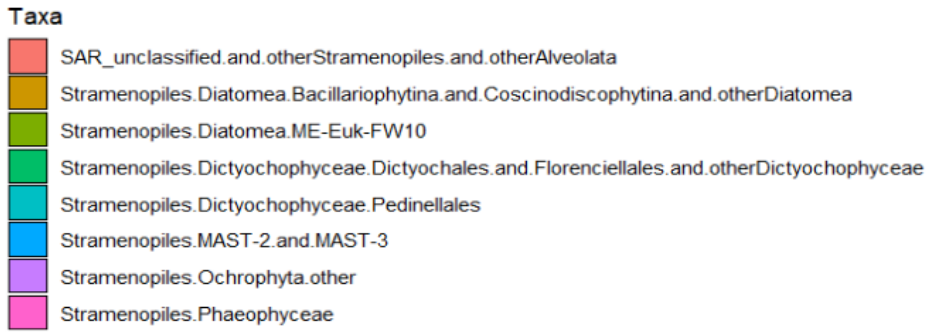
Date


Taxa
SAR_unclassified.and.otherStramenopiles.and.otherAlveolata
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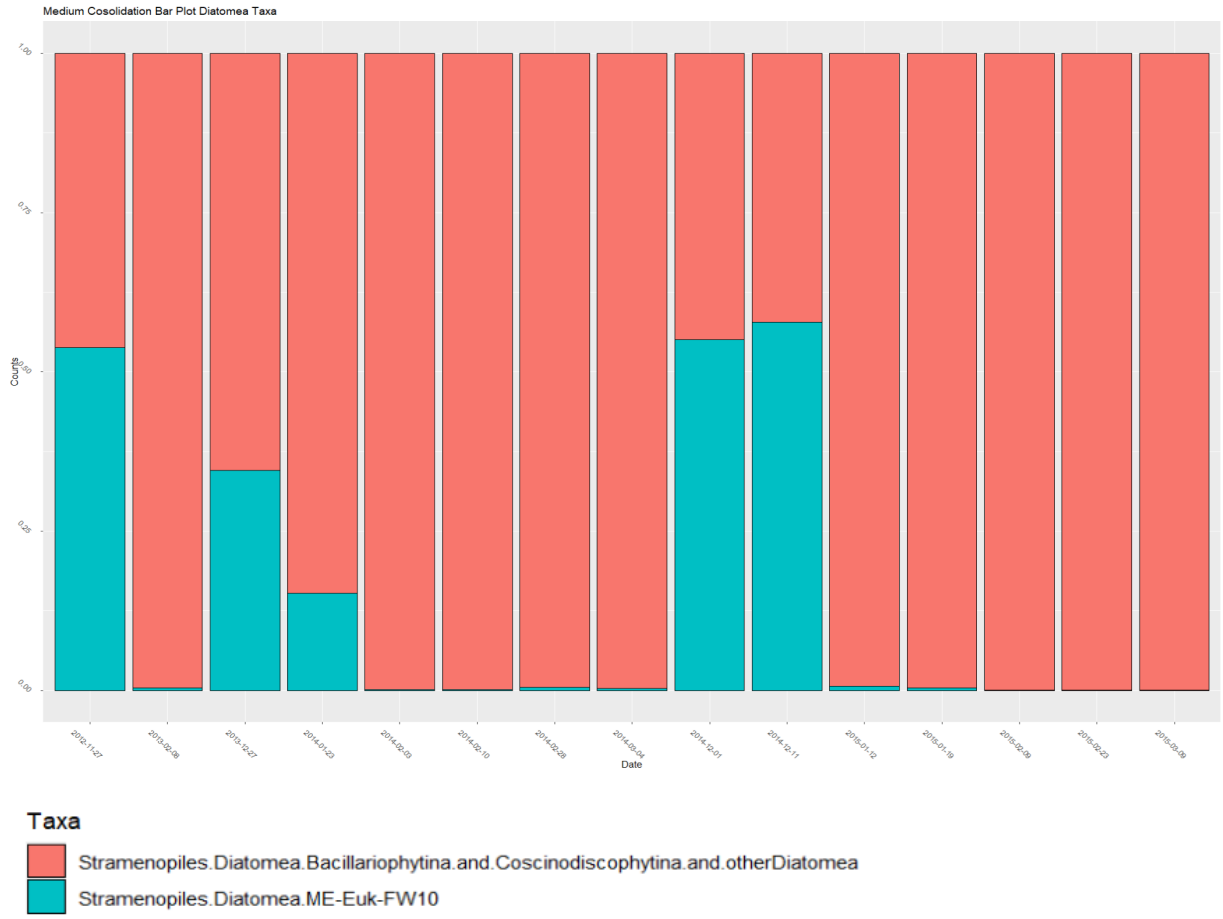
Stramenopiles.Dictyochophyceae.Pedinellales
Stramenopiles.MAST-2.and.MAST-3
Stramenopiles.Ochrophyta.other
Stramenopiles.Phaeophyceae

C.


Medium Cosolidation Bar Plot Diatomea Taxa
Counts
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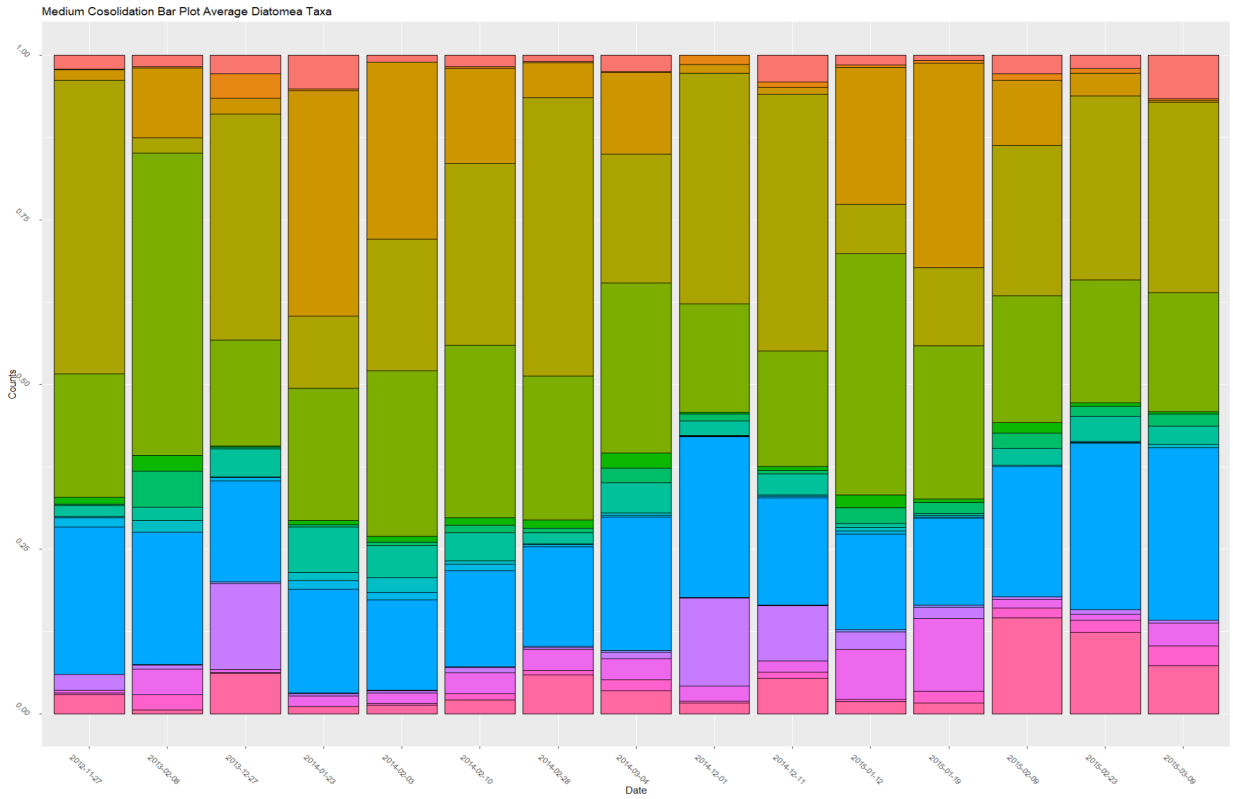
0.75
0.50
0.25
0.00
2012-11-27
2013-02-08
2013-10-27
2014-01-23
2014-02-03
2014-02-10
2014-02-28
2014-09-04
2014-12-01
2014-12-11
2015-01-12
2015-01-19
2015-02-09
2015-02-23
2015-03-09
Date
Taxa
Stramenopiles.Diatomea.Bacillariophytina.and.Coscinodiscophytina.and.otherDiatomea
Stramenopiles.Diatomea.ME-Euk-FW10

D.



Taxa

- Alveolata.Dinoflagellata
- Archaeplastida.Chlorophyta.and.otherArchaeplastida
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- Diatomea
- Eukaryota;other
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- Haptophyta.Phaeocystis
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- Stramenopiles.Phaeophyceae

E.

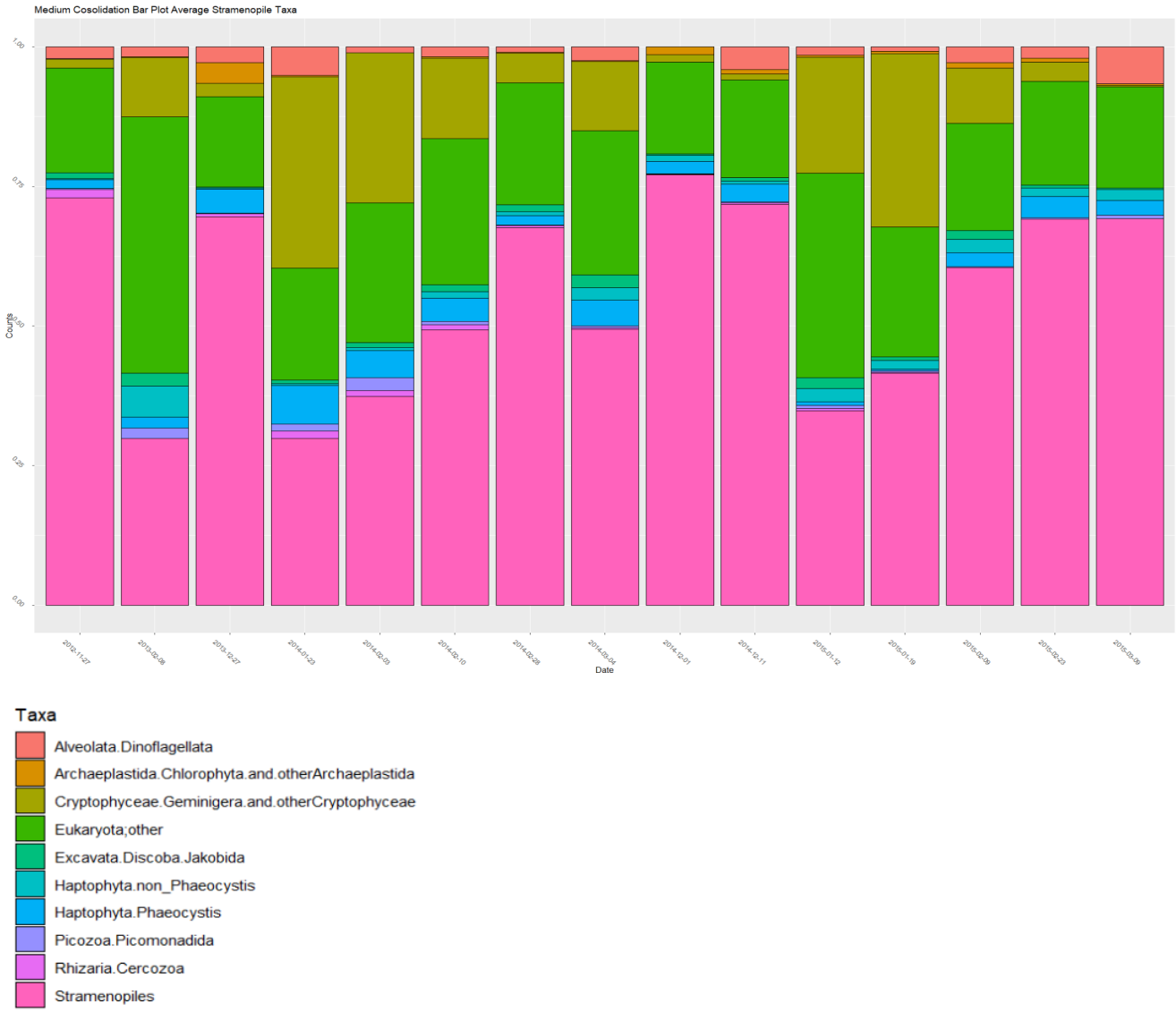


Figure 1: Stacked bar plots of identified OTUs with medium consolidation. Observed population percentage counts on a given date sampled. A. consists of all OTUs with no further grouping. B. only the Stramenopile OTU populations from the overall population, each date is normalized to the total Stramenopile population on the given sample date. C. Only the diatom OTU populations from the overall population, each date is normalized to the total diatom population on the given sample date. D. All OTUs with the diatom population percent counts were summed up and created the diatom OTU. E. All OTUs with the Stramenopile population percent counts were summed up and created the Stramenopile OTU.

In early summer of 2012, the microbial eukaryotic population was observed to comprise 44.6% Diatomea, 28.3% non-Diatomea Stramenopiles, and 27% other Eukaryota. Out of the total 28.3% of Stramenopiles, 22.4% were the ***SAR_unclassified.&.other.Stramenopiles.&.other.Alveolata*** group (Fig. 6).

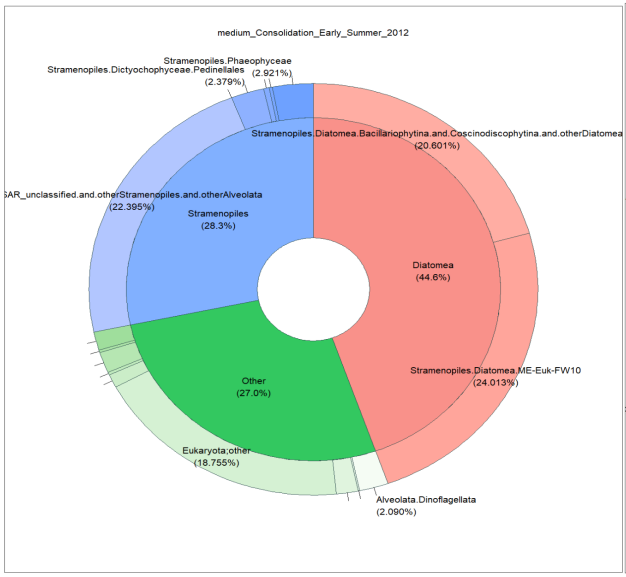
In late summer of 2013, the Diatomea grouping fell sharply from the observed 44.6% in early summer of 2012, to only 2% in late summer. Stramenopiles remained largely unchanged, losing only 0.3% of their population. The ***SAR_unclassified.&.other.Stramenopiles.&.other.Alveolata*** OTU remained the dominant Stramenopiles at 20% of the total population and 71.7% of the Stramenopile population. By mid-summer of 2013-2014, the microbial eukaryotic population had largely stabilized. The Diatomea only mildly fluctuated in population percentage from this point forward, consisting of 22.6% in mid-summer of 2013-2014, 27.4% in late summer of 2014, 23.3% in mid-summer of 2014-2015, and 26.6% in late summer of 2015. The late summer of 2013 appears to be an outlier as, similarly to the diatoms, the Stramenopiles and the “Other” grouping stabilized after late summer 2013.

Yearly changes were observed in population percentage distributions. In the summer of 2012, the Stramenopiles comprised 28% of the population, the Diatomea comprised 23.5% of the population, and the “Other” microbial eukaryotes comprised 48.6% of the population. In the summer of 2013, the population closely resembled the population distribution of the previous summer. The Stramenopiles comprised 24.7% of the population, the Diatomea comprised 25.8% of the population, and the “Other” microbial eukaryotes comprised 49.5% of

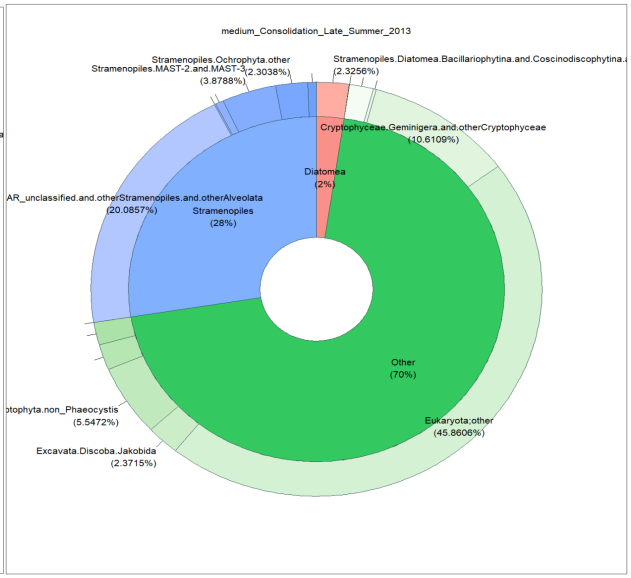
the population. In the summer of 2014, the population percent distributions shifted with the Stramenopiles observed to have increased their population percentage to 35.8%, the Diatomea aligning with previous years at 24.7% of the population, and the "Other" microbial eukaryotes sharply falling to of 39.4% of the population. Overall, during the austral summer months, Stramenopiles are 30.3% of the population with the ***SAR_unclassified.&.other.Stramenopiles.&.other Alveolata*** OTU consisting of 60.88% of the Stramenopiles population. Diatoms were observed to be 25% of the total population, 78.64% of the Diatomea population being ***Stramenopiles.Diatomea.Bacillariophytina.&.Coscinodiscophytina.&.otherDiatomea*** and the remaining 21.36% being ***Stramenopiles.Diatomea.ME-Euk-FW10***. Finally, 44.7% of the population were observed to be comprised of other eukaryotes such as ***Cryptophyceae.Geminigera.&.otherCryptophyceae*** which composed 26.2% of the other population.

Figure 2.

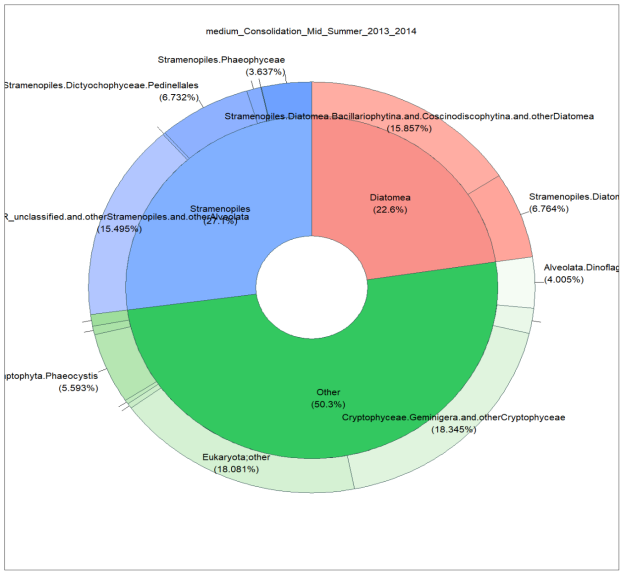
A.



B.



C.



D.

E. F.

Figure 2: Donut charts of identified OTUs with medium consolidation: blue consists of non-diatom Stramenopiles OTUs, red consists of only diatom OTUs, and green consists of non-Stramenopile OTUs. A. Early summer of 2012 (November 2012) B. Late summer of 2012 (February, March 2013) C. Mid-summer of 2013 (December 2013, January 2014) D. Late summer of 2013 (February, March 2014) E. Mid-summer of 2014 (December 2014, January 2015) F. Late summer of 2014 (February, March 2015)

Figure 3.

A. B.

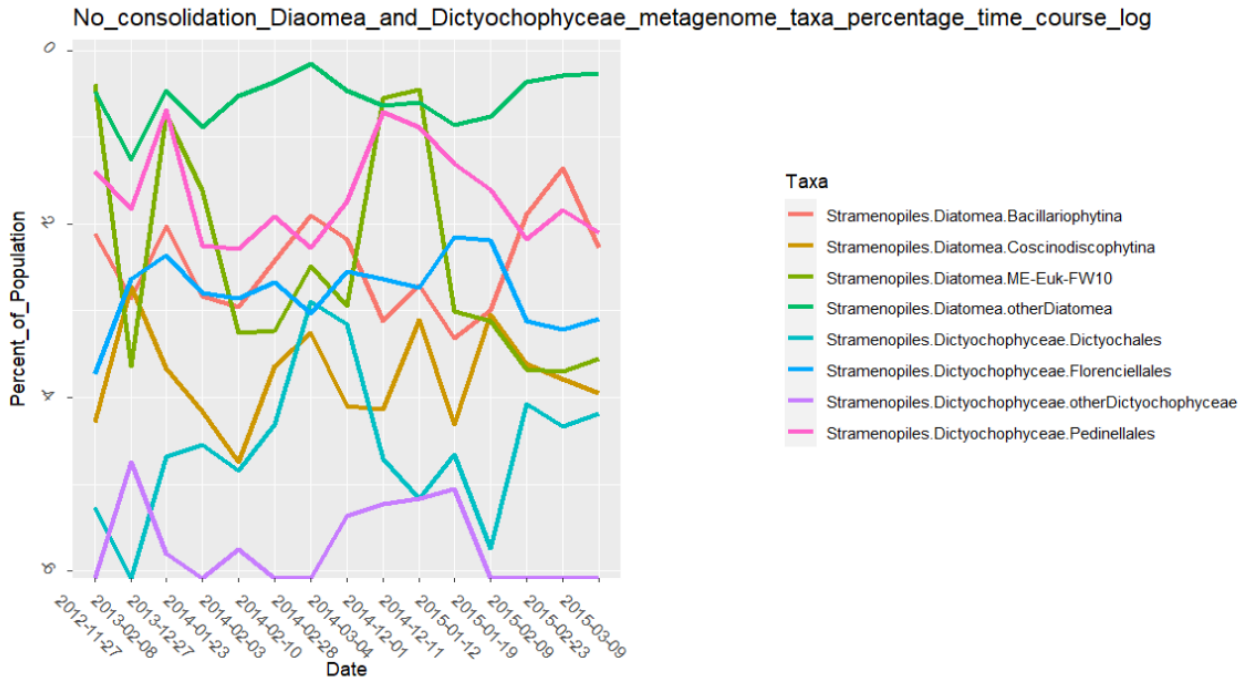
C. D.

Figure 3. Donut charts of identified OTUs with medium consolidation, blue consists of non-diatom Stramenopiles OTUs, red consists of only diatom OTUs, and green consists of non-Stramenopile OTUs. A. Summer of 2012 B. Summer of 2013 C. Summer of 2014 D. Summer of 2012, 2013 and, 2014

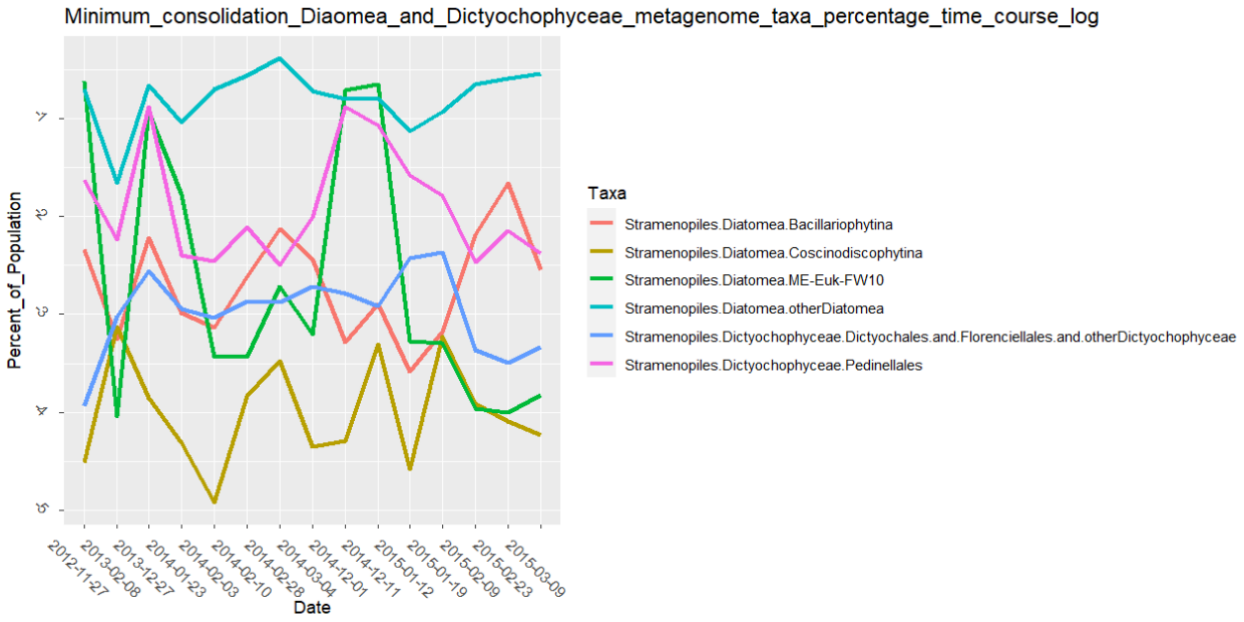
OTUs were consolidated into three degrees of stringency to reduce noise and generate more comprehensible data. To determine the optimal degree of consolidation stringency, a subset of diatoms and Dictyochophyceae were investigated through a time course as seen in Figure 4. No consolidation contains 8 OTUs with a high degree of noise. Maximum consolidation yielded 2 OTUs with a very low degree of OTU separation. Minimum consolidation resulted in 6 OTUs, and medium consolidation resulted in 4. While minimum consolidation had a decrease in noise, it was determined not to be sufficient for meaningful analysis. As such, medium consolidation was determined to have the appropriate noise reduction, while not sacrificing important OTU delineation.

Figure 4.

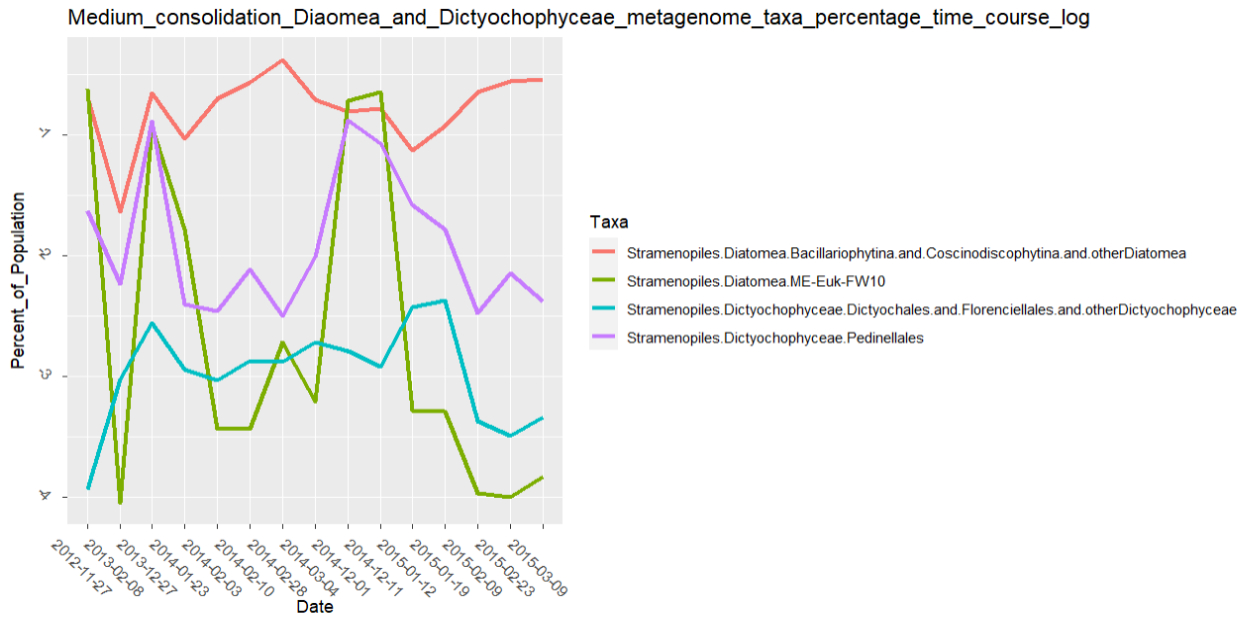
A.



B.


Minimum_consolidation_Diaomea_and_Dictyochophyceae_metagenome_taxa_percentage_time_course_log
Percent_of_Population
Date
2012-11-27
2013-02-08
2013-12-27
2014-01-23
2014-02-03
2014-02-10
2014-02-28
2014-03-04
2014-12-01
2014-12-11
2015-01-12
2015-01-19
2015-02-09
2015-02-23
2015-03-09
Taxa
Stramenopiles.Diatomea.Bacillariophytina
Stramenopiles.Diatomea.Coscinodiscophytina
Stramenopiles.Diatomea.ME-Euk-FW10
Stramenopiles.Diatomea.otherDiatomea
Stramenopiles.Dictyochophyceae.Dictyochales.and.Florenciellales.and.otherDictyochophyceae
Stramenopiles.Dictyochophyceae.Pedinellales

C.


Medium_consolidation_Diaomea_and_Dictyochophyceae_metagenome_taxa_percentage_time_course_log
Percent_of_Population
Date
2012-11-27
2013-02-08
2013-12-27
2014-01-23
2014-02-03
2014-02-10
2014-02-28
2014-03-04
2014-12-01
2014-12-11
2015-01-12
2015-01-19
2015-02-09
2015-02-23
2015-03-09
Taxa
Stramenopiles.Diatomea.Bacillariophytina.and.Coscinodiscophytina.and.otherDiatomea
Stramenopiles.Diatomea.ME-Euk-FW10
Stramenopiles.Dictyochophyceae.Dictyochales.and.Florenciellales.and.otherDictyochophyceae
Stramenopiles.Dictyochophyceae.Pedinellales

D.

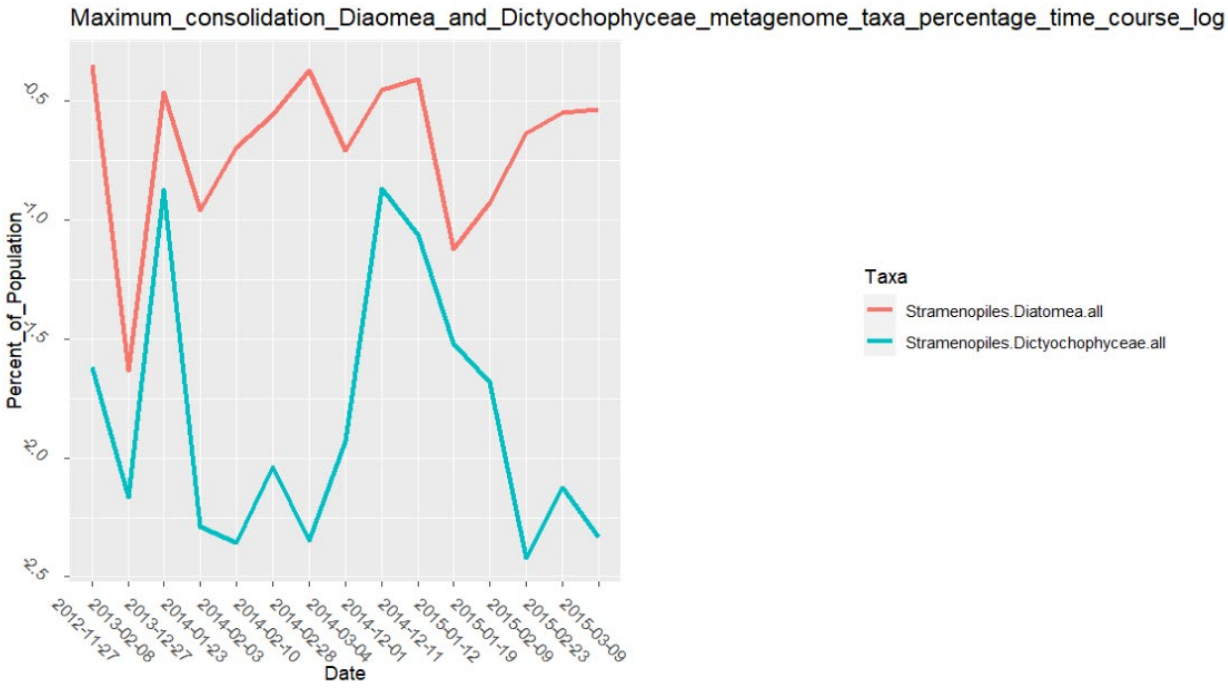


Figure 4. Time course of percentage population as a function of time in diatoms and Dictyochophyceae across no, minimum, medium, and maximum consolidation.

Figure 5.

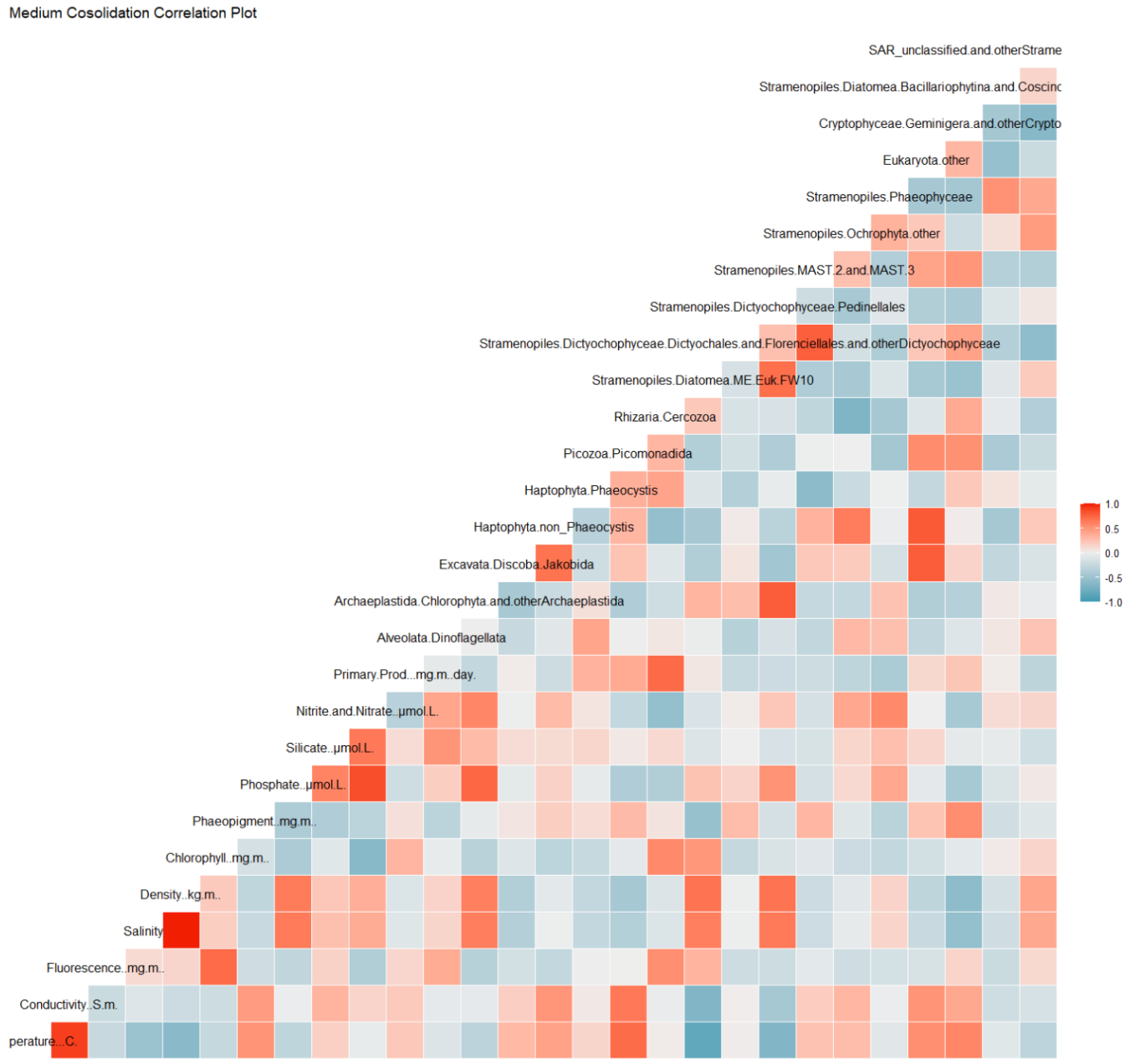


Figure 5. Correlation plot of environmental factors and medium consolidation OTUs using Pearson correlation. Red denotes a positive correlation while blue denotes a negative correlation.

Using the medium consolidation OTUs, those with a Pearson correlation values above 0.65 or below -0.65 were grouped together into four separate partitions as seen in Table 2 (Supplementary Table 2.). Negative correlations were only found between the ***Rhizaria.Cercozoa*** and ***Stramenopiles.Ochrophyta.other*** OTUs.

Table 2.

Group 1	Archaeplastida.Chlorophyta.and.otherArchaeplastida
	Stramenopiles.Diatomea.ME_Euk_FW10
	Stramenopiles.Dictyochophyceae.Pedinellales
Group 2	Excavata.Discoba.Jakobida
	Haptophyta.non_Phaeocystis
	Eukaryota_other
Group 3	Picozoa.Picomonadida
Group 4	Stramenopiles.Dictyochophyceae.Dictyochales. and.Florenciellales.and.otherDictyochophyceae
	Stramenopiles.MAST_2.and.MAST_3
	Stramenopiles.Ochrophyta.other

Table 2. table of grouped OTUs with high correlation determined using Supplementary Table 2.

Principal component analysis of Group 1 confirms the correlation established using the Pearson correlation and explains 63.19% of the data. Fig. 6.A also suggests that salinity has a strong positive correlation with ***Stramenopiles.Dictyochophyceae.Pedinellales***, while lightly correlating with ***Stramenopiles.Diatomea.ME_Euk_FW10*** and ***Archaeplastida.Chlorophyta.and.otherArchaeplastida***. Temperature had a strong negative correlation with ***Stramenopiles.Diatomea.ME_Euk_FW10*** and a slight negative correlation with ***Stramenopiles.Dictyochophyceae.Pedinellales***. The PCA also suggests that ***Archaeplastida.Chlorophyta.and.otherArchaeplastida*** strongly correlates with phosphate, in line with the Pearson correlation results.

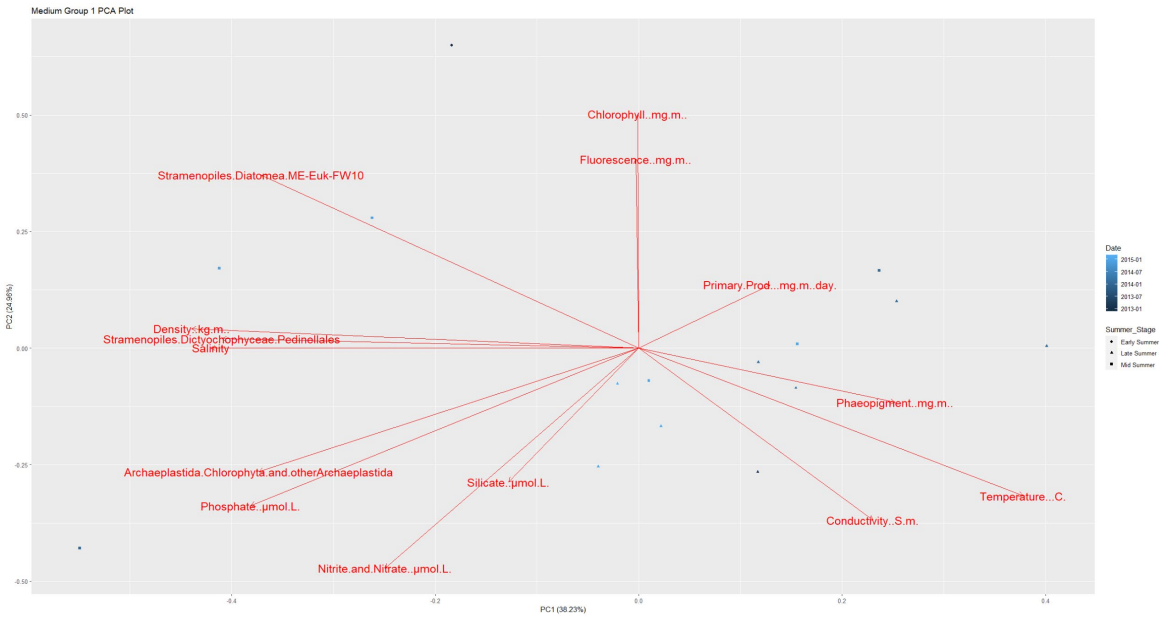
The PCA for Group 2 accounts for 56.09% of the data. The correlation plot did not find a Pearson correlation between Group 2 and environmental factors. However, the PCA indicates a correlation between all Group 2 members, conductivity, and temperature.

PCA of Group 3 accounts for 53.61% of the Group 3 data and corroborates a correlation between ***Picozoa.Picomonadida*** and temperature.

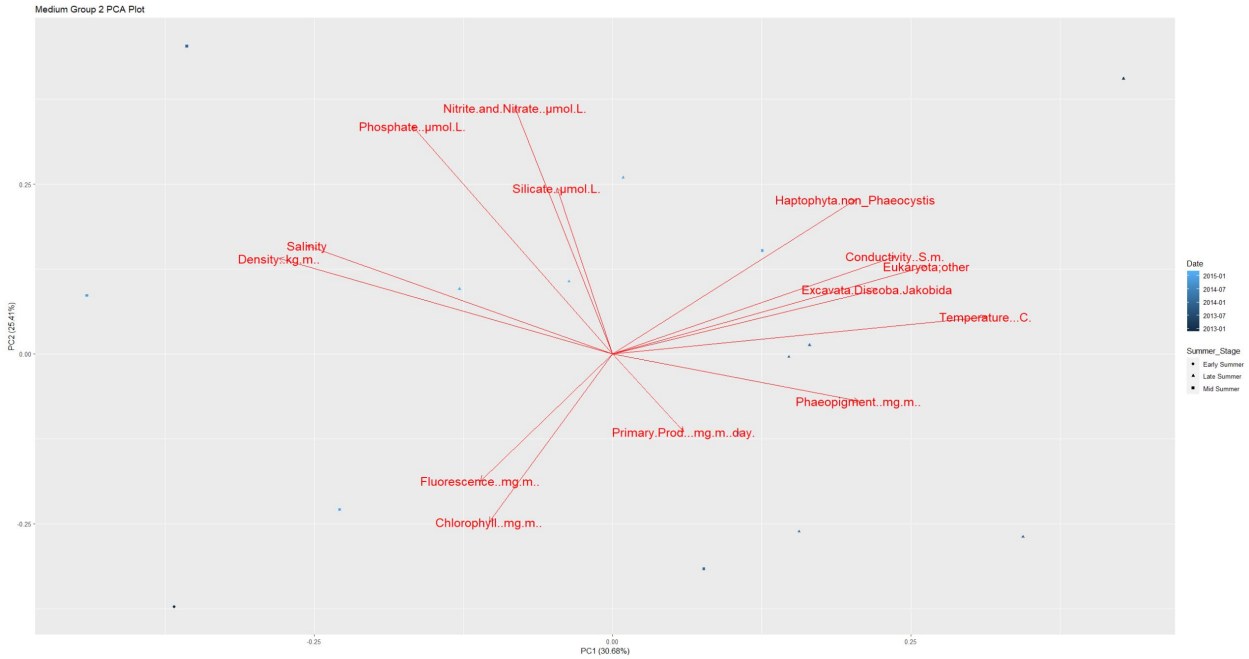
PCA of Group 4 accounts for 51.55% of the data and corroborates a correlation between ***Rhizarai.Cerozoa*** and primary production.

Figure 6.

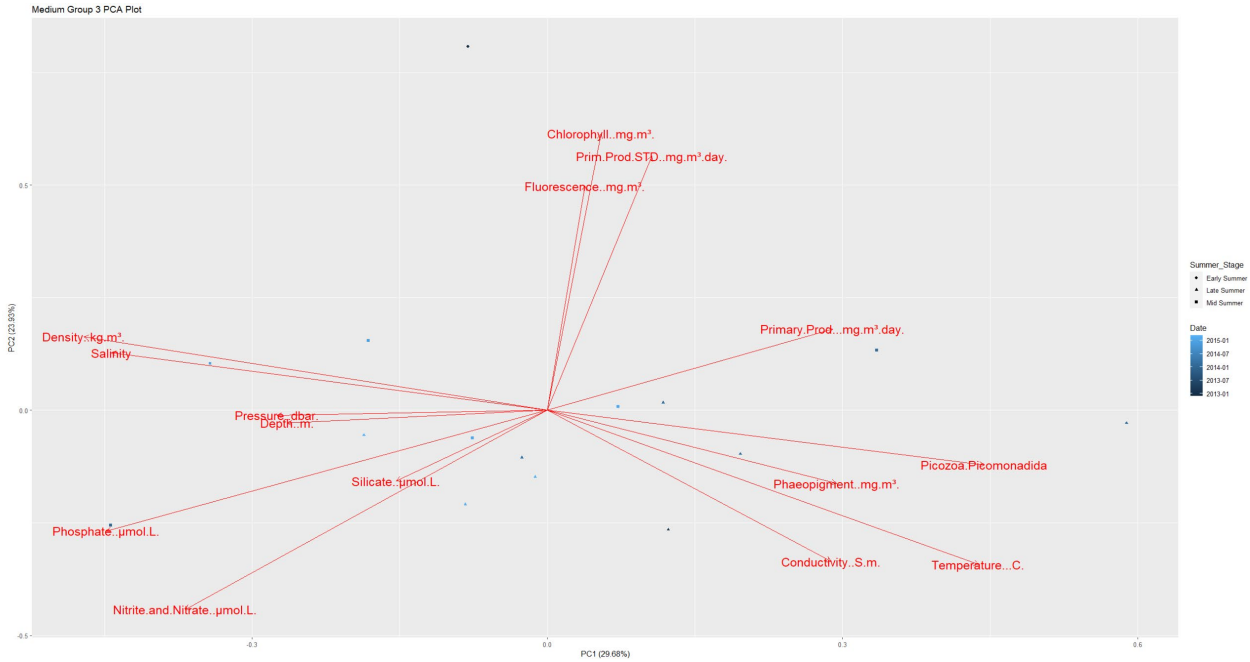
A.



B.


Medium Group 2 PCA Plot
Nitrite.and.Nitrate..µmol.L.
Phosphate..µmol.L.
Silicate..µmol.L.
Haptophyta.non_Phaeocystis
Salinity
Density..kg.m..
Conductivity..S.m.
Eukaryota.other
Excavata.Discoba.Jakobida
Temperature...C.
Phaeopigment..mg.m..
Primary.Prod...mg.m..day.
Fluorescence..mg.m..
Chlorophyll..mg.m..
PC2 (25.41%)
PC1 (30.68%)
Date
2015-01
2014-07
2014-01
2013-07
2013-01
Summer_Stage
Early Summer
Late Summer
Mid Summer

C.


Medium Group 3 PCA Plot
Chlorophyll..mg.m³.
Prim.Prod.STD..mg.m³.day.
Fluorescence..mg.m³.
Density..kg.m³.
Salinity
Primary.Prod...mg.m³.day.
Pressure..dbar.
Depth..m.
Picozoa.Picomonadida
Silicate..µmol.L.
Phaeopigment..mg.m³.
Phosphate..µmol.L.
Conductivity..S.m.
Temperature...C.
Nitrite.and.Nitrate..µmol.L.
PC2 (23.93%)
PC1 (29.68%)
Summer_Stage
Early Summer
Late Summer
Mid Summer
Date
2015-01
2014-07
2014-01
2013-07
2013-01

D.

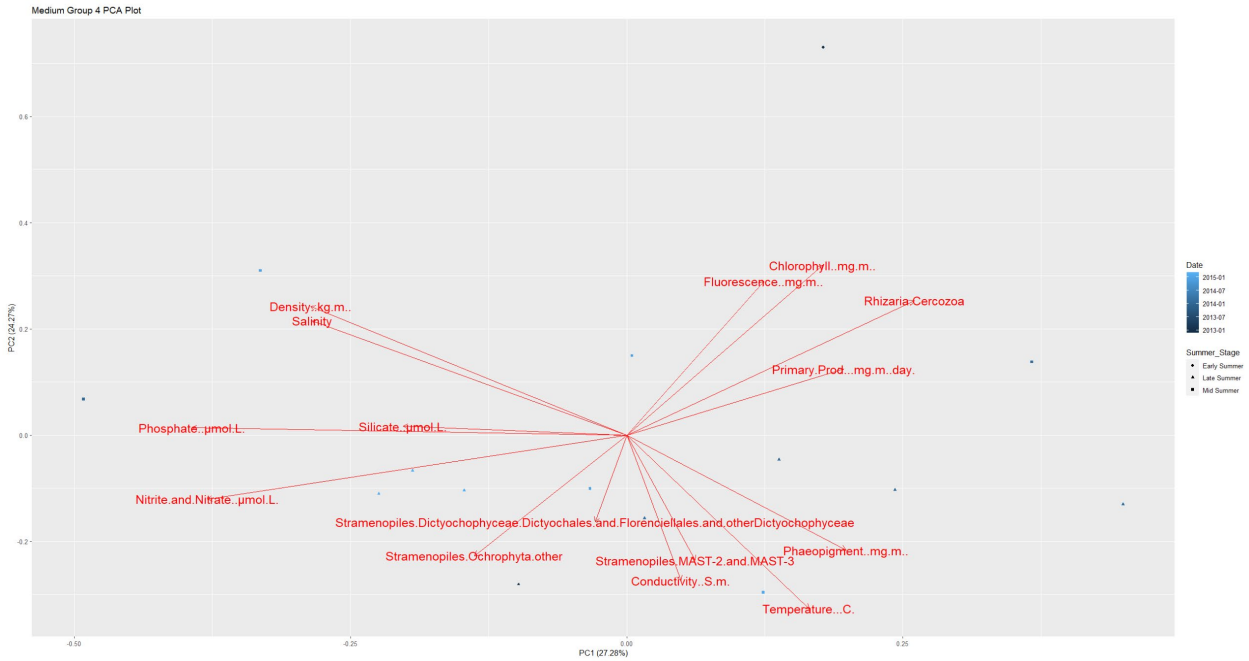


Figure 6. Principal Component Analysis of environmental factors and medium consolidated OTUs and groupings from table 2. A. Group 1 OTUs B. Group 2 OTUs C. Group 3 OTU D. Group 4 OTUs

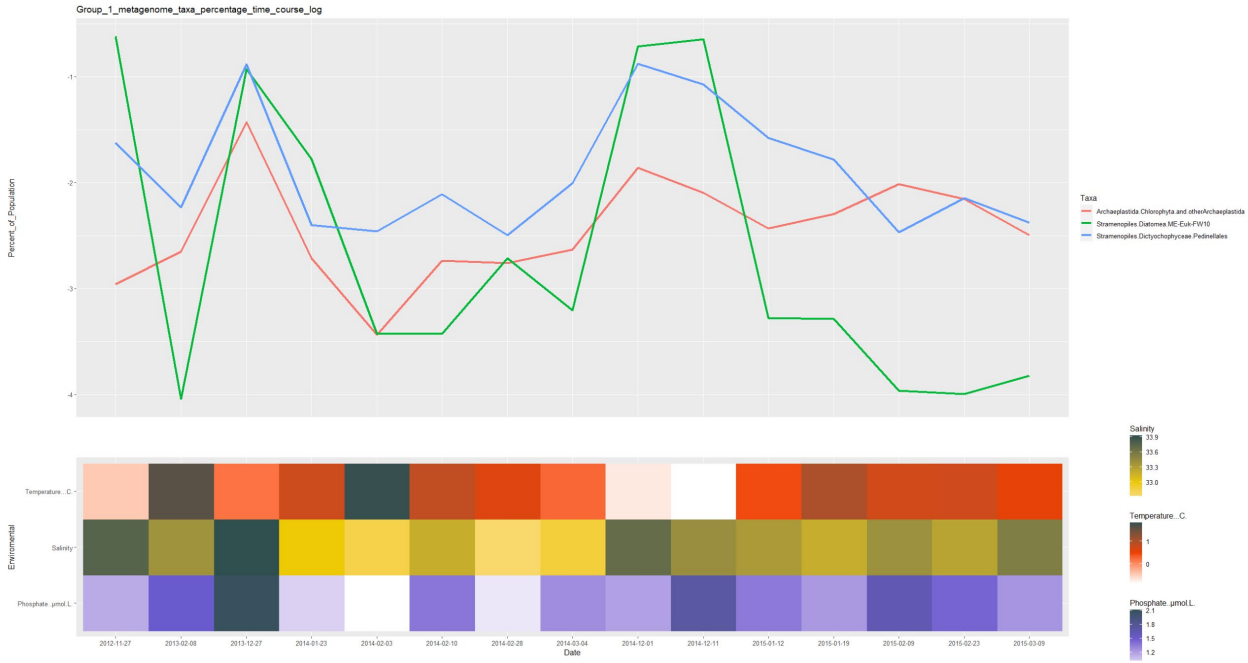
As seen in Fig. 6.A and Fig. 5, Group 1 has a negative correlation with temperature. In particular, ***Stramenopiles.Diatomea.ME_Euk_FW10*** has a strong negative correlation with temperature. There also exists a strong positive correlation between phosphate and ***Archaeplastida.Chlorophyta.and.otherArchaeplastida***. PCA of Group 1 suggests a strong correlation between ***Stramenopiles.Dictyochophyceae.Pedinellales*** and salinity, while the Pearson correlation suggests a weak correlation between the two.

While the Pearson correlation concluded that the correlation of Group 2 to an environmental factor was between -0.65 and 0.65 (Fig. 5), the PCA of Fig. 6.B suggested Group 2 has a correlation with both conductivity and temperature.

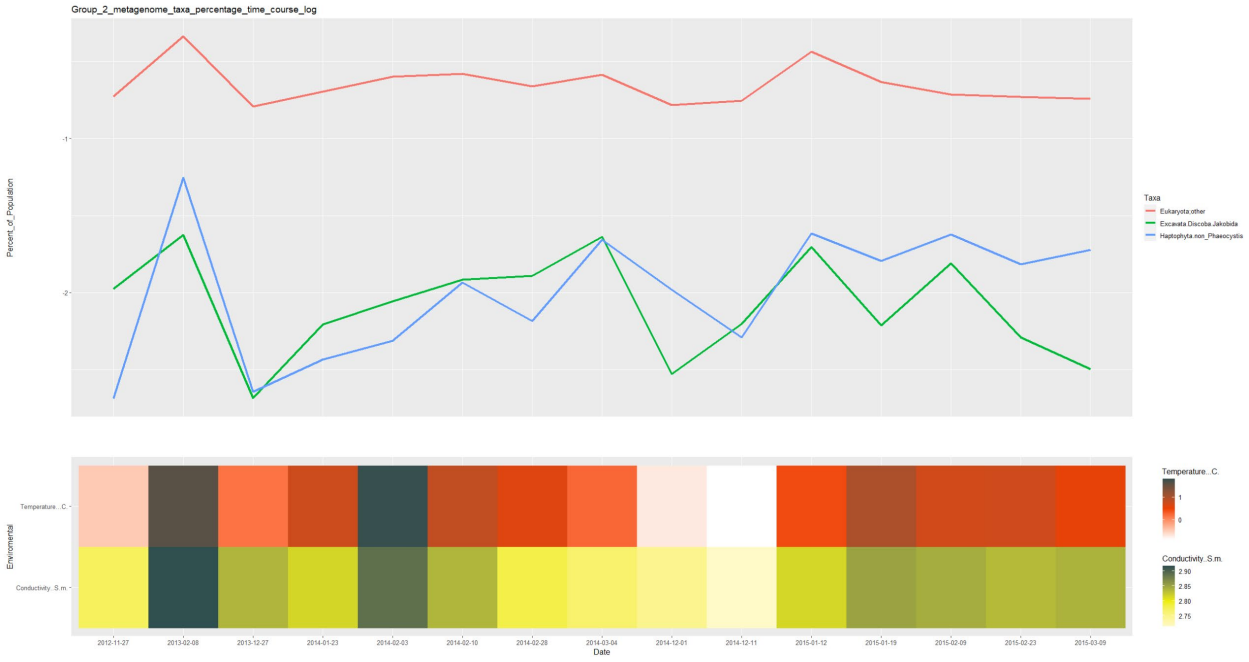
Picazoa.Picomonadida had a strong Pearson correlation with temperature, although displayed a weaker correlation via PCA (Fig. 5 and Fig. 6.C).

Figure 7.

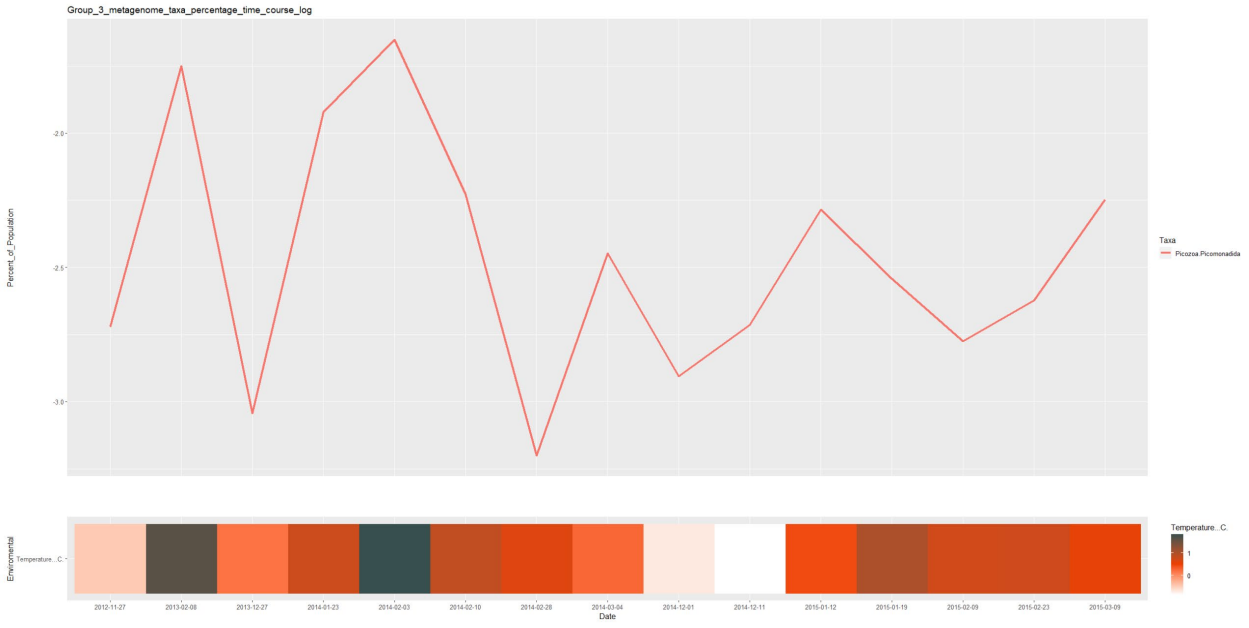
A.



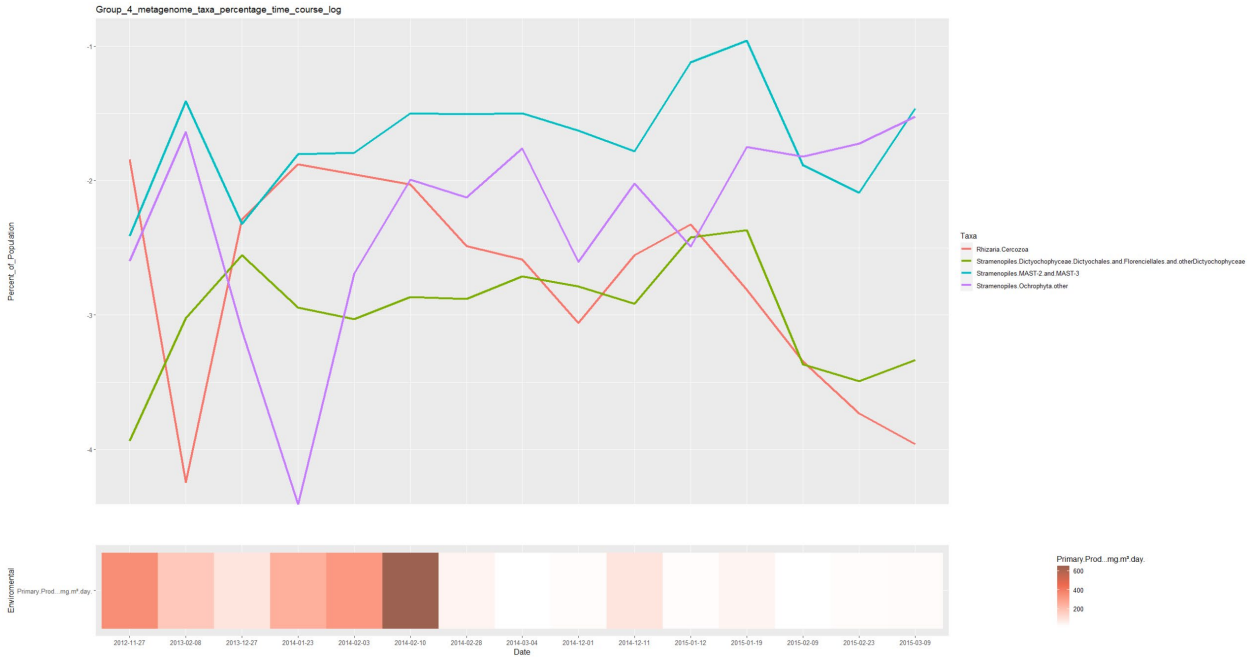
B.


Group_2_metagenome_taxa_percentage_time_course_log
Percent_of_Population
Taxa
Eukaryota.other
Excavata.Discoba.Jakobida
Haptophyta.non_Phaeocystis
Enviromental
Temperature...C.
Conductivity..S.m.
Date

C.


Group_3_metagenome_taxa_percentage_time_course_log
Percent_of_Population
Taxa
Picozoa.Picomonadida
Enviromental
Temperature...C.
Date

D.



E.

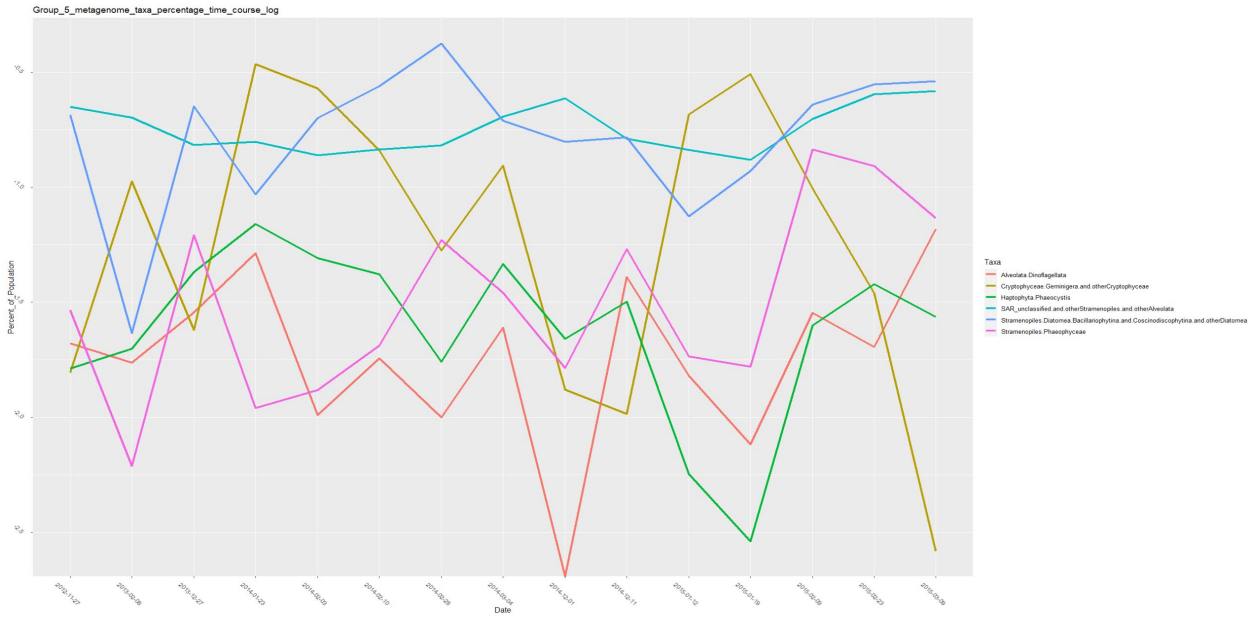


Figure 7. Time course of medium consolidation OTUs group percentages as a function of time. A. Group 1 OTUs with a heat map of Temperature, salinity, and phosphate. B. Group 2 OTUs with a heat map of Temperature and Conductivity. C. Group 3 OTUs with

a heat map of Temperature D. Group 4 OTUs with a heat map of Primary Production. E. Time course of Group 5, all non-correlated OTUs

Discussion

Our results suggest that there is not just one life cycle strategy across microbial eukaryotes during austral summers in the WAP; rather both persistence presence and boom-and-bust were observed in different eukaryotic OTUs. ***Haptophyta_Phaeocystis***, ***Eukaryota;other***, ***SAR_unclassified.and.otherStramenopiles.and.otherAlveolata***, and ***Archaeplastida.Chlorophyta.and.otherArchaeplastida*** have been observed to be persistent in the population through the summer months. This suggests that these OTUs are more resistant to environmental stresses. However, this comes with the drawback of reduced capitalization of favorable environmental conditions. Alternatively, ***Stramenopiles.Diatomea.ME-Euk-FW10***, ***Stramenopiles.Diatomea.Bacillariophytina.and.Coscinodiscophytina.and.otherDiatomea***, ***Cryptophyceae.Geminigera.and.otherCryptophyceae***, ***Stramenopiles.Phaeophyceae***, and ***Stramenopiles.Dictyochophyceae.Dictyochales.and.Florenciellales.and.otherDictyochophycea e*** were observed to have larger variations in their population percentages, suggesting that these OTUs are boom-and-bust.

Both OTUs of the diatom community were observed to be boom-and-bust, suggesting that diatoms in WAP are more susceptible to environmental changes and may be sensitive to climate change. Additionally, it was observed that the diatom population percentage was lower in midsummer during January but increased by 300-400% in the late summer months (Fig. 1.A).

The exception to this trend is the summer of 2012, which experienced an abnormally cold early summer followed by a warm mid-summer and late summer. The diatom population comprised 44.6% of the population in early summer of 2012 and drastically fell to only 2% by later summer. In combination with the Pearson correlation of Fig. 5 and the PCA Fig 6.A, this drastic shift suggests that temperature is a major factor of the diatom population. If temperature is the primary factor at play, then the observed trend favoring the mid-summer over late summer could be caused by a biological affinity for colder weather. Alternatively, it could be caused indirectly by an environmental factor that was not measured, which itself could be a consequence of a lower temperature.

Despite the boom-and-bust nature of the diatoms, they maintained a consistent presence year over year, suggesting that their populations are stable in the long run. The summer of 2014 saw a large shift in populations with Stramenopiles increasing to 35.8% of the population from 24.7% in the previous year. The yearly consistency of the diatoms suggests that the observed shift was due to both an increase in Stramenopiles and a decrease in other eukaryotes.

Salinity, temperature, and light have been shown to positively affect photosynthesis efficiency. It was expected that photosynthesis producing microbial eukaryotes, such as diatoms, would have an increase in population percentages during warmer temperatures and higher salinity. In contrast to those expectations, ***Stramenopiles.Diatomea.ME-Euk-FW10***, ***Archaeplastida.Chlorophyta.and.otherArchaeplastida***, and ***Stramenopiles.Dictyochophyceae.Pedinellales*** instead had a negative correlation with

temperature. While salinity did have a positive correlation, the strong negative correlation between temperature and population percentage suggests that the effects observed are not necessarily due to environmental impacts on photosynthesis or a strong underlying biological factor. It is likely that ocean stratification plays a greater role in microbial eukaryotic populations than previously thought. As outlined by Seyitmuhammedov et al., WAP ocean upwelling is primarily caused by glacial water melt, which induces density shifts and results in water turbulence (Seyitmuhammedov et al., 2022). Ducklow et al. have noted that algae blooms were more frequent and more intense following colder winters and colder austral summers (Ducklow et al., 2006). Additionally, Saba et al. and Higgins Álvarez et al. outlined how higher glaciers, longer periods of ice covers, and reduced wind speeds at sea level decrease ocean wave intensity. In turn, this decreases water column mixing and decreases the water stratified columns to about 20m (Saba et al., 2014; Higgins Álvarez et al., 2022). Venables et al. also suggested that diatom density and count increase as a result of shallower water columns (Venables et al., 2013). This may explain why we observed a decrease in Group 1 population percentage when temperatures rose, particularly when above freezing, and an increase when temperatures fell below freezing. Additionally, this would explain the positive correlation between Group 1 population percentage and salinity. As glacial and sea ice melt increases, the salinity decreases. This causes oceanic upwelling and a decrease in Group 1 population percentages.

To our knowledge, this is the first report that links the population percentage of ***Stramenopiles.Diatomea.ME-Euk-FW10*** to temperature and potentially water column

stratification. The ***Stramenopiles.Diatomea.ME-Euk-FW10*** OTU, as observed in the results, is a large portion of the diatom population and is likely a key species in the marine WAP ecosystem. Better identification, and further research of this ***Stramenopiles.Diatomea.ME-Euk-FW10***, could prove to be critical in understanding the marine WAP ecosystem.

Picozoa.Picomondida is a microbial eukaryote that was once thought to be photosynthetic but has since been discovered to feed off small organic particles. Its strong correlation to temperature is also unexpected. This potentially adds support to the model of ocean stratification having a greater effect on microbial eukaryotic populations than previously expected. Oceanic upwelling brings with it small organic particles that have drifted down the water columns. Increased oceanic turbidity and upwelling could increase the availability of food that Picozoa requires.

More research still needs to be done in the field in order to solidify these conclusions more confidently. For example, clustering using NMDS and PCA were not able to be established. In order to achieve clustering, more water samples must be collected across a wider range of dates. Additionally, in order to confirm stratification as a model for microbial population distribution, future research should collect, sequence, and identify additional microbial eukaryotic populations in relation to water at varying depths and water column depths. Doing this will complicate the analysis but allow for a deeper understanding of the effects of ocean stratification. Further empirical data on glacial size, temperatures, water melt, and sea ice cover would also help in understanding these interactions. Lastly, our work did not

touch upon predation for the populations observed. This is another complex level of interactions as viruses, bacteria, krill, fish, and penguins all play a role.

Citations

Ducklow, H., Vernet, M. and Prezelin, B. (2019) *Dissolved inorganic nutrients including 5 macro nutrients: silicate, phosphate, nitrate, nitrite, and ammonium from water column bottle samples collected between October and April at Palmer Station, 1991 - 2019.*, *Data Portal - Data Package Summary | Environmental Data Initiative (EDI)*. Environmental Data Initiative. Available at: https://portal.edirepository.org/nis/mapbrowse?packageid=knb-lter-pal.211.9 (Accessed: November 24, 2022).

Christaki, U. *et al.* (2005) "Grazing impact of different heterotrophic nanoflagellates on eukaryotic (Ostreococcus Tauri) and prokaryotic picoautotrophs (Prochlorococcus and Synechococcus)," *Environmental Microbiology*, 7(8), pp. 1200–1210. Available at: https://doi.org/10.1111/j.1462-2920.2005.00800.x.

Strassert, J.F. *et al.* (2016) "Moramonas marocensis gen. nov., sp. nov.: a jakobid flagellate isolated from desert soil with a bacteria-like, but bloated mitochondrial genome," *Open Biology*, 6(2), p. 150239. Available at: https://doi.org/10.1098/rsob.150239.

Venables, H.J., Clarke, A. and Meredith, M.P. (2013) "Wintertime controls on summer stratification and productivity at the western Antarctic Peninsula," *Limnology and Oceanography*, 58(3), pp. 1035–1047. Available at: https://doi.org/10.4319/lo.2013.58.3.1035.

Altschul, S.F. *et al.* (1990) "Basic local alignment search tool," *Journal of Molecular Biology*, 215(3), pp. 403–410. Available at: https://doi.org/10.1016/s0022-2836(05)80360-2.

Yilmaz, P. *et al.* (2013) "The Silva and 'all-species living tree project (LTP)' taxonomic frameworks," *Nucleic Acids Research*, 42(D1). Available at: https://doi.org/10.1093/nar/gkt1209.

Quast, C. *et al.* (2012) "The Silva ribosomal RNA gene database project: Improved Data Processing and web-based tools," *Nucleic Acids Research*, 41(D1). Available at: https://doi.org/10.1093/nar/gks1219.

Schloss, P.D. *et al.* (2009) "Introducing mothur: Open-source, platform-independent, community-supported software for describing and comparing microbial communities," *Applied and Environmental Microbiology*, 75(23), pp. 7537–7541. Available at: https://doi.org/10.1128/aem.01541-09.

Meyer, A. *et al.* (2010) "Fast evolving 18s rrna sequences from Solenogastres (mollusca) resist standard PCR amplification and give new insights into mollusk substitution rate

heterogeneity," *BMC Evolutionary Biology*, 10(1). Available at: https://doi.org/10.1186/1471-2148-10-70.

Suzuki, M.T. *et al.* (1997) "Bacterial diversity among small-subunit rrna gene clones and cellular isolates from the same seawater sample," *Applied and Environmental Microbiology*, 63(3), pp. 983–989. Available at: https://doi.org/10.1128/aem.63.3.983-989.1997.

Frölicher, T.L. *et al.* (2015) "Dominance of the Southern Ocean in anthropogenic carbon and heat uptake in CMIP5 models," *Journal of Climate*, 28(2), pp. 862–886. Available at: https://doi.org/10.1175/jcli-d-14-00117.1.

Sabine, C.L. *et al.* (2004) "The oceanic sink for anthropogenic co 2," *Science*, 305(5682), pp. 367–371. Available at: https://doi.org/10.1126/science.1097403.

Kock, K.-H. *et al.* (2007) "Fisheries in the Southern Ocean: An ecosystem approach," *Philosophical Transactions of the Royal Society B: Biological Sciences*, 362(1488), pp. 2333–2349. Available at: https://doi.org/10.1098/rstb.2006.1954.

Saba, G.K. *et al.* (2014) "Winter and spring controls on the summer food web of the coastal West Antarctic Peninsula," *Nature Communications*, 5(1). Available at: https://doi.org/10.1038/ncomms5318.

Seyitmuhammedov, K. *et al.* (2022) "The distribution of Fe across the shelf of the western Antarctic Peninsula at the start of the phytoplankton growing season," *Marine Chemistry*, 238, p. 104066. Available at: https://doi.org/10.1016/j.marchem.2021.104066.

Ducklow, H.W. *et al.* (2006) "Marine pelagic ecosystems: The West Antarctic Peninsula," *Philosophical Transactions of the Royal Society B: Biological Sciences*, 362(1477), pp. 67–94. Available at: https://doi.org/10.1098/rstb.2006.1955.

Smith, R. *et al.* (1995) "The Palmer LTER: A long-term ecological research program at Palmer Station, Antarctica," *Oceanography*, 8(3), pp. 77–86. Available at: https://doi.org/10.5670/oceanog.1995.01.

Hernández-Ruiz, M. *et al.* (2018) "Seasonal succession of small planktonic Eukaryotes inhabiting surface waters of a coastal upwelling system," *Environmental Microbiology*, 20(8), pp. 2955–2973. Available at: https://doi.org/10.1111/1462-2920.14313.

Higgins Álvarez, A. *et al.* (2022) "The effect of waves in hydrodynamics, stratification, and salt wedge intrusion in a microtidal estuary," *Frontiers in Marine Science*, 9. Available at: https://doi.org/10.3389/fmars.2022.974163.

Mock, T. *et al.* (2017) "Evolutionary genomics of the cold-adapted Diatom Fragilariopsis Cylindrus," *Nature*, 541(7638), pp. 536–540. Available at: https://doi.org/10.1038/nature20803.

Assmy, P. *et al.* (2013) "Thick-shelled, grazer-protected Diatoms decouple ocean carbon and silicon cycles in the iron-limited Antarctic Circumpolar current," *Proceedings of the National Academy of Sciences*, 110(51), pp. 20633–20638. Available at: https://doi.org/10.1073/pnas.1309345110.

Thomsen, H.A. *et al.* (1991) "Fine structure and biology of *cryothecomonas* gen. nov. (Protista incertae sedis) from the ice biota," *Canadian Journal of Zoology*, 69(4), pp. 1048–1070. Available at: https://doi.org/10.1139/z91-150.

Jungblut, A.D., Vincent, W.F. and Lovejoy, C. (2012) "Eukaryotes in Arctic and Antarctic cyanobacterial mats," *FEMS Microbiology Ecology*, 82(2), pp. 416–428. Available at: https://doi.org/10.1111/j.1574-6941.2012.01418.x.

Ruggiero, M.A. *et al.* (2015) "Correction: A higher level classification of all living organisms," *PLOS ONE*, 10(6). Available at: https://doi.org/10.1371/journal.pone.0130114.

Cassar, N. *et al.* (2015) "The relation of mixed-layer net community production to phytoplankton community composition in the Southern Ocean," *Global Biogeochemical Cycles*, 29(4), pp. 446–462. Available at: https://doi.org/10.1002/2014gb004936.

Lin, Y. *et al.* (2017) "Specific eukaryotic plankton are good predictors of net community production in the Western Antarctic Peninsula," *Scientific Reports*, 7(1). Available at: https://doi.org/10.1038/s41598-017-14109-1.

Douglas, S.E. and Penny, S.L. (1999) "The plastid genome of the cryptophyte alga, Guillardia Theta: Complete sequence and conserved synteny groups confirm its common ancestry with red algae," *Journal of Molecular Evolution*, 48(2), pp. 236–244. Available at: https://doi.org/10.1007/pl00006462.

Magalhães, K. *et al.* (2021) "Hemiselmis Aquamarina sp. nov. (Cryptomonadales, Cryptophyceae), a cryptophyte with a novel phycobiliprotein type (CR-PC 564)," *Protist*, 172(4), p. 125832. Available at: https://doi.org/10.1016/j.protis.2021.125832.

Sogawa, S. *et al.* (2022) "DNA metabarcoding reveals vertical variation and hidden diversity of Alveolata and rhizaria communities in the western North Pacific," *Deep Sea Research Part I: Oceanographic Research Papers*, 183, p. 103765. Available at: https://doi.org/10.1016/j.dsr.2022.103765.

Queiroz, K.de, Cantino, P.D. and Gauthier, J.A. (2020) "Alveolata T. Cavalier-Smith 1991 [A. G. B. Simpson, B. S. Leander, and M. Dunthorn] converted clade name," *Phylonyms*, pp. 85–90. Available at: https://doi.org/10.1201/9780429446276-13.

Parfrey, L.W. *et al.* (2011) "Estimating the timing of early eukaryotic diversification with multigene molecular clocks," *Proceedings of the National Academy of Sciences*, 108(33), pp. 13624–13629. Available at: https://doi.org/10.1073/pnas.1110633108.

Randlett, M.-È. *et al.* (2014) "Alkenone distribution in Lake Van Sediment over the last 270 ka: Influence of temperature and haptophyte species composition," *Quaternary Science Reviews*, 104, pp. 53–62. Available at: https://doi.org/10.1016/j.quascirev.2014.07.009.

Renaud, S.M. *et al.* (1995) "Effect of temperature on the growth, total lipid content and fatty acid composition of recently isolated tropical microalgae isochrysis sp., Nitzschia Closterium, nitzschia paleacea, and commercial species isochrysis sp. (clone T.ISO)," *Journal of Applied Phycology*, 7(6), pp. 595–602. Available at: https://doi.org/10.1007/bf00003948.

Cuvelier, M.L. *et al.* (2010) "Targeted metagenomics and ecology of globally important uncultured eukaryotic phytoplankton," *Proceedings of the National Academy of Sciences*, 107(33), pp. 14679–14684. Available at: https://doi.org/10.1073/pnas.1001665107.

Seenivasan, R. *et al.* (2013) "Picomonas Judraskeda gen. et sp.. nov.: The first identified member of the picozoa phylum Nov., a widespread group of PicoEukaryotes, formerly known as 'picobiliphytes,'" *PLoS ONE*, 8(3). Available at: https://doi.org/10.1371/journal.pone.0059565.

Moreira, D. and López-García, P. (2014) "The rise and fall of picobiliphytes: How assumed autotrophs turned out to be heterotrophs," *BioEssays*, 36(5), pp. 468–474. Available at: https://doi.org/10.1002/bies.201300176.

O'Kelly, C.J. (1993) "The JAKOBID flagellates: Structural features of jakoba, reclinomonas and Histiona and implications for the early diversification of Eukaryotes," *The Journal of Eukaryotic Microbiology*, 40(5), pp. 627–636. Available at: https://doi.org/10.1111/j.1550-7408.1993.tb06120.x.

Mardones, J.I. *et al.* (2022) "From molecules to ecosystem functioning: Insight into new approaches to taxonomy to monitor harmful algae diversity in Chile," *Advances in Phytoplankton Ecology*, pp. 119–154. Available at: https://doi.org/10.1016/b978-0-12-822861-6.00011-x.

Skjelbred, B. *et al.* (2011) "Toxicity of the ichthyotoxic marine flagellate Pseudochattonella (Dictyochophyceae, Heterokonta) assessed by six bioassays," *Harmful Algae*, 10(2), pp. 144–154. Available at: https://doi.org/10.1016/j.hal.2010.08.007.

Eckford-Soper, L. and Daugbjerg, N. (2016) "The ichthyotoxic genus pseudochattonella (Dictyochophyceae): Distribution, toxicity, enumeration, ecological impact, succession and life history – a review," *Harmful Algae*, 58, pp. 51–58. Available at: https://doi.org/10.1016/j.hal.2016.08.002.

Lengyel, E. *et al.* (2019) "Annual hydrological cycle of environmental variables in astatic soda pans (Hungary)," *Journal of Hydrology*, 575, pp. 1188–1199. Available at: https://doi.org/10.1016/j.jhydrol.2019.06.015.

Lakshmi, N.J., Bhattacharjya, R. and Tiwari, A. (2022) "Impact of 17-β estradiol on growth and metabolism of marine Diatom Thalassiosira weissflogii," *Environmental Advances*, 9, p. 100291. Available at: https://doi.org/10.1016/j.envadv.2022.100291.

Sethi, D. *et al.* (2020) "Diatoms for carbon sequestration and bio-based manufacturing," *Biology*, 9(8), p. 217. Available at: https://doi.org/10.3390/biology9080217.

Zhou, D. *et al.* (2022) "Diatom frustules based dissolved oxygen sensor with superhydrophobic surface," *Sensors and Actuators B: Chemical*, 371, p. 132549. Available at: https://doi.org/10.1016/j.snb.2022.132549.

Lengyel, E. *et al.* (2020) "Climate change projections for Carpathian soda pans on the basis of photosynthesis evidence from typical Diatom species," *Science of The Total Environment*, 710, p. 136241. Available at: https://doi.org/10.1016/j.scitotenv.2019.136241.

Han, K.Y. *et al.* (2019) "Dictyochophyceae plastid genomes reveal unusual variability in their organization," *Journal of Phycology*, 55(5), pp. 1166–1180. Available at: https://doi.org/10.1111/jpy.12904.

Cho, A. *et al.* (2022) "Monophyly of diverse bigyromonadea and their impact on phylogenomic relationships within Stramenopiles," *Molecular Phylogenetics and Evolution*, 171, p. 107468. Available at: https://doi.org/10.1016/j.ympev.2022.107468.

Keeling, P.J. and Burki, F. (2019) "Progress towards the tree of Eukaryotes," *Current Biology*, 29(16). Available at: https://doi.org/10.1016/j.cub.2019.07.031.

Blaby-Haas, C.E. and Merchant, S.S. (2019) "Comparative and functional algal Genomics," *Annual Review of Plant Biology*, 70(1), pp. 605–638. Available at: https://doi.org/10.1146/annurev-arplant-050718-095841.

Tikhonenkov, D.V. (2020) “Predatory flagellates – the new recently discovered deep branches of the eukaryotic tree and their evolutionary and ecological significance,” *Protistology*, 14(1). Available at: https://doi.org/10.21685/1680-0826-2020-14-1-2.

Ball, S. *et al.* (2011) “The evolution of glycogen and starch metabolism in Eukaryotes gives molecular clues to understand the establishment of plastid endosymbiosis,” *Journal of Experimental Botany*, 62(6), pp. 1775–1801. Available at: https://doi.org/10.1093/jxb/erq411.

Supplementary Tables and Figures

Table 1.A Minimum consolidation

Chlorophyta & other Archaeplastida	Archaeplastida. Chlorophyta & otherArchaeplastida
Cryptophyte	Cryptophyceae.Cryptomonadales.Geminigera
Cryptophyte	Cryptophyceae.otherCryptophyceae
Jakobida	Excavata.Discoba.Jakobida
Haptophyte	Haptophyta.Phaeocystis
Haptophyte	Haptophyta.non-Phaeocystis
Picozoa	Picozoa.Picomonadida
Dinoflagellate	Alveolata.Dinoflagellata
Other Alveolata	Alveolata.otherAlveolata
Rhizaria	Rhizaria.Cercozoa
MAST (Marine stramenopile)	Stramenopiles.MAST-2 & MAST-3
Diatom	Stramenopiles.Diatomea.Bacillariophytina
Diatom	Stramenopiles.Diatomea.Coscinodiscophytina
Diatom	Stramenopiles.Diatomea.otherDiatomea
Diatom	Stramenopiles.Diatomea.ME-Euk-FW10
Dictyochophyceae	Stramenopiles.Dictyochophyceae.Dictyochales & Florenciellales & otherDictyochophyceae
Dictyochophyceae	Stramenopiles.Dictyochophyceae.Pedinellales
Ochrophyta	Stramenopiles.Ochrophyta.other
Phaeophyceae	Stramenopiles.Phaeophyceae
Other Stramenopiles	Stramenopiles.otherStramenopiles
SAR_unclassified	SAR_unclassified
other Eukaryote	Eukaryota;other

Table 1.B Maximum Consolidation

Chlorophyta & other Archaeplastida	Archaeplastida. Chlorophyta & otherArchaeplastida
Cryptophyte	Cryptophyceae..Geminigera & otherCryptophyceae
Jakobida	Excavata.Discoba.Jakobida
Haptophyte	Haptophyta.Phaeocystis & non-Phaeocystis
Picozoa	Picozoa.Picomonadida
Dinoflagellate	Alveolata.Dinoflagellata
Rhizaria	Rhizaria.Cercozoa

MAST (Marine stramenopile)	Stramenopiles.MAST-2 & MAST-3
Diatom	Stramenopiles.Diatomea (all)
Dictyochophyceae	Stramenopiles.Dictyochophyceae (all)
SAR unclassified	SAR_unclassified & other Stramenopiles & other Alveolata & Ochrophyta & Phaeophyceae
other Eukaryote	Eukaryota;other

Minimum Consolidation

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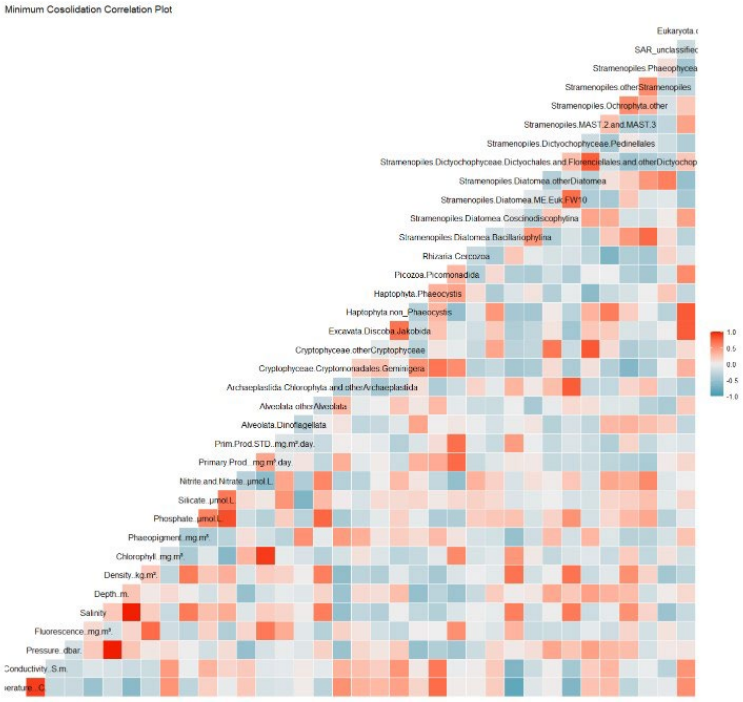

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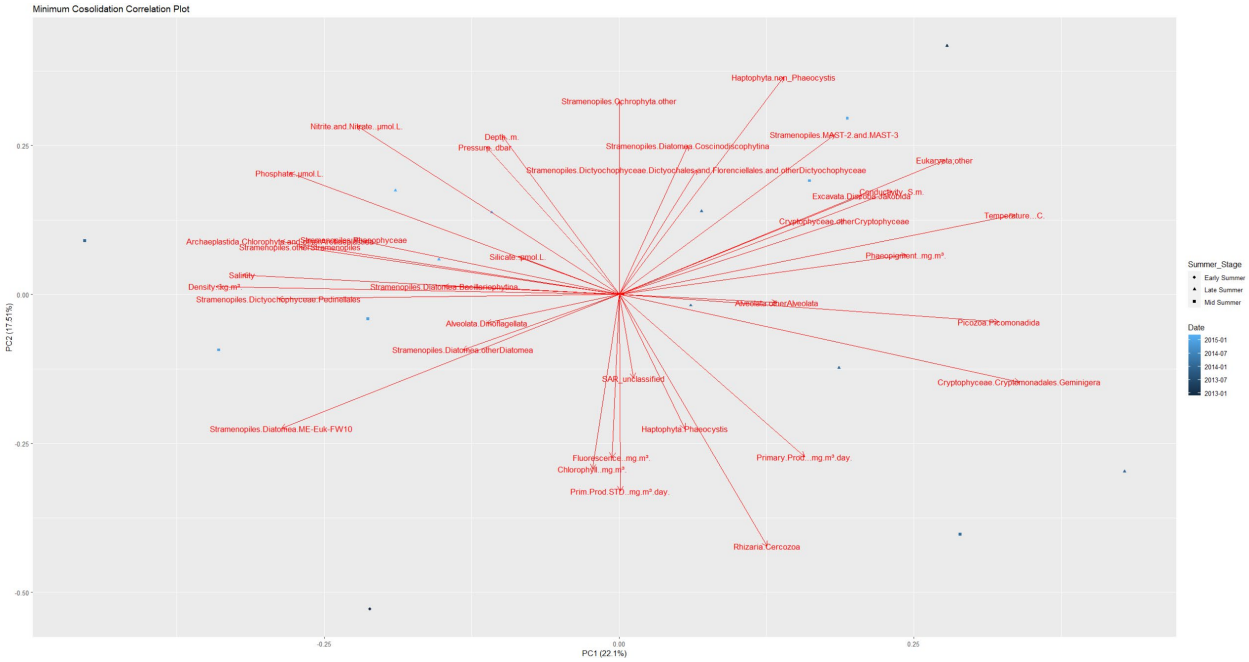

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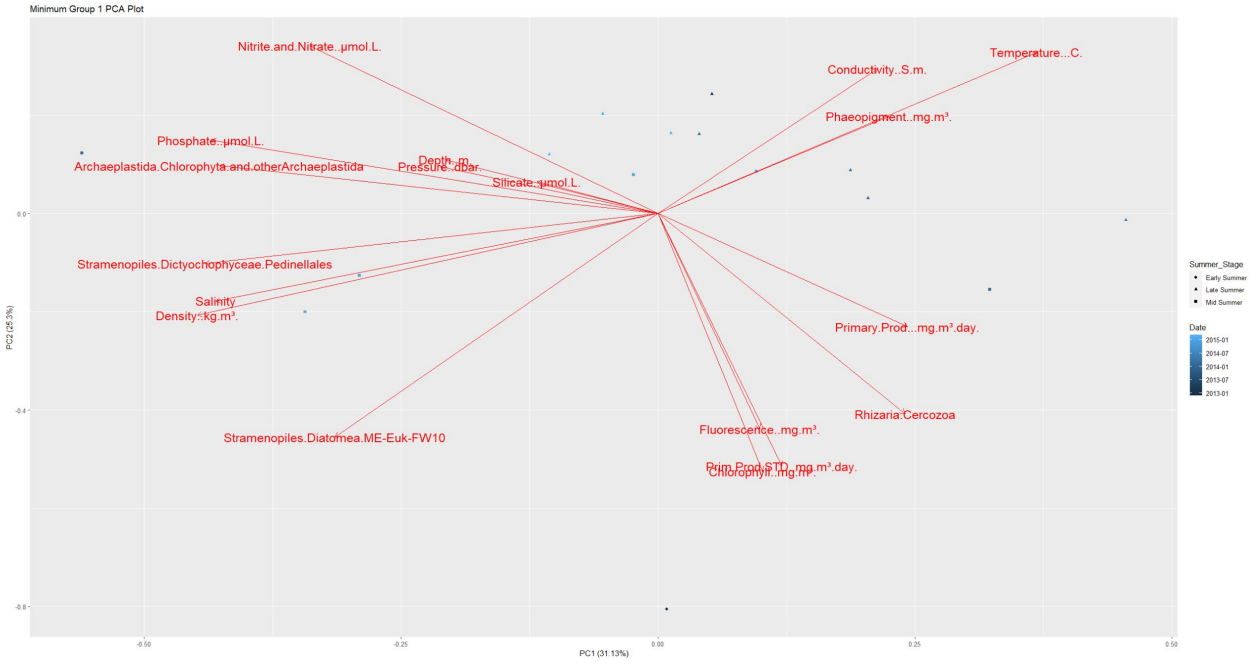

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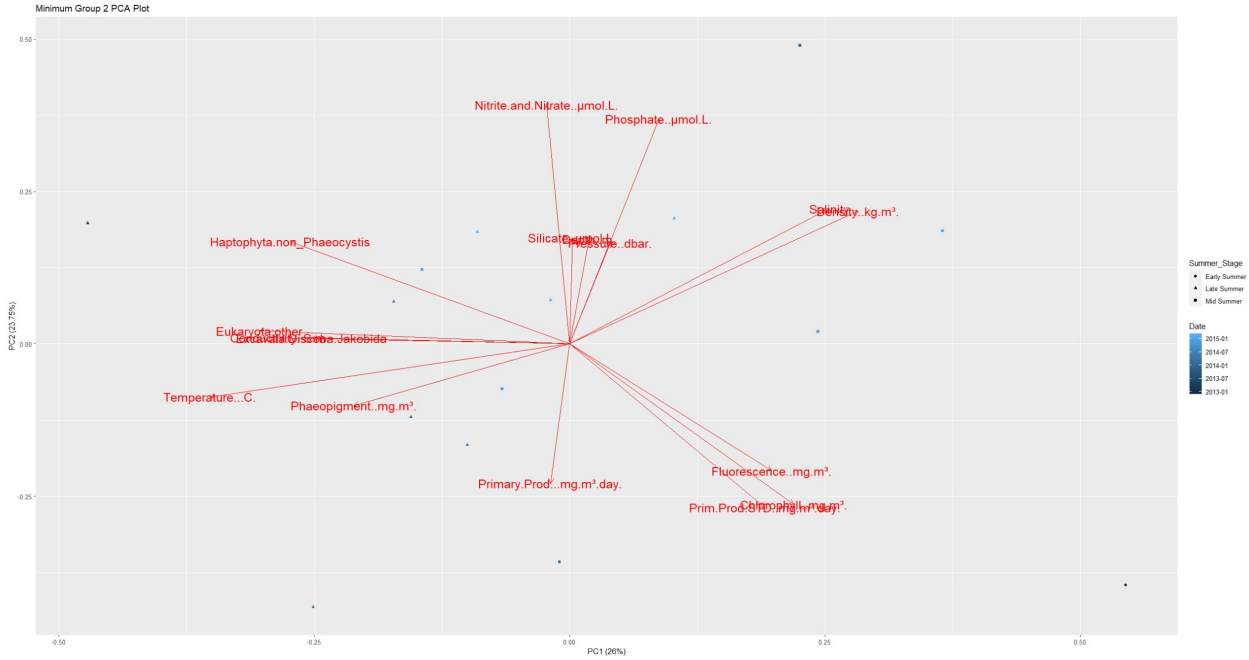

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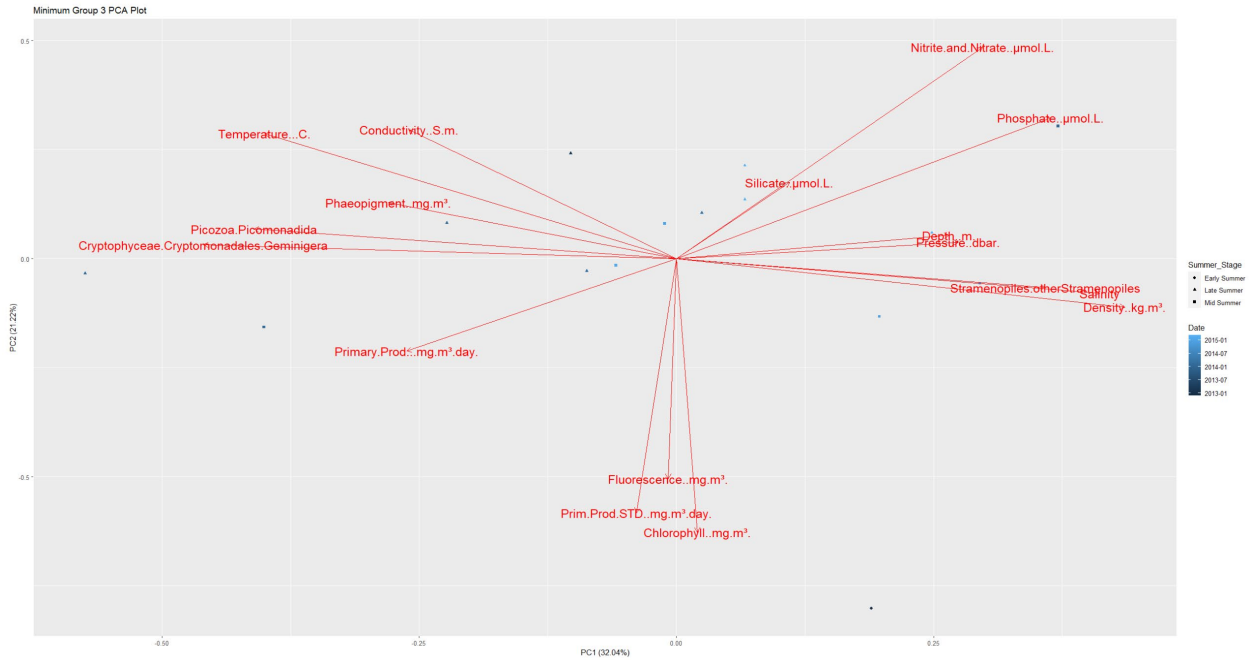

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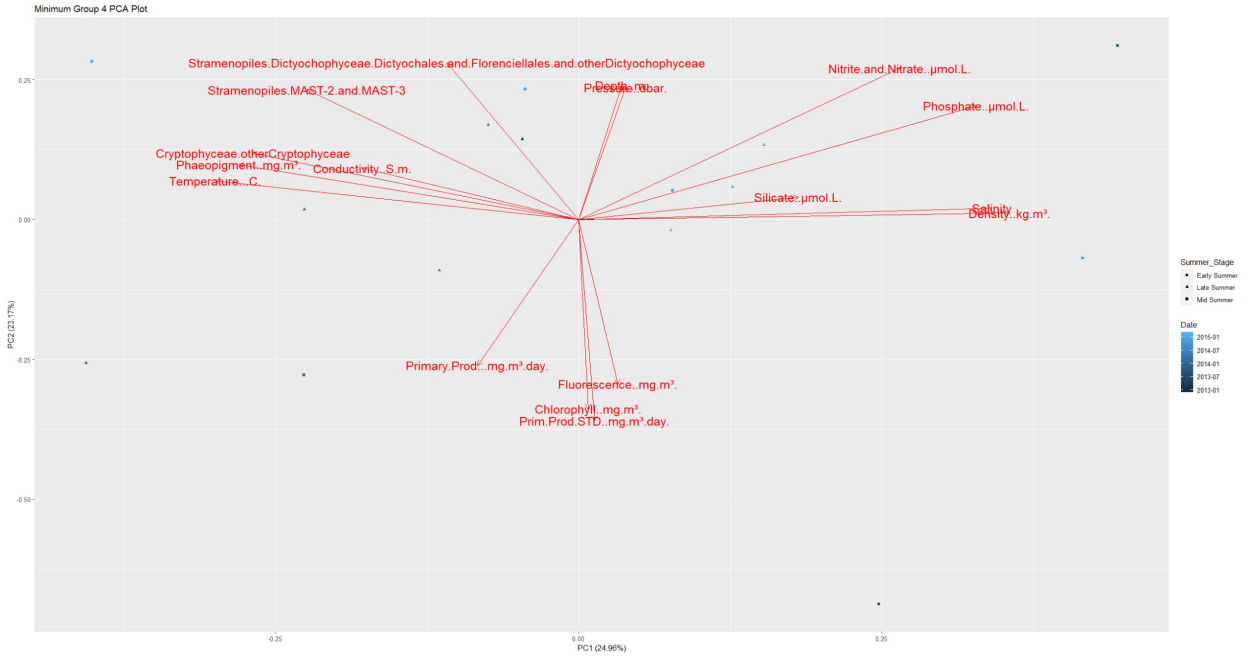

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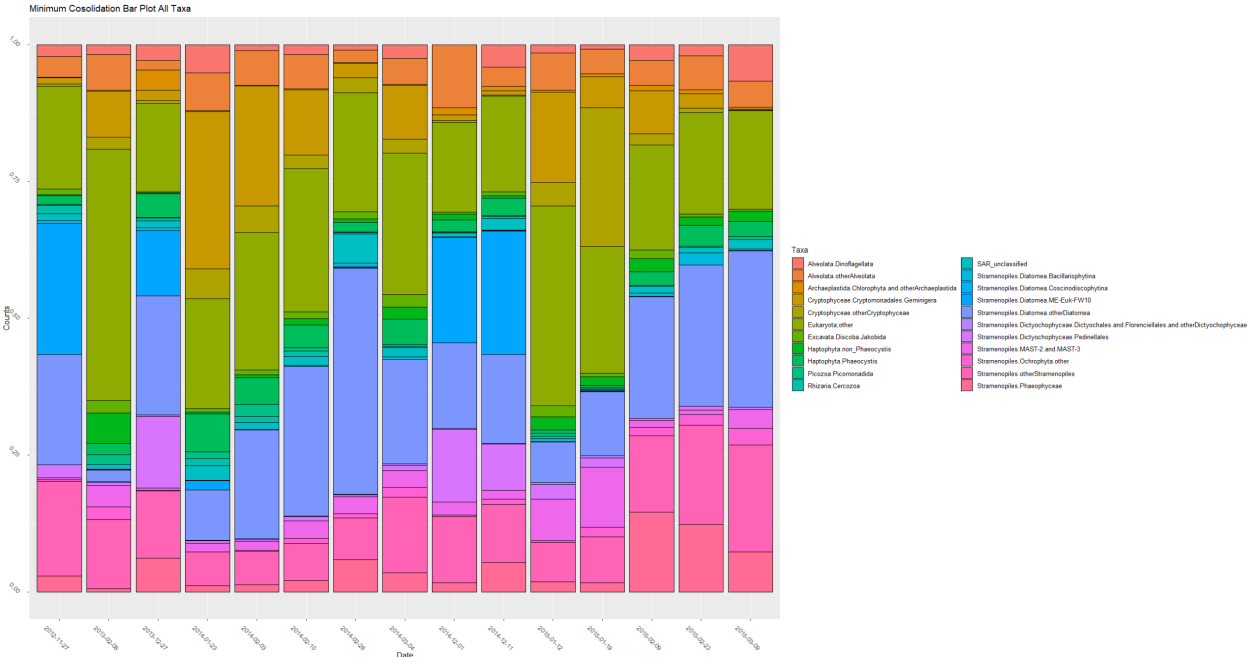

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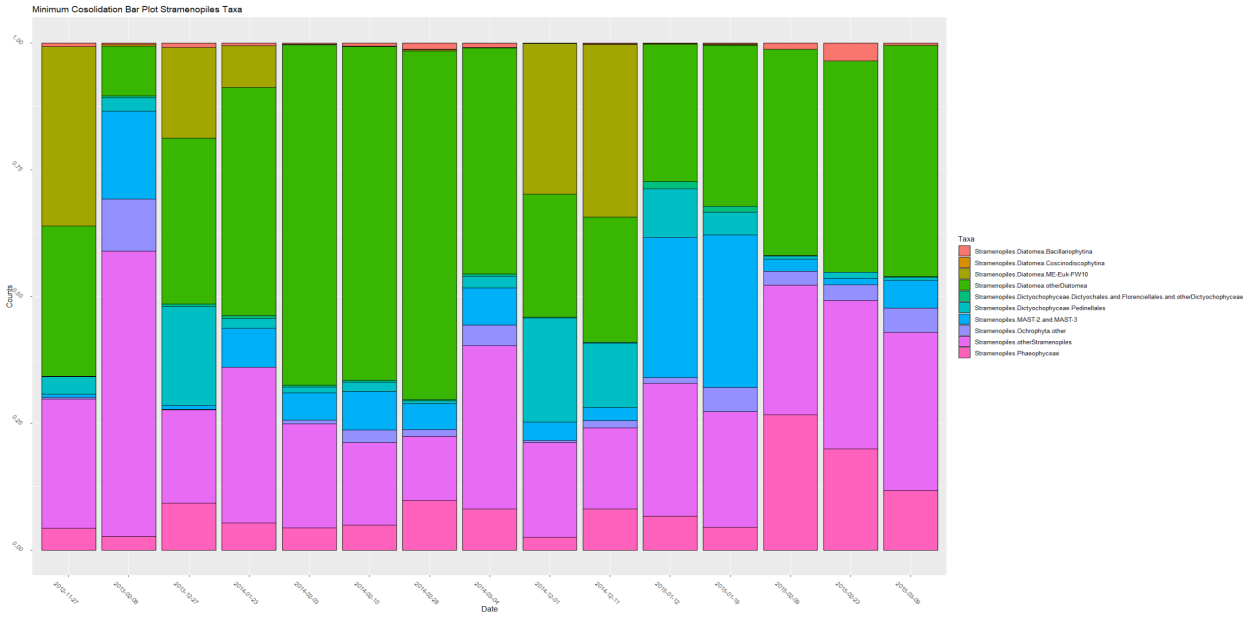

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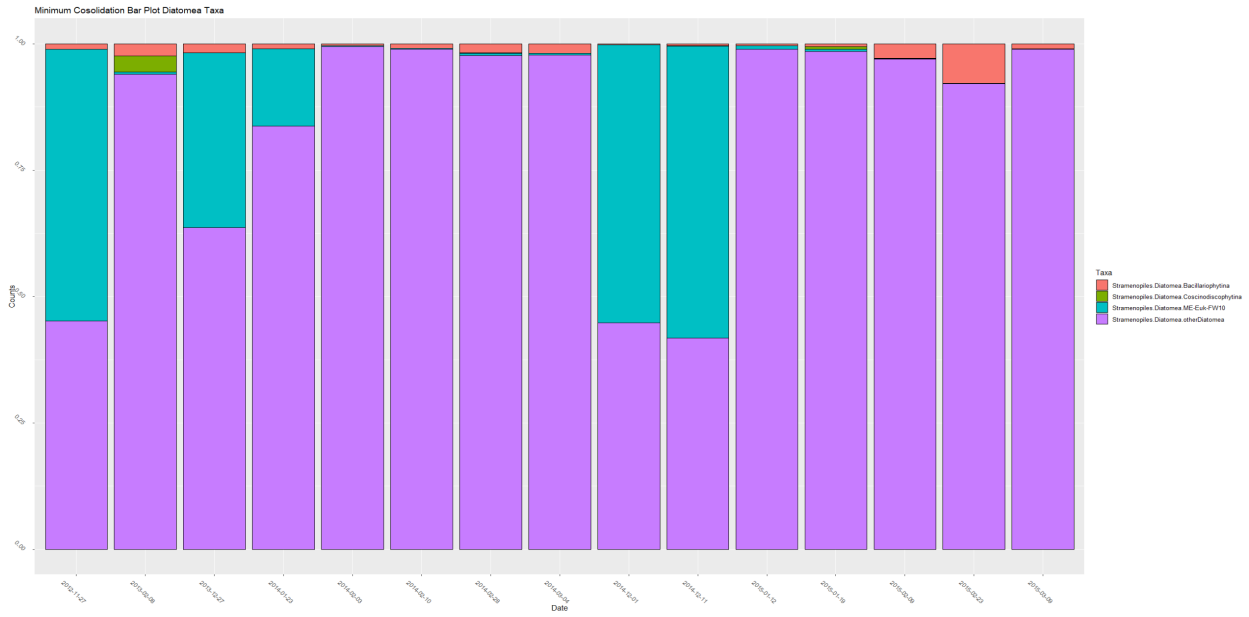

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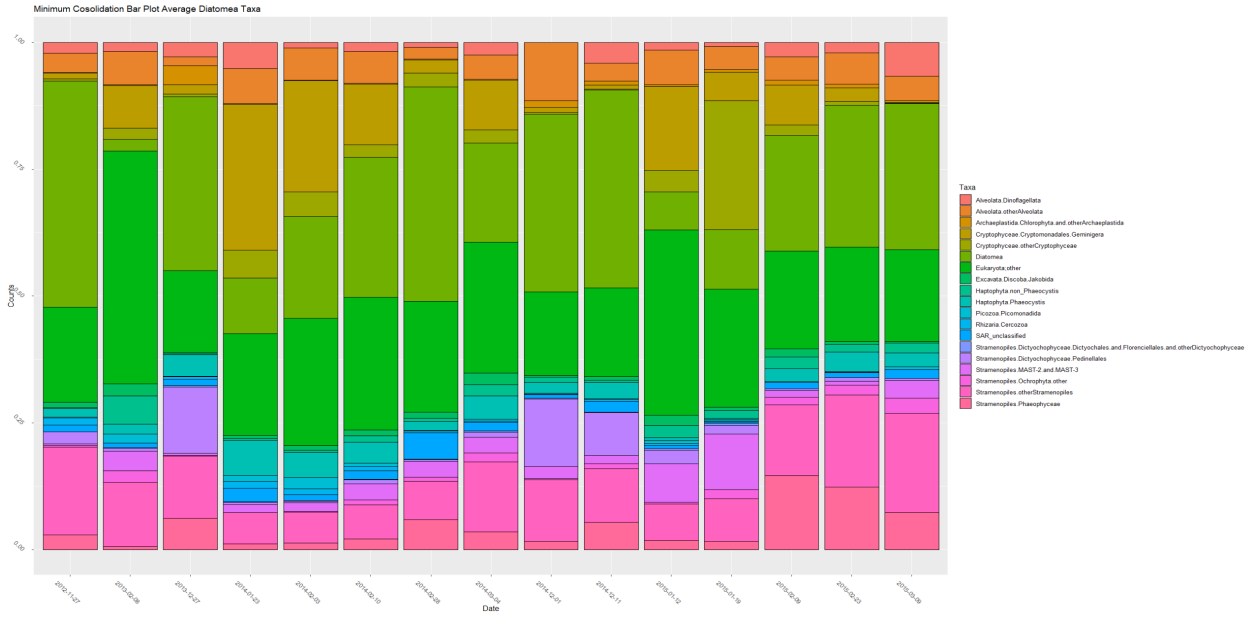

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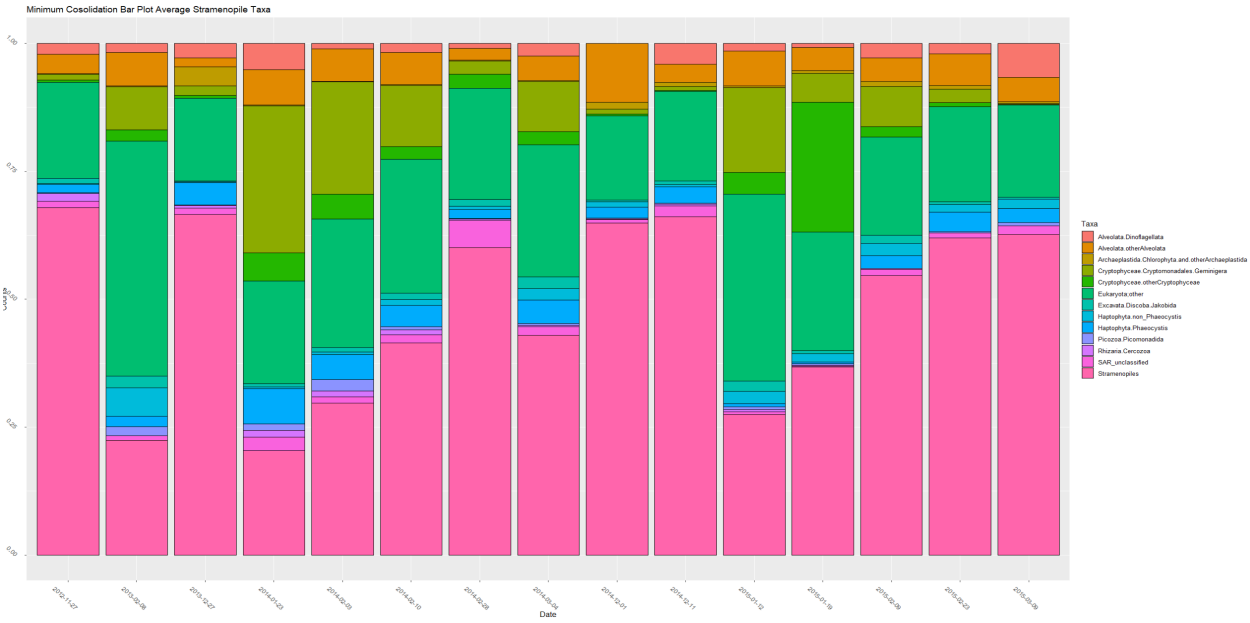

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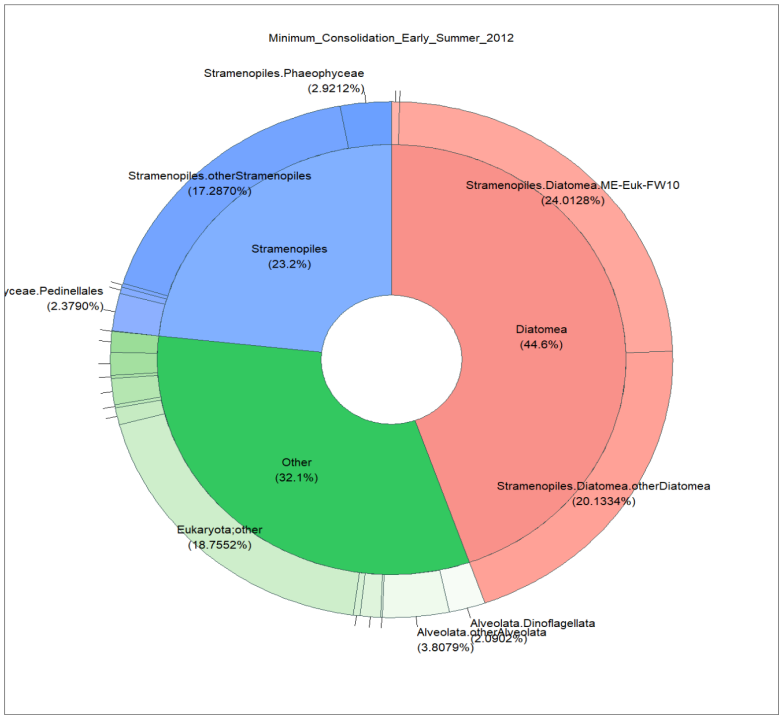

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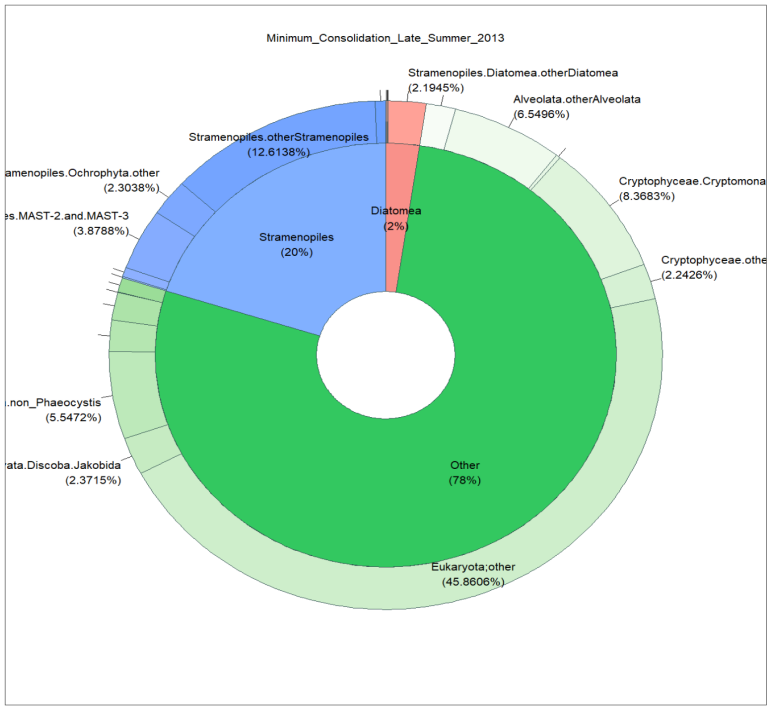

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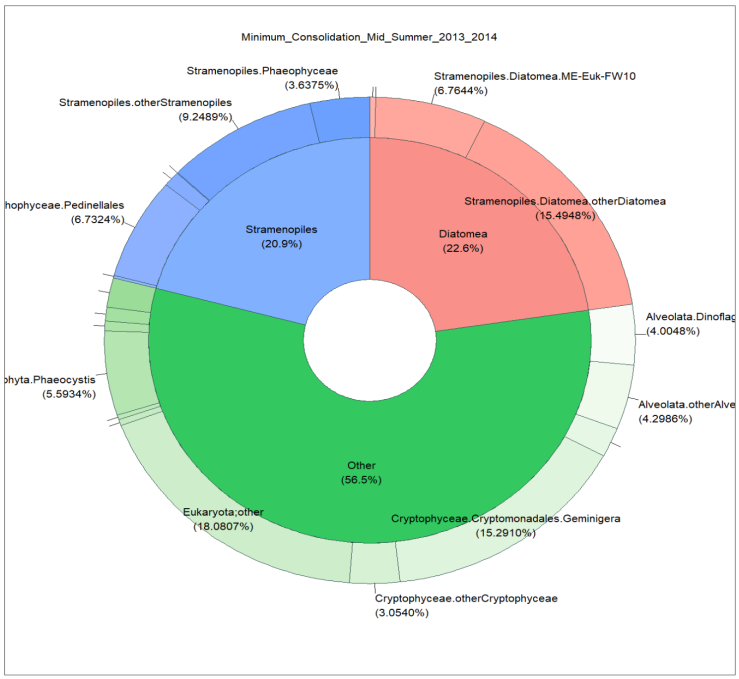

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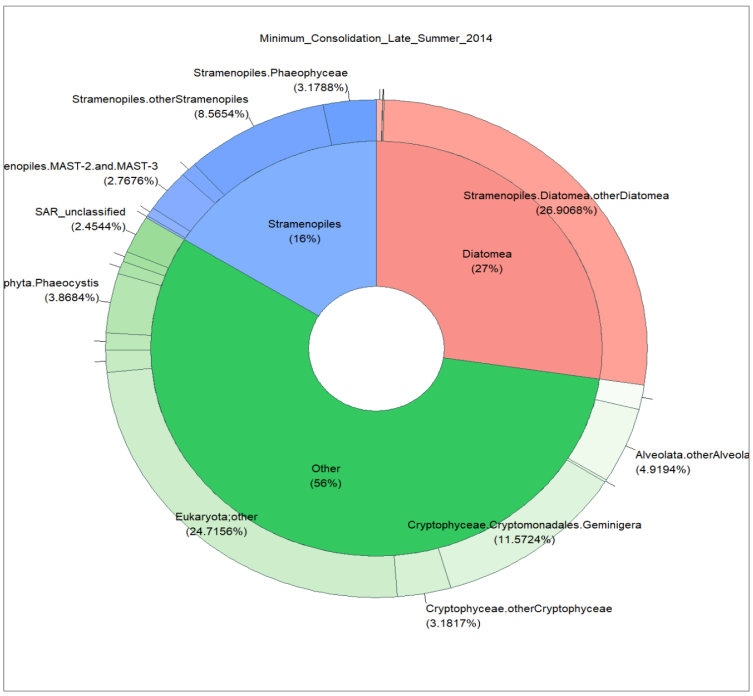

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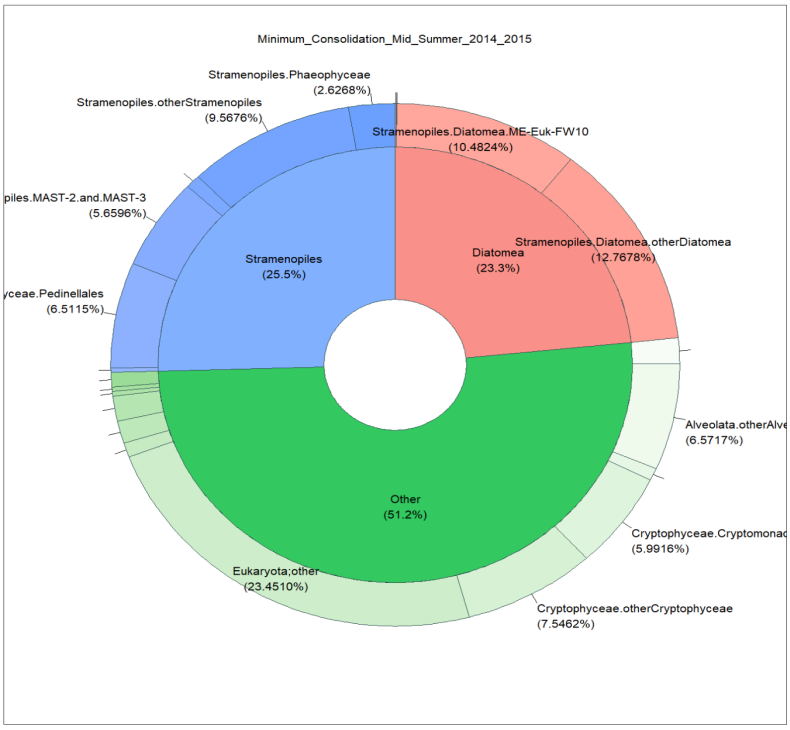

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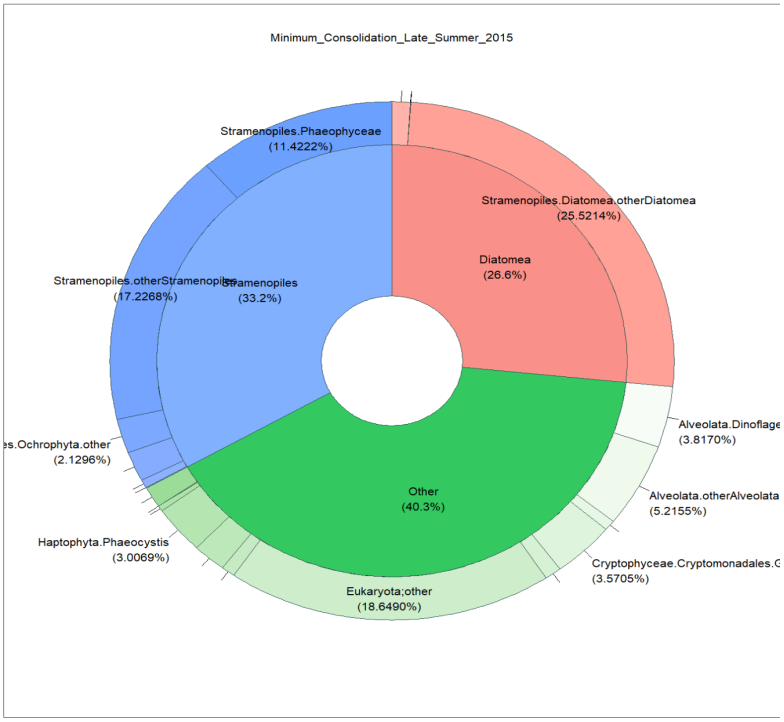

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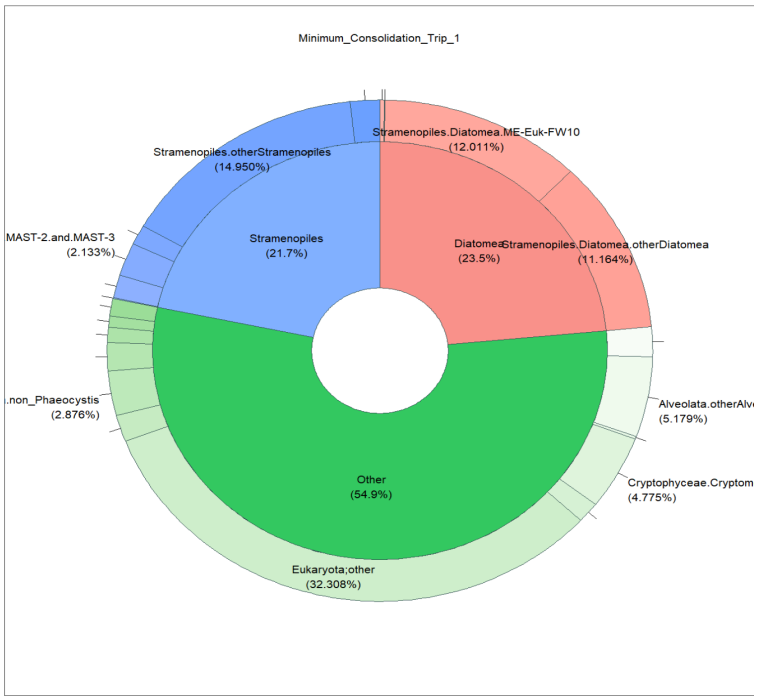

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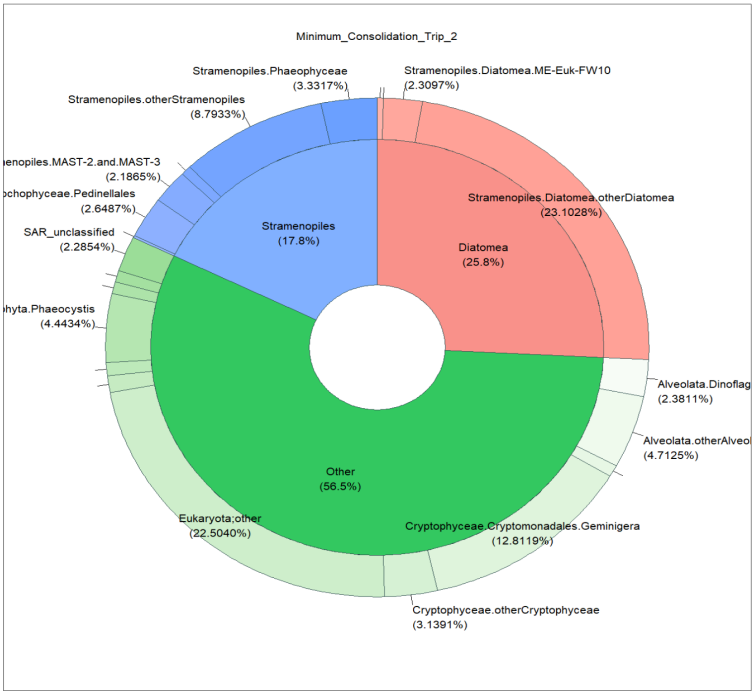

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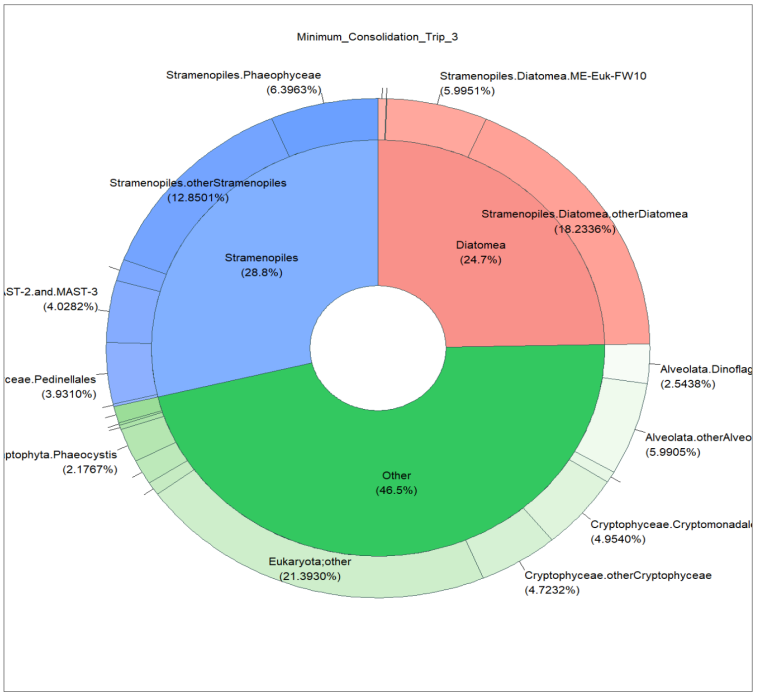

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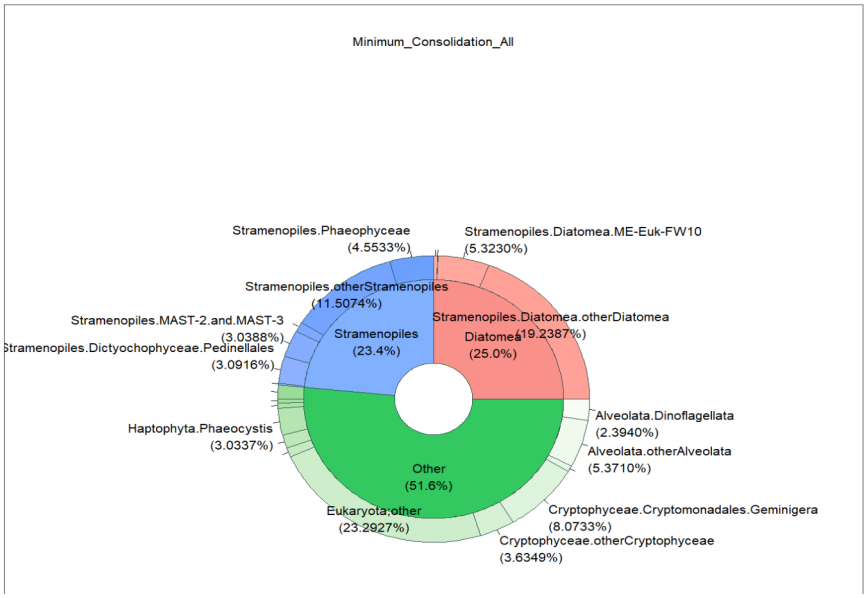

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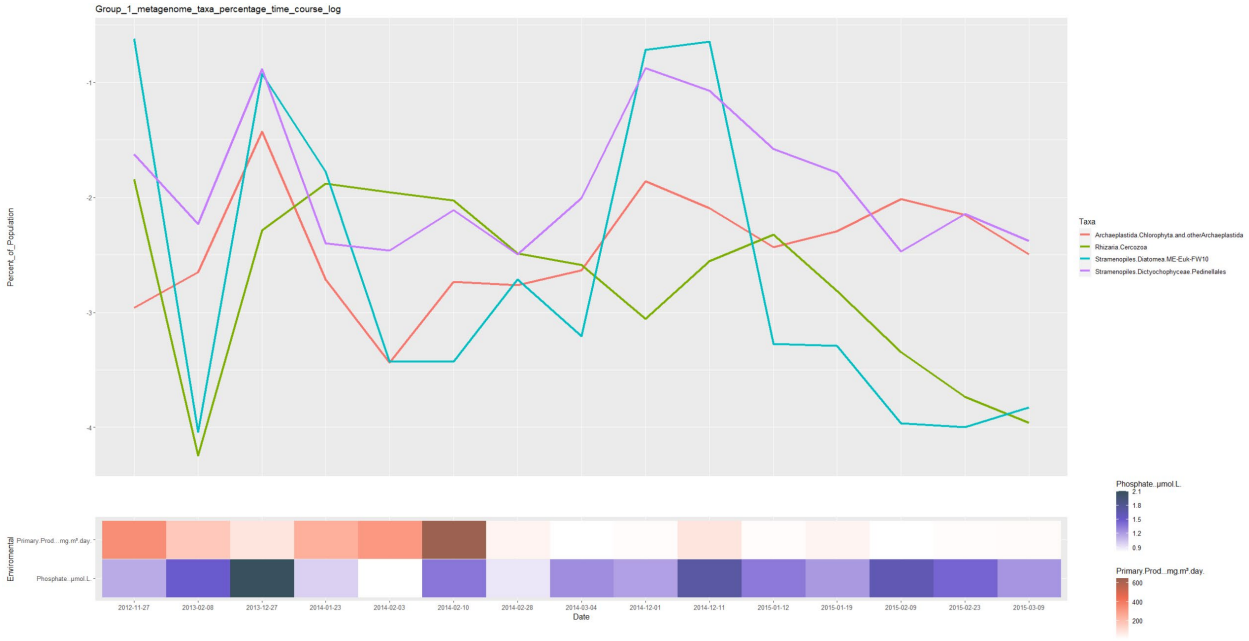

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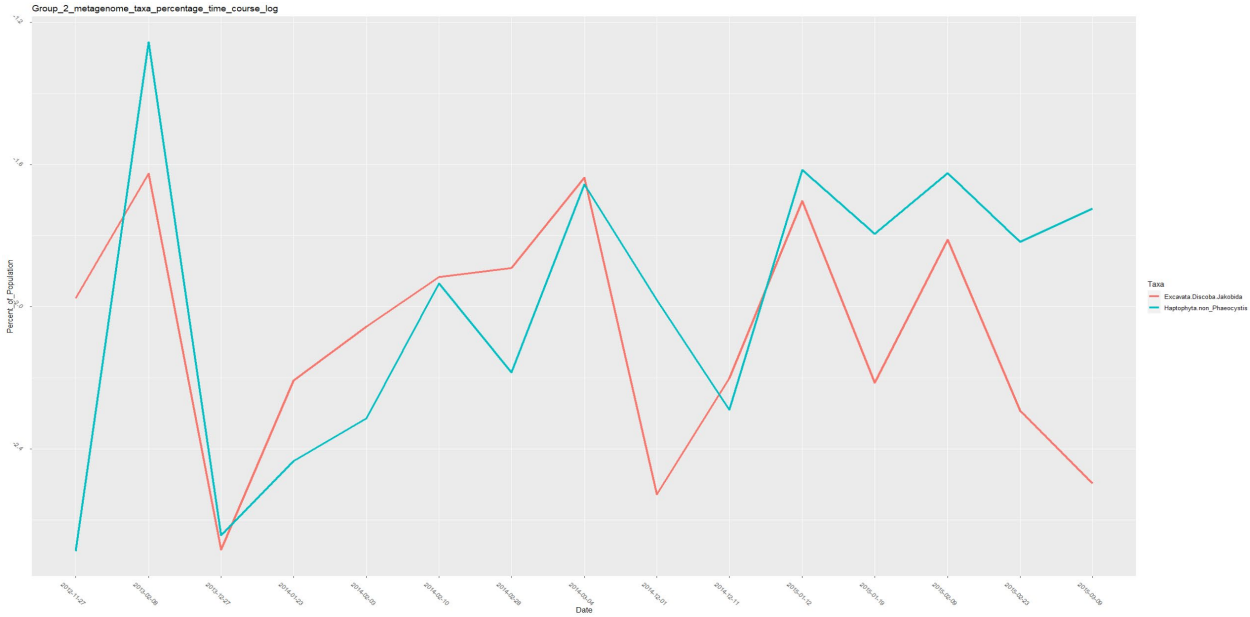

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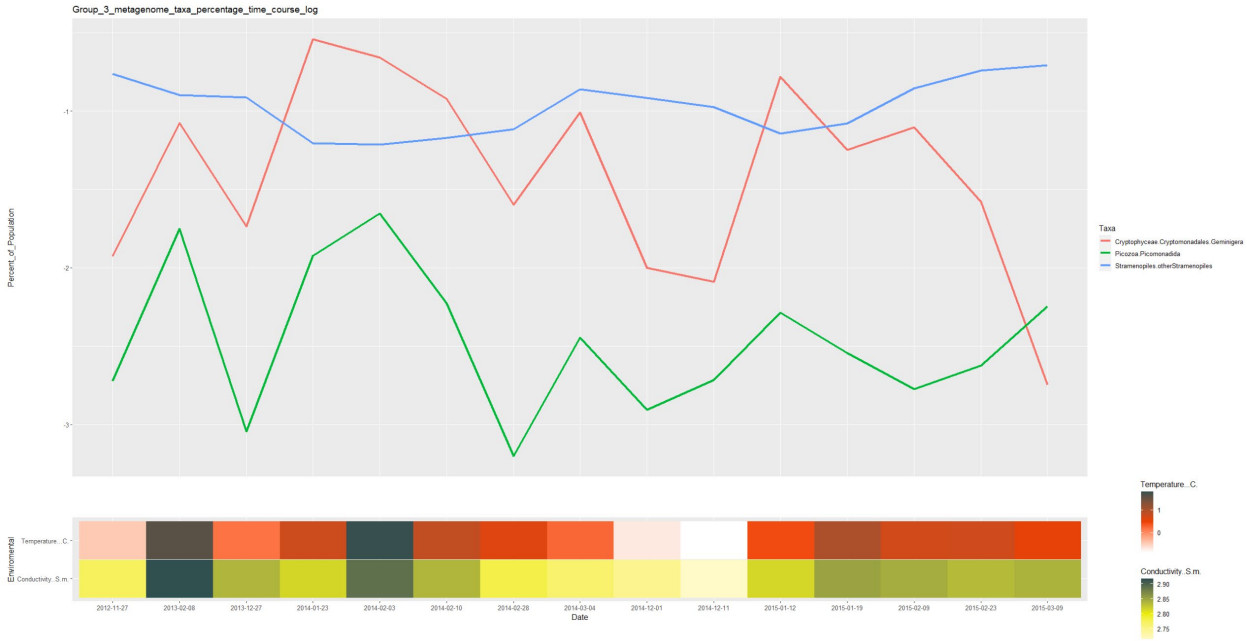

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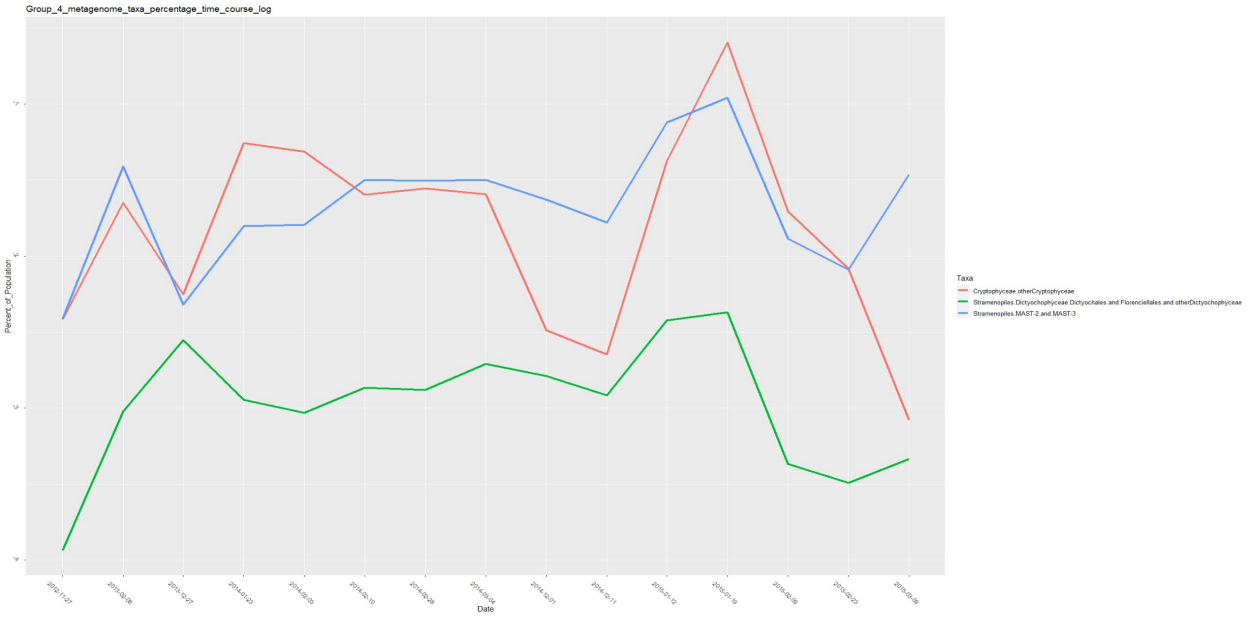

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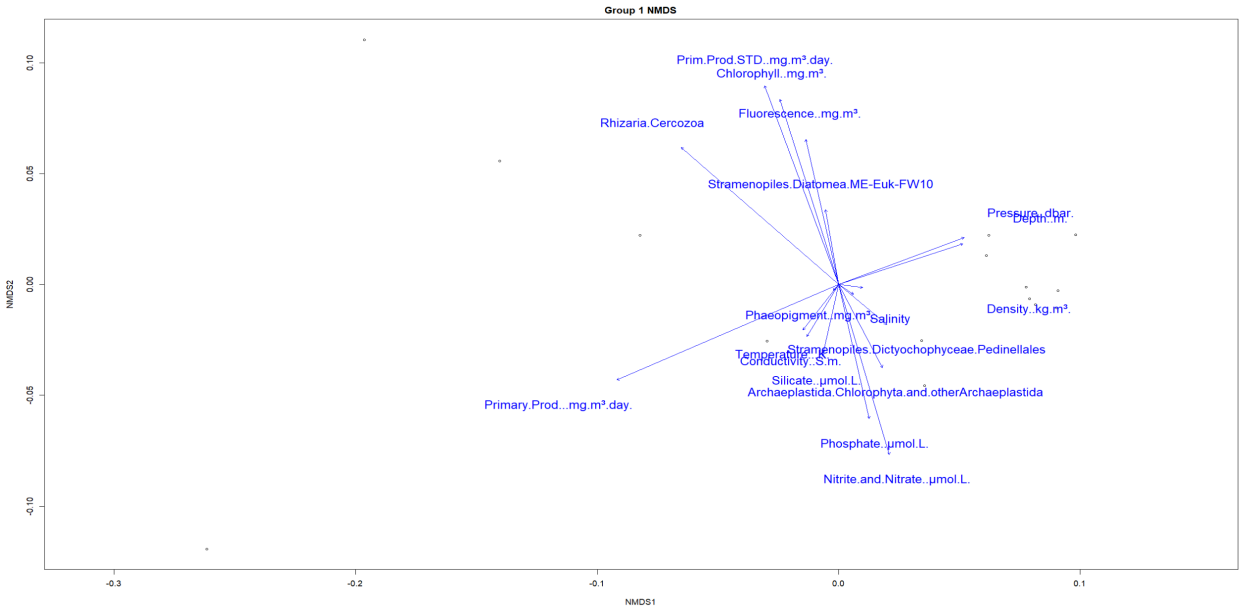

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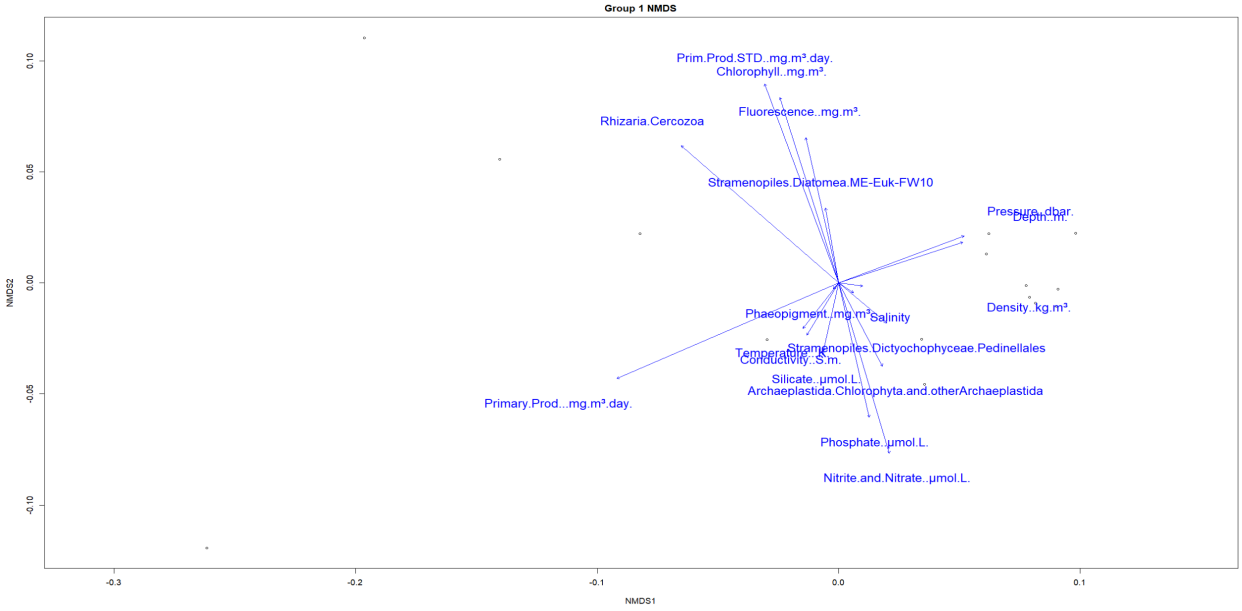

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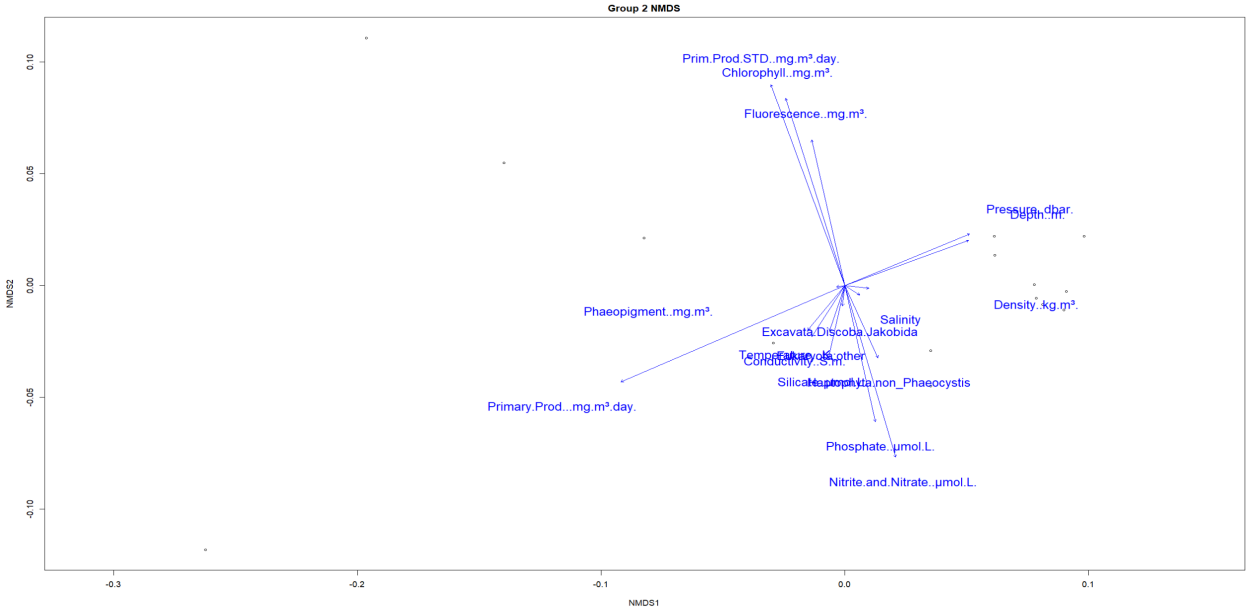


Figure 6.D

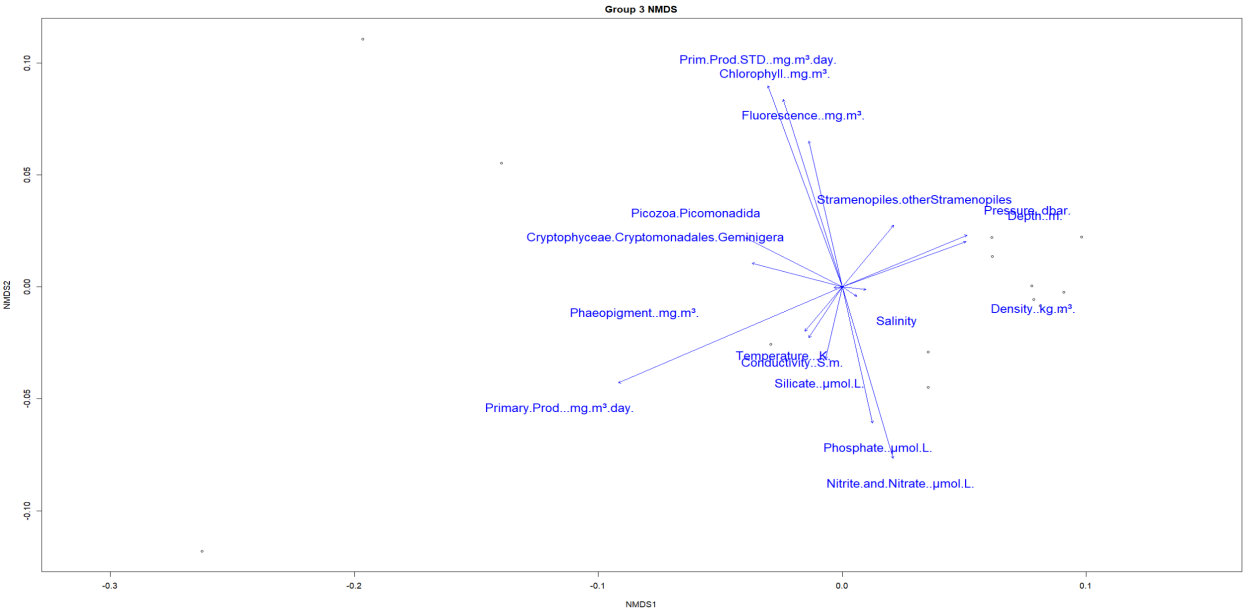
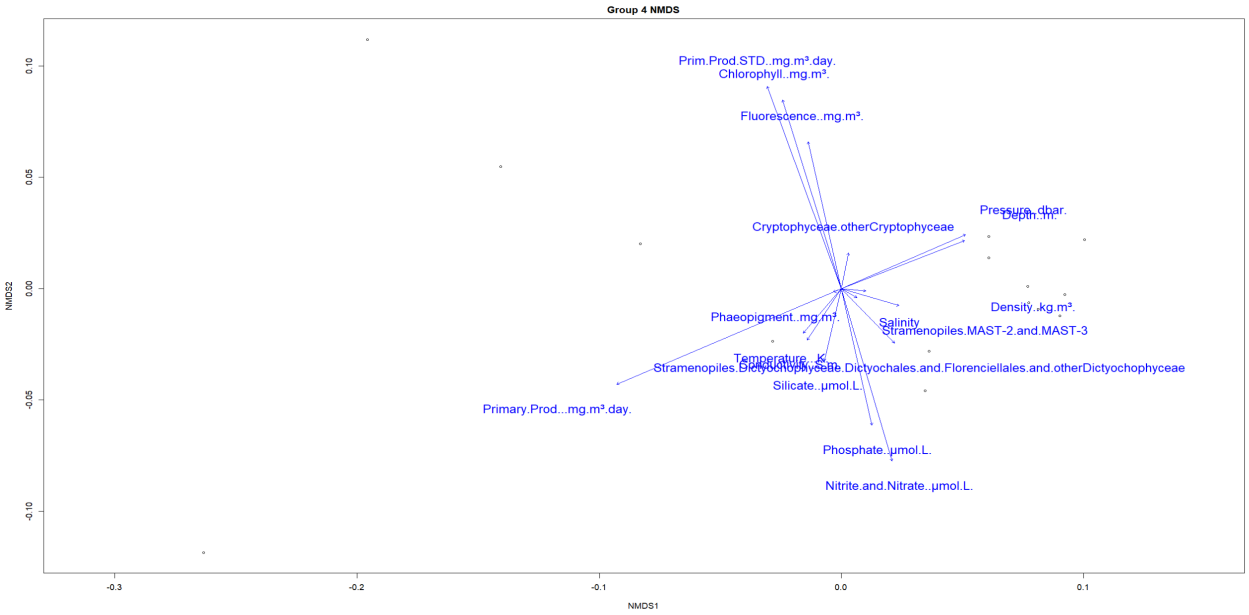


Figure 6.E



Medium Consolidation

Table 2.

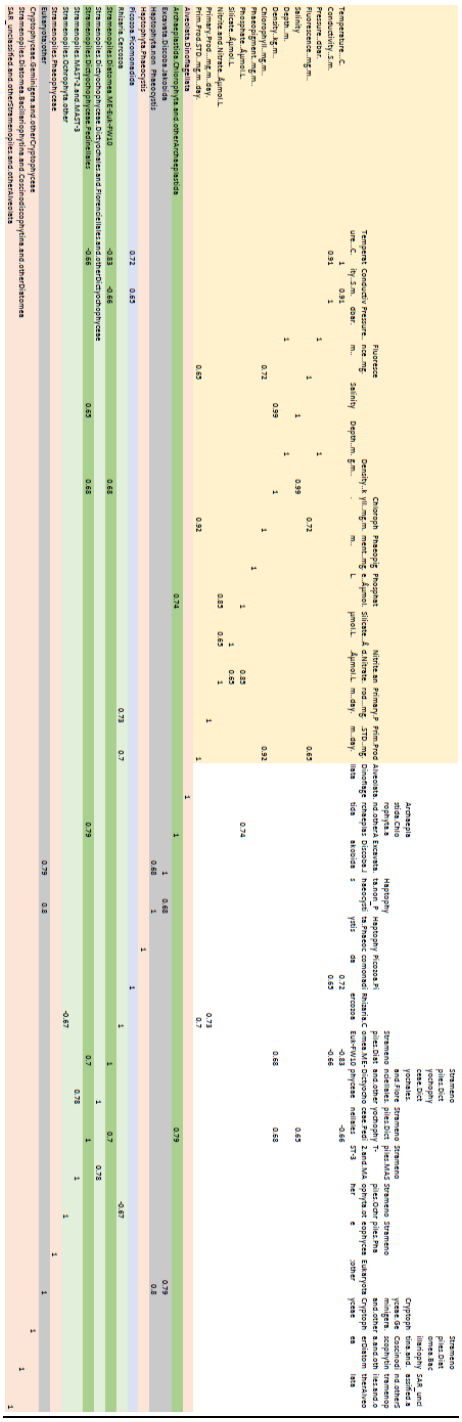


Table 2. Pearson correlation values of medium consolidation OTUs above 0.65 or below -0.65

Figure 7.A

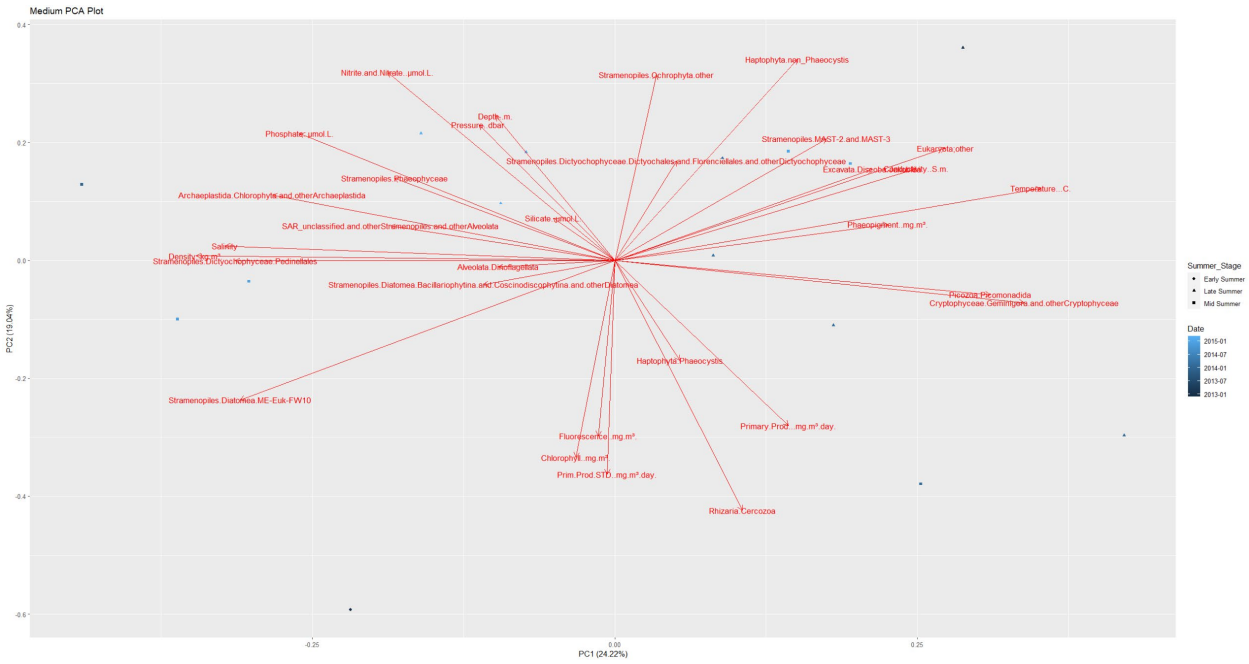


Figure 7.B

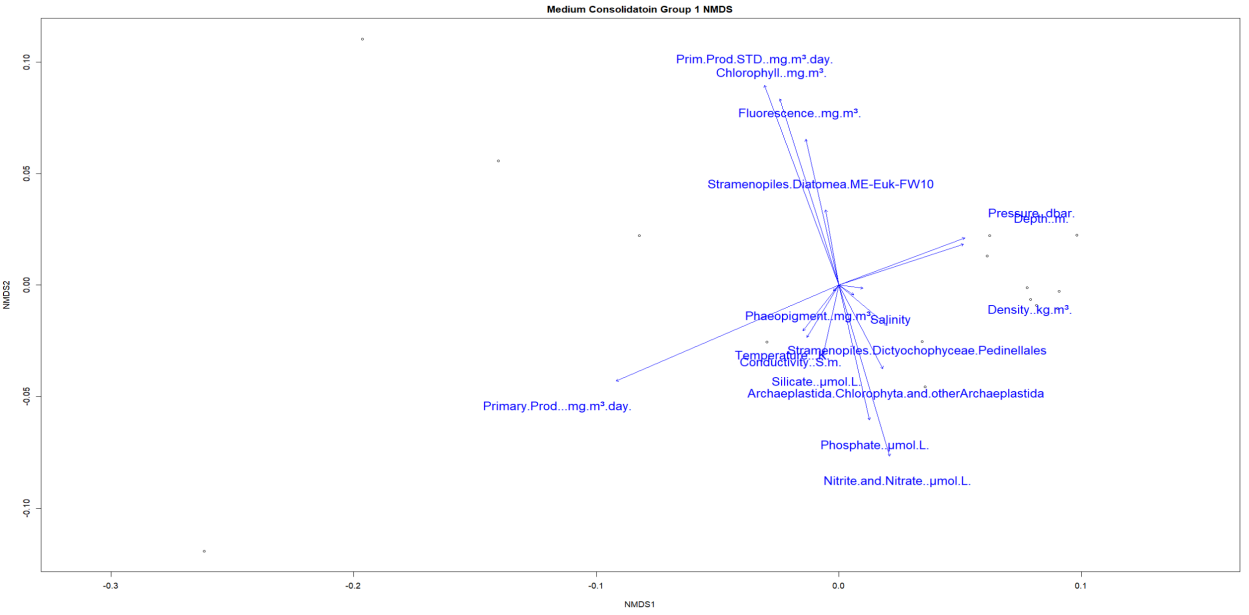


Figure 7.C

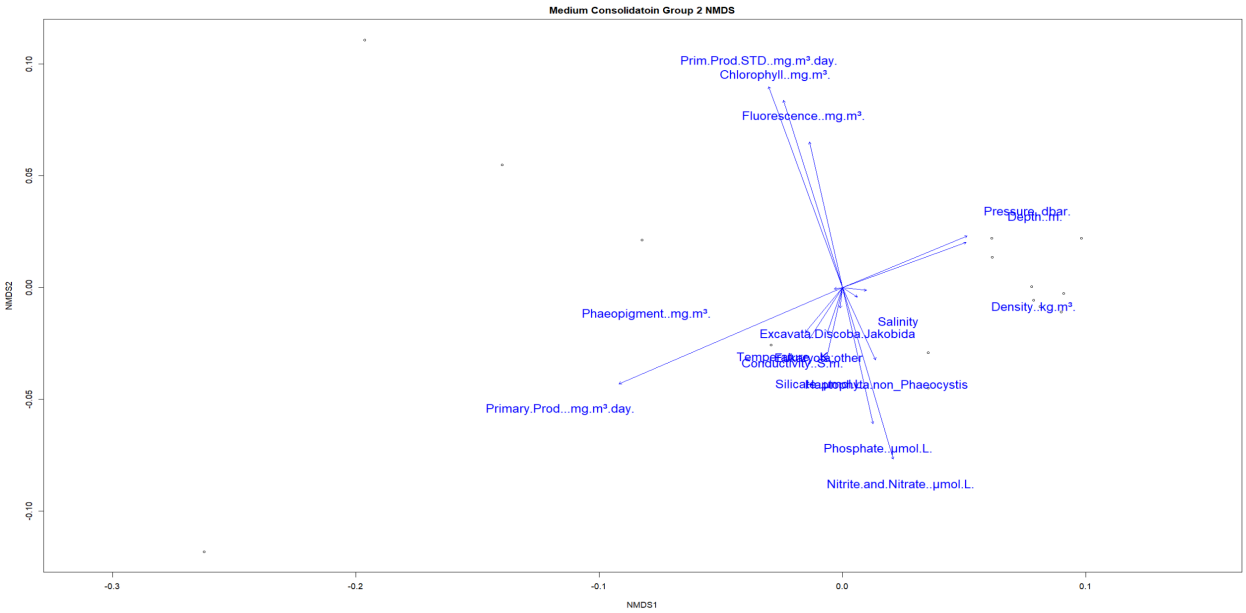


Figure 7.D

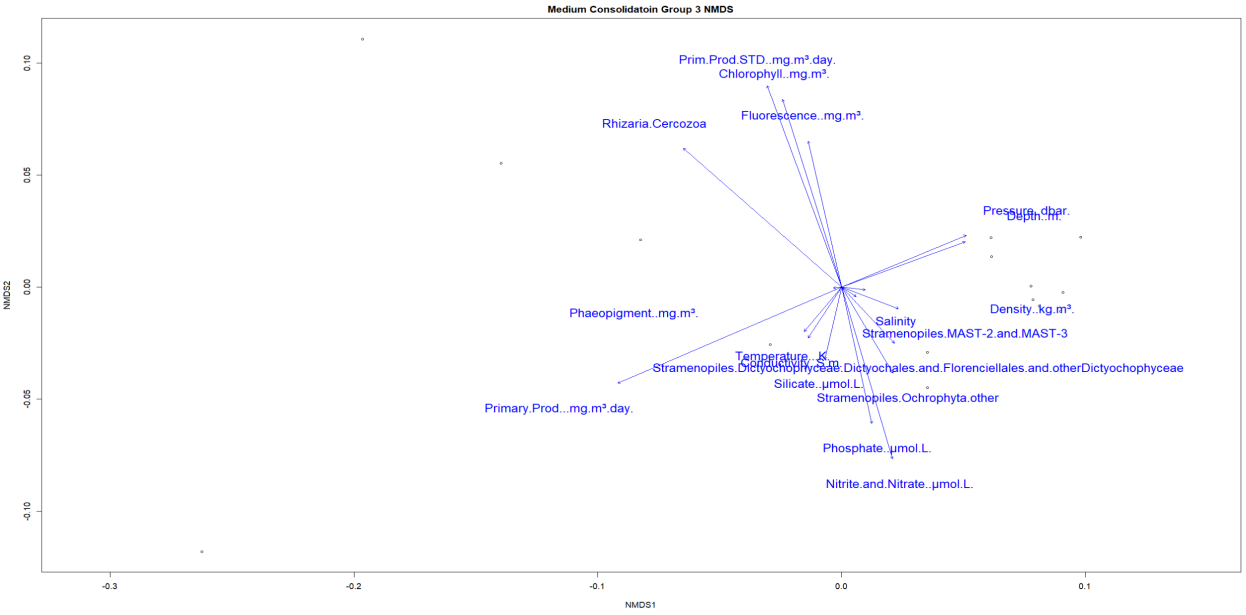
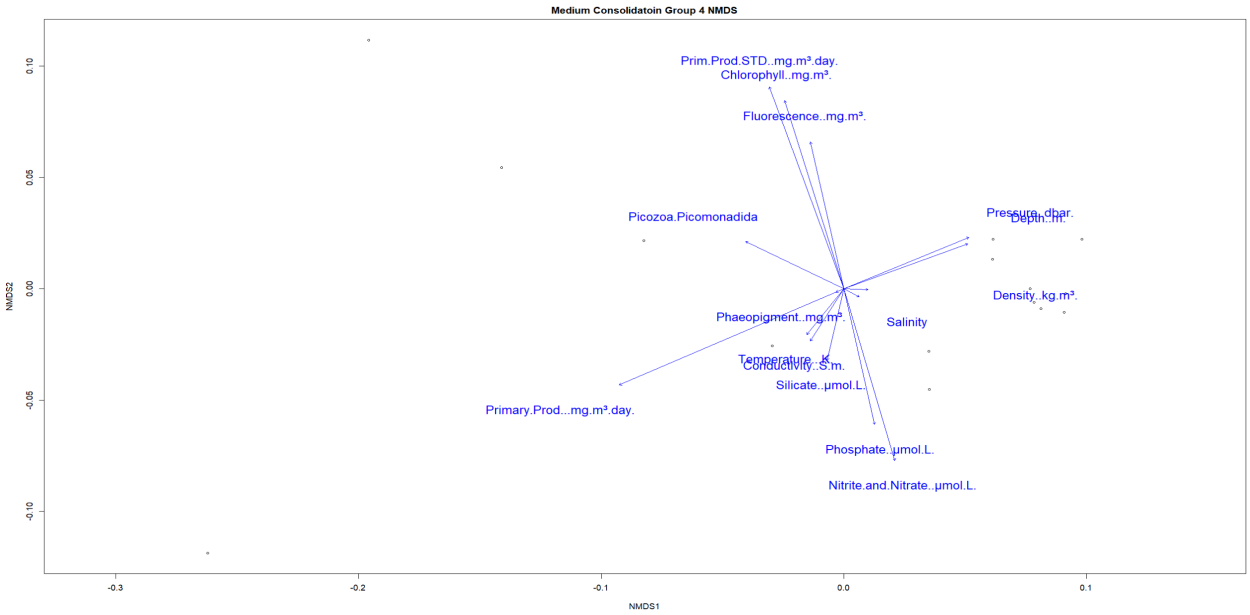


Figure 7.E



Maximum Consolidation

Figure 8.

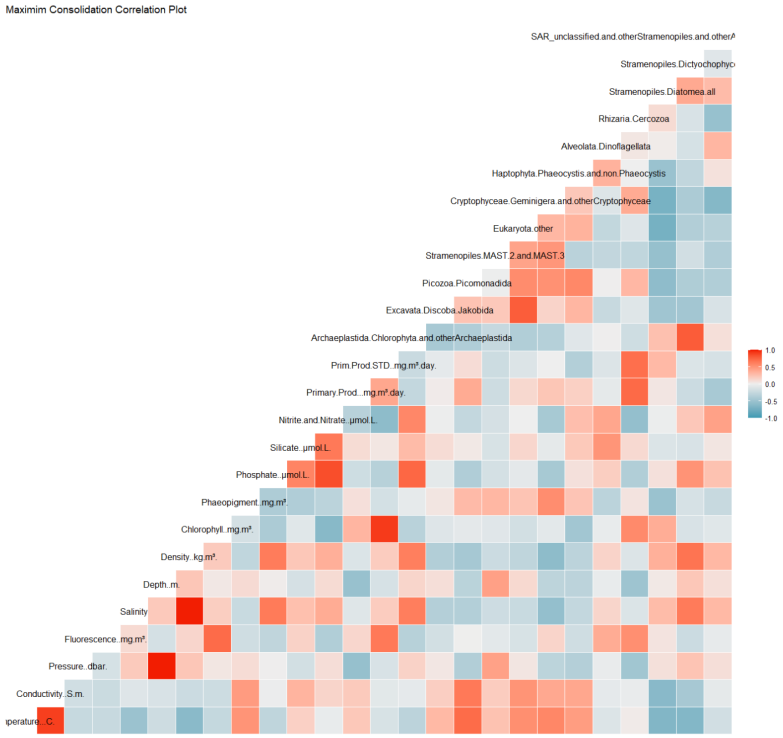


Figure 9.A

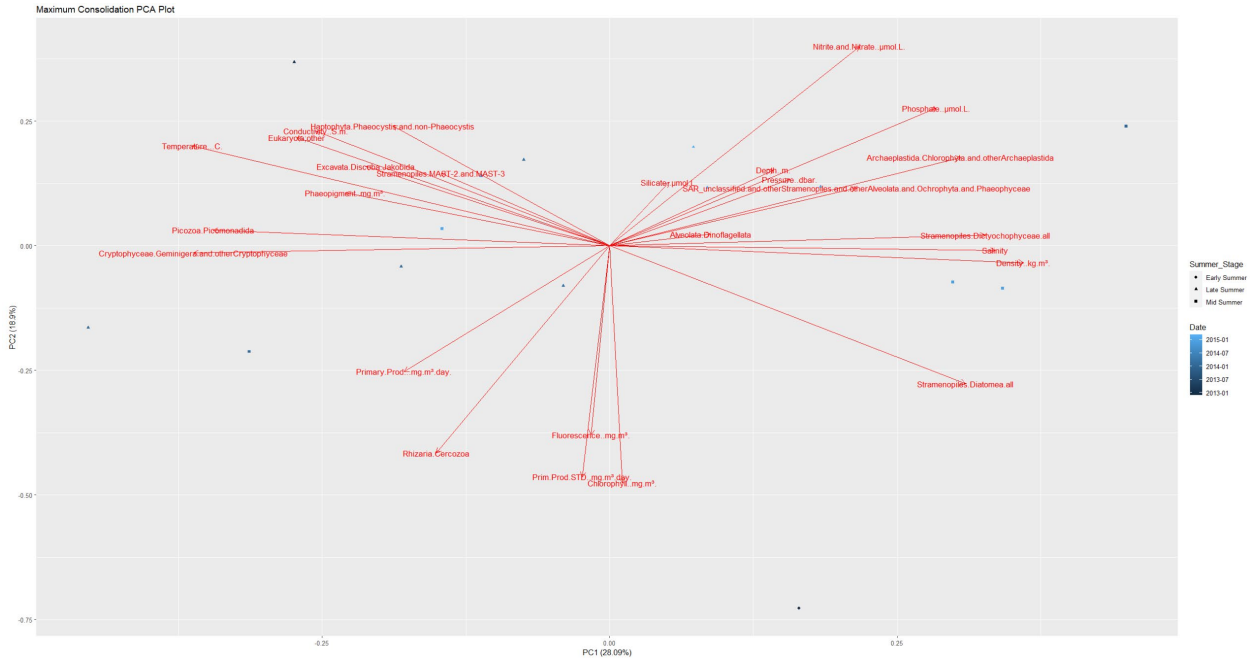


Figure 9.B

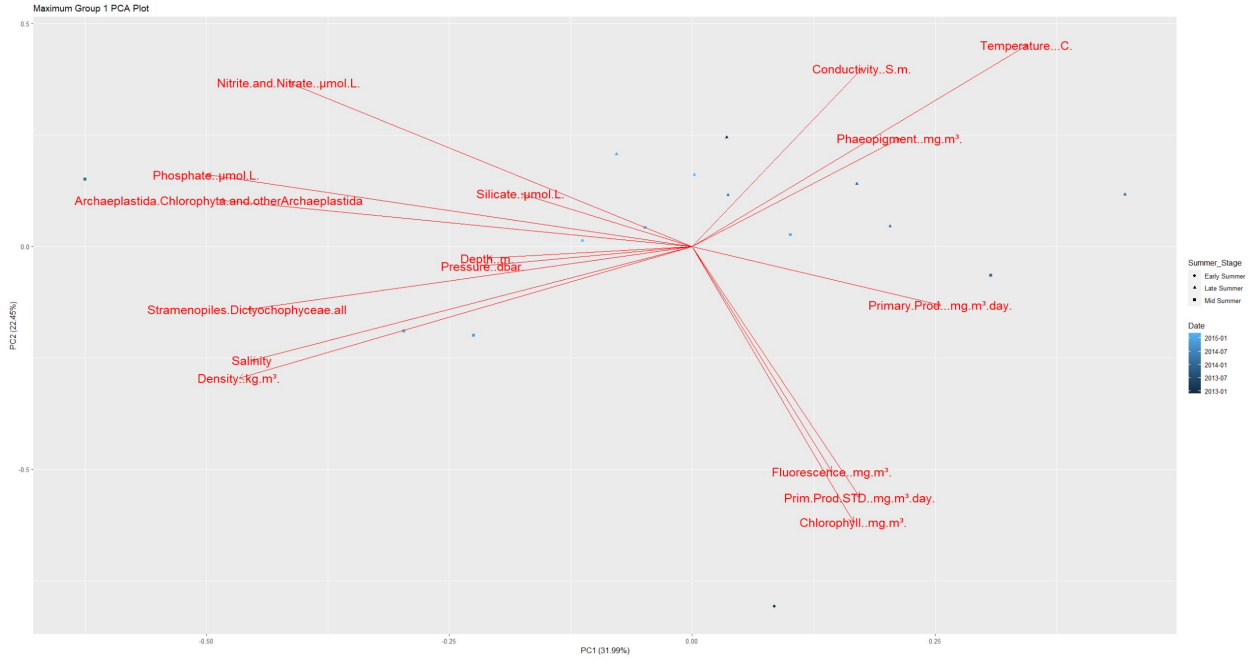


Figure 9.C

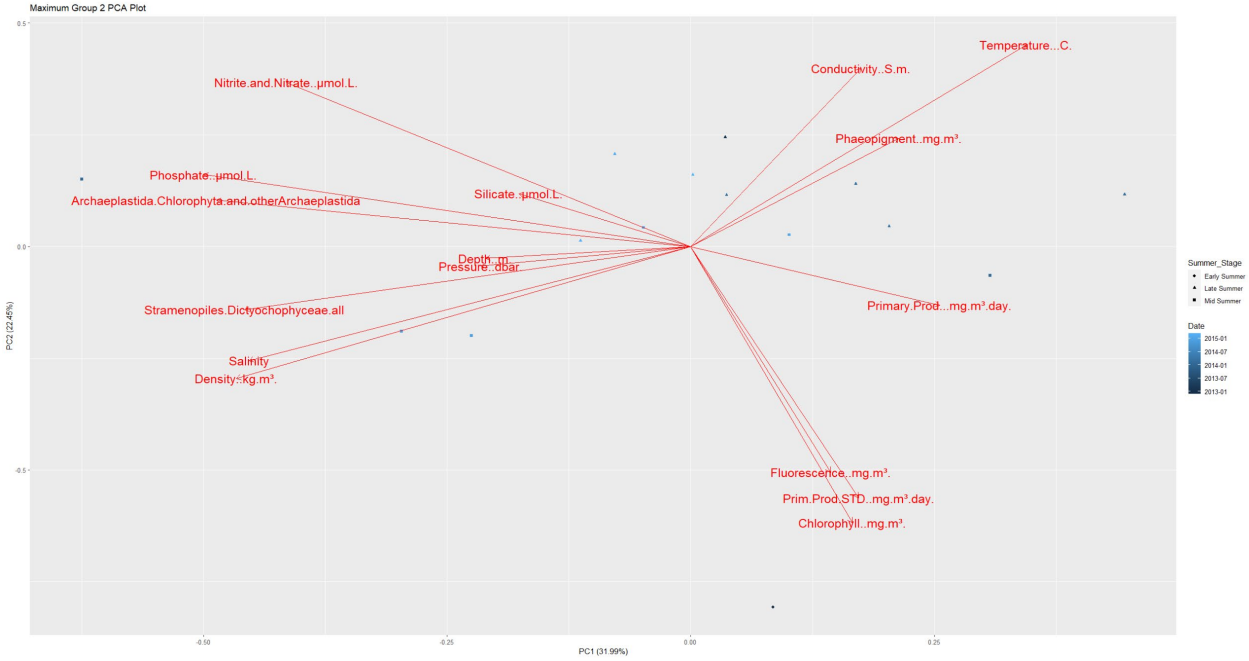


Figure 9.D

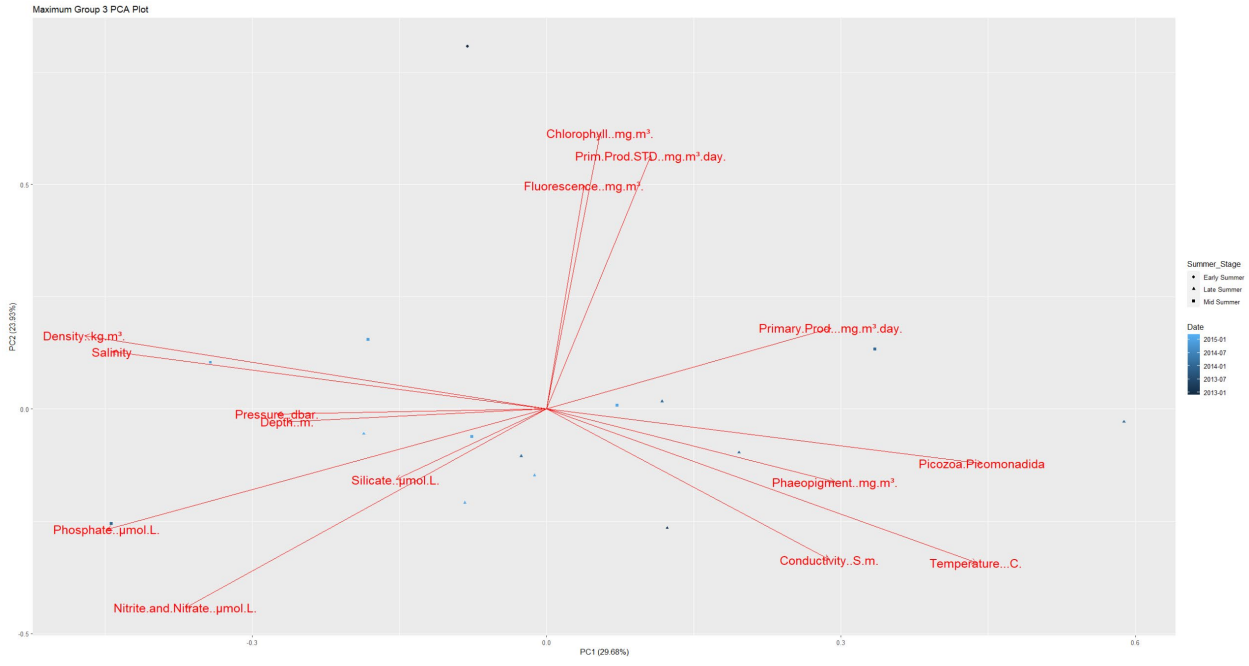


Figure 9.E

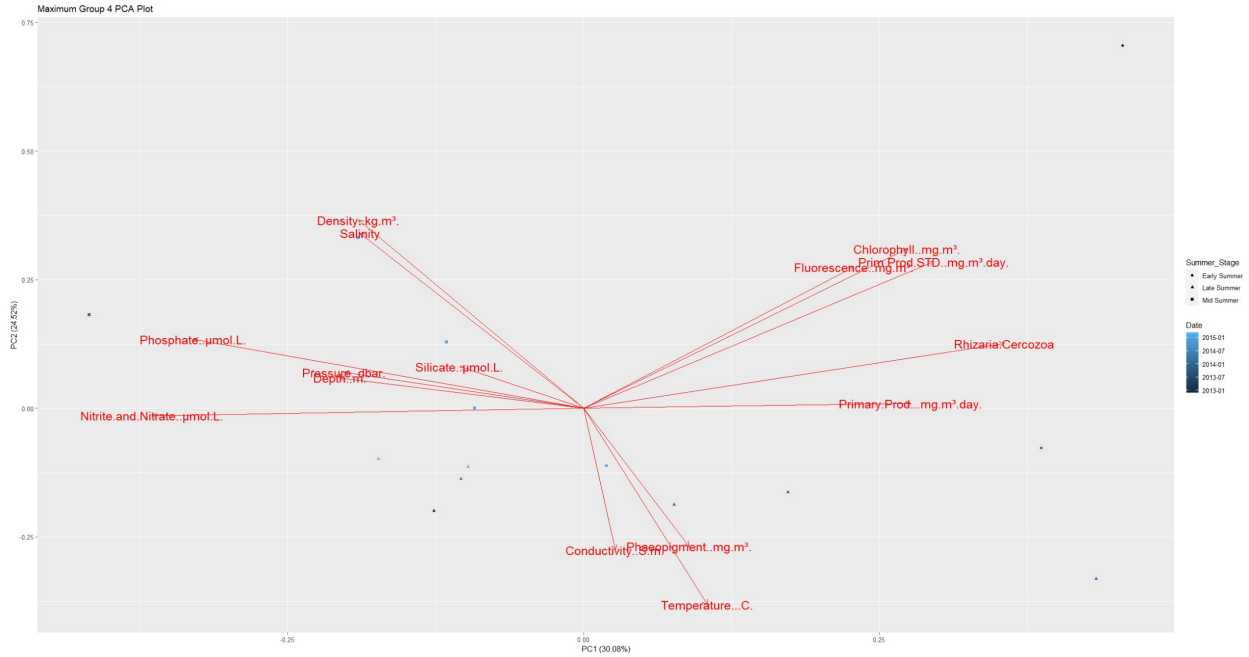


Figure 10.A

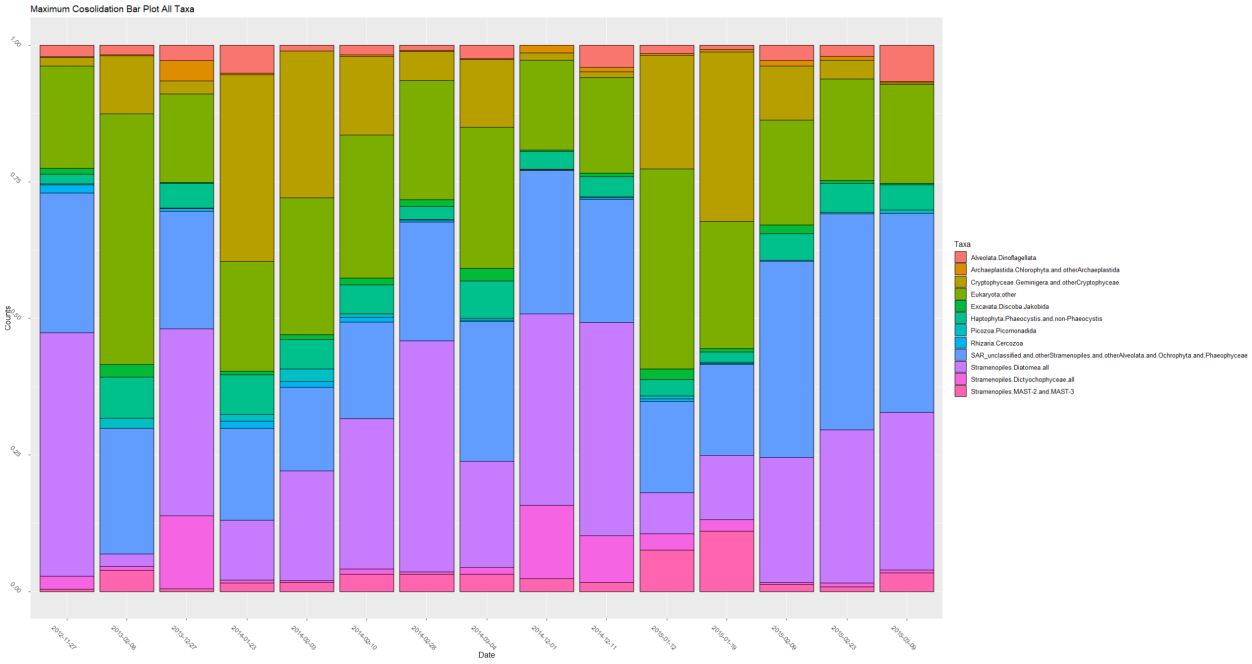


Figure 10.B

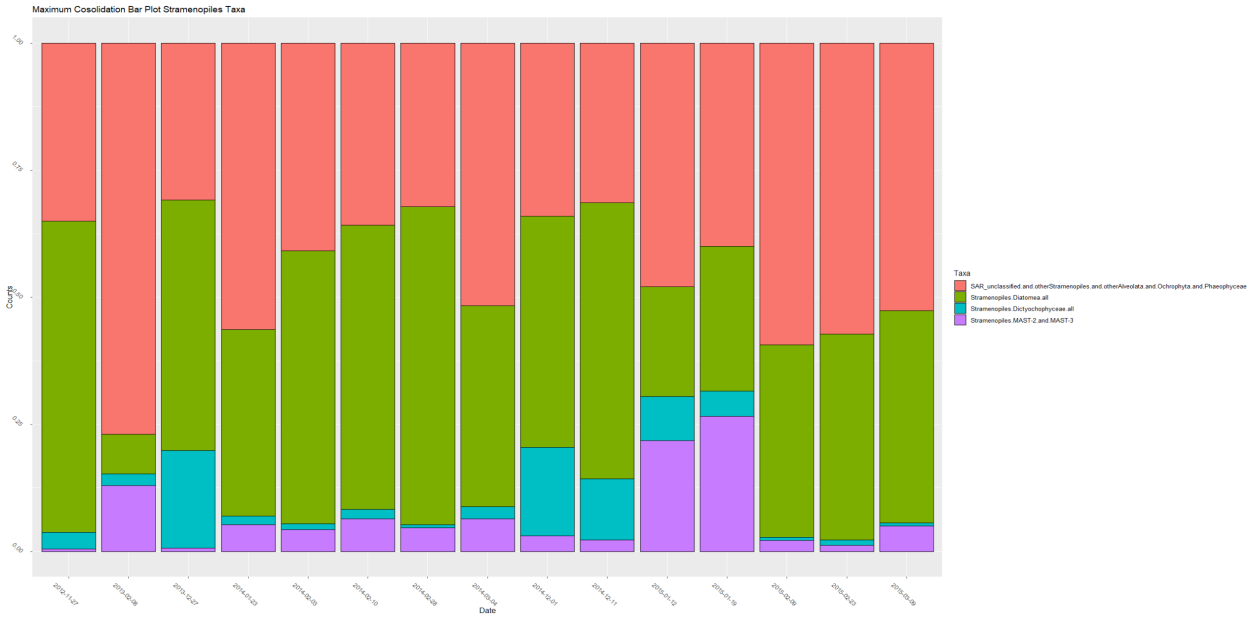


Figure 10.C

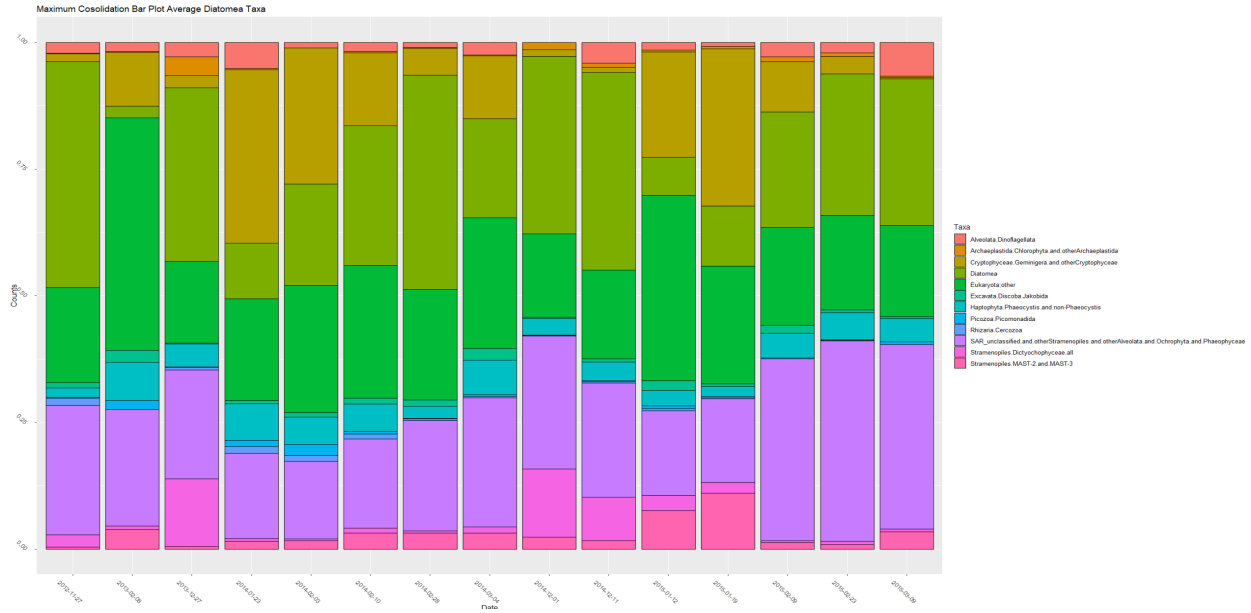


Figure 10.D

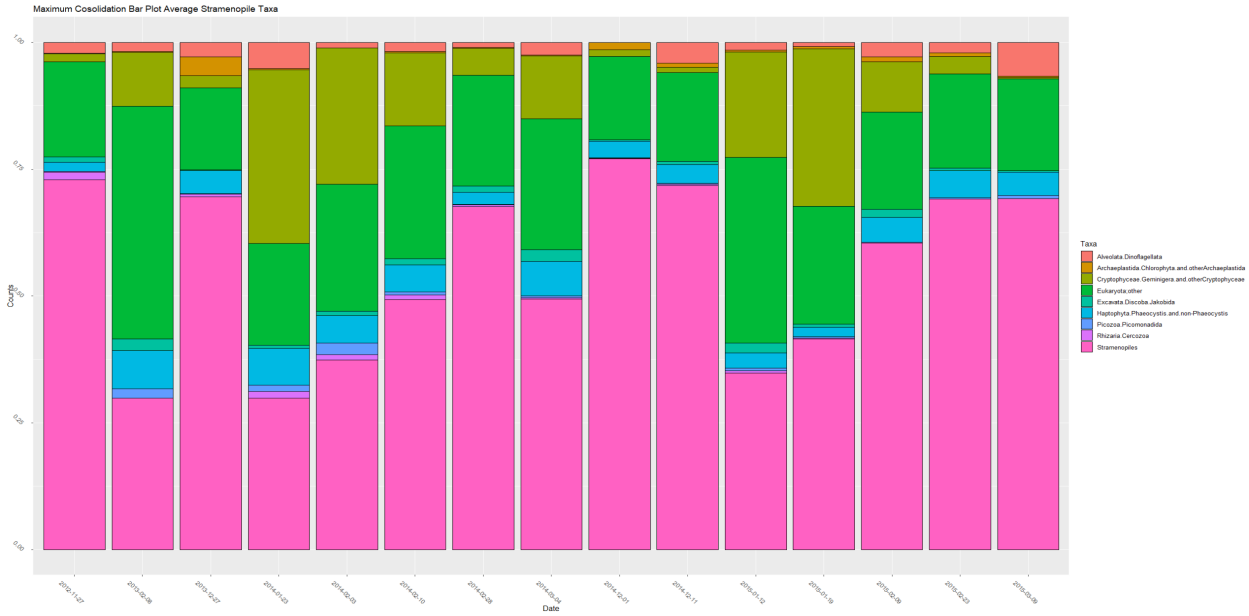


Figure 11.A

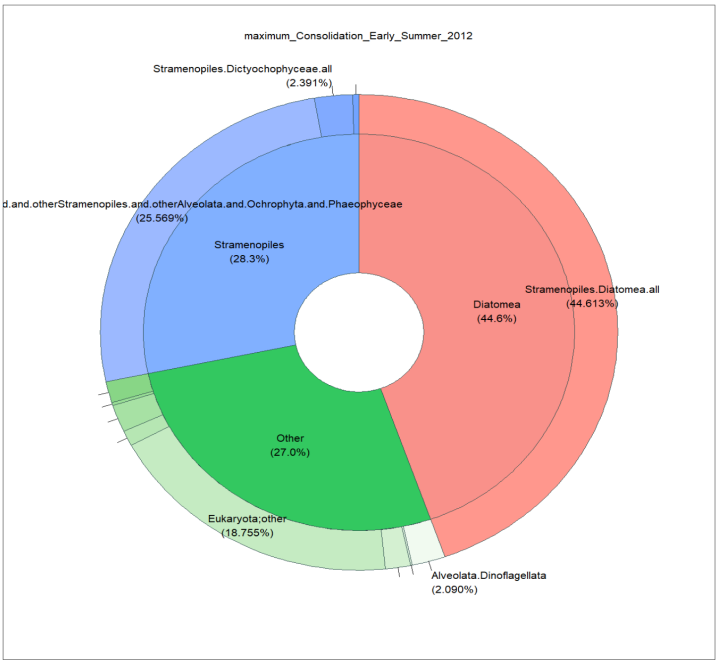


Figure 11.B

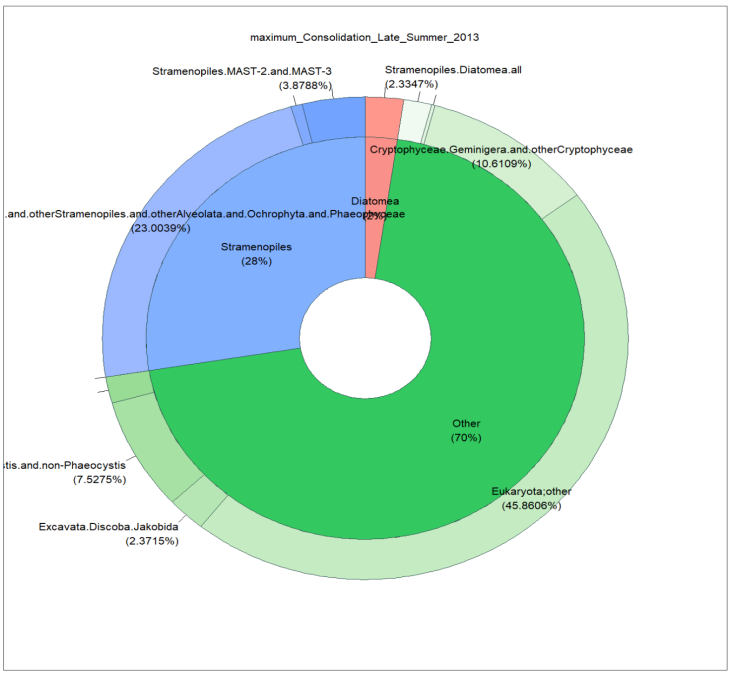


Figure 11.C

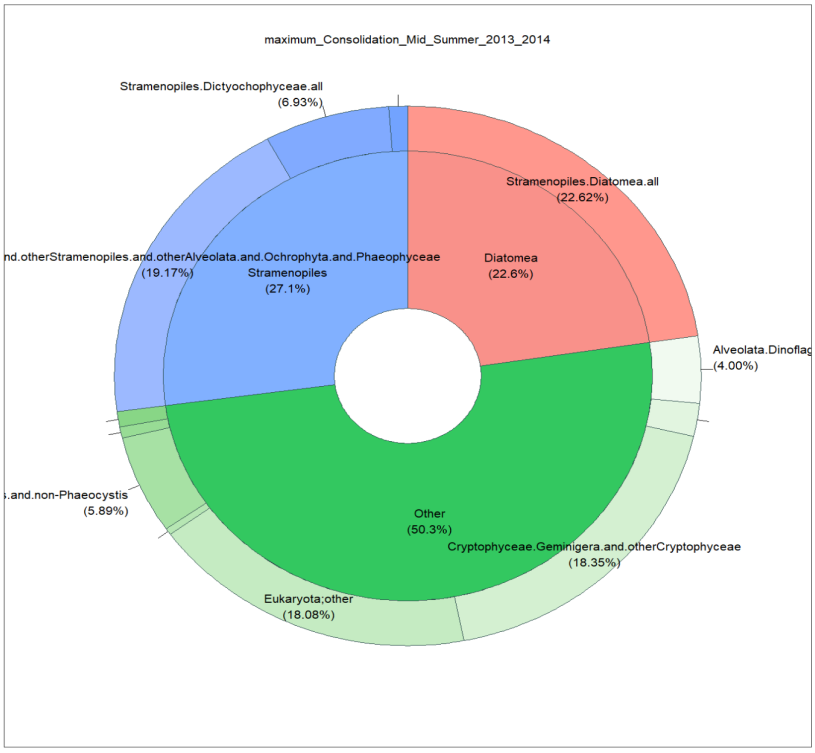


Figure 11.D

Figure 11.E

Figure 11.F

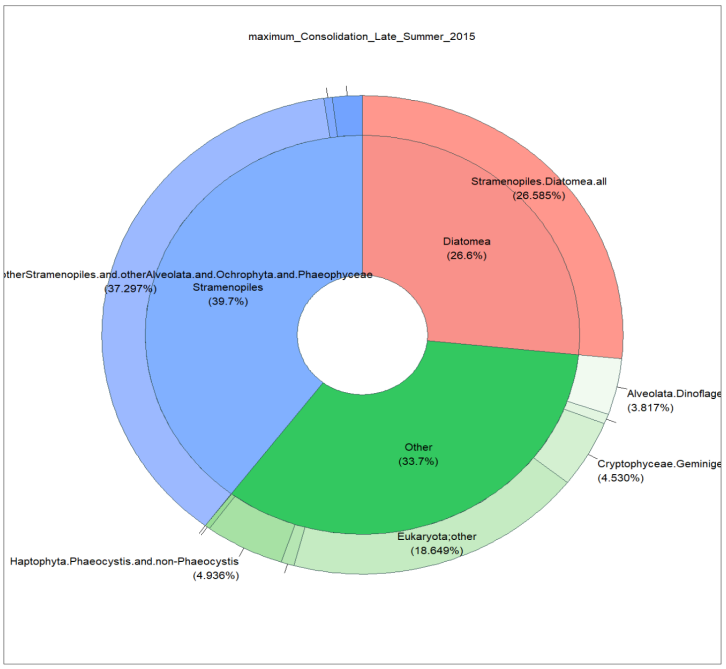


Figure 11.G

Figure 11.H

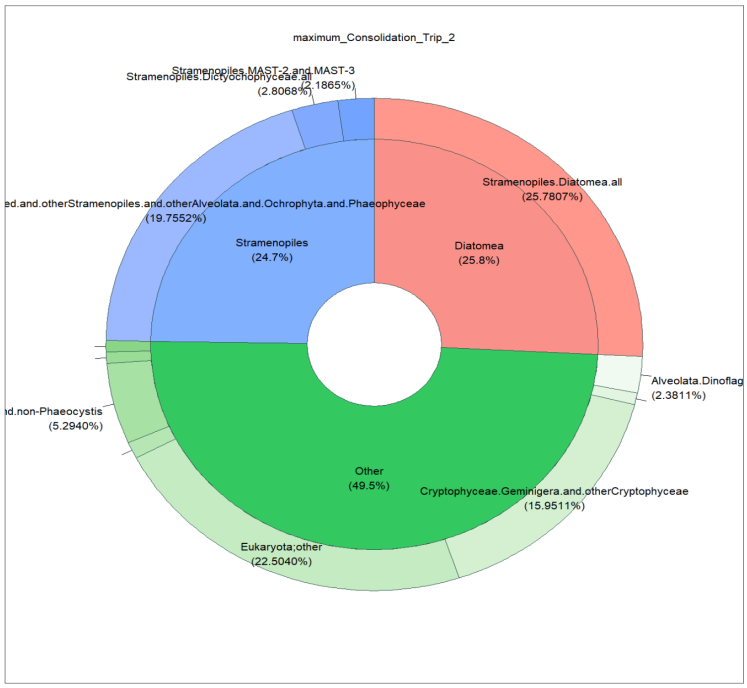


Figure 11.I

Figure 11.J

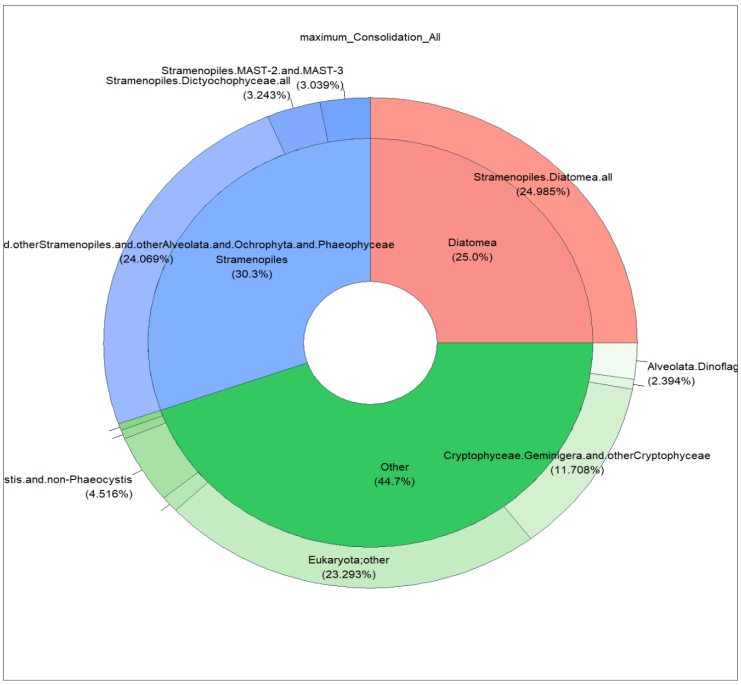


Figure 12.A

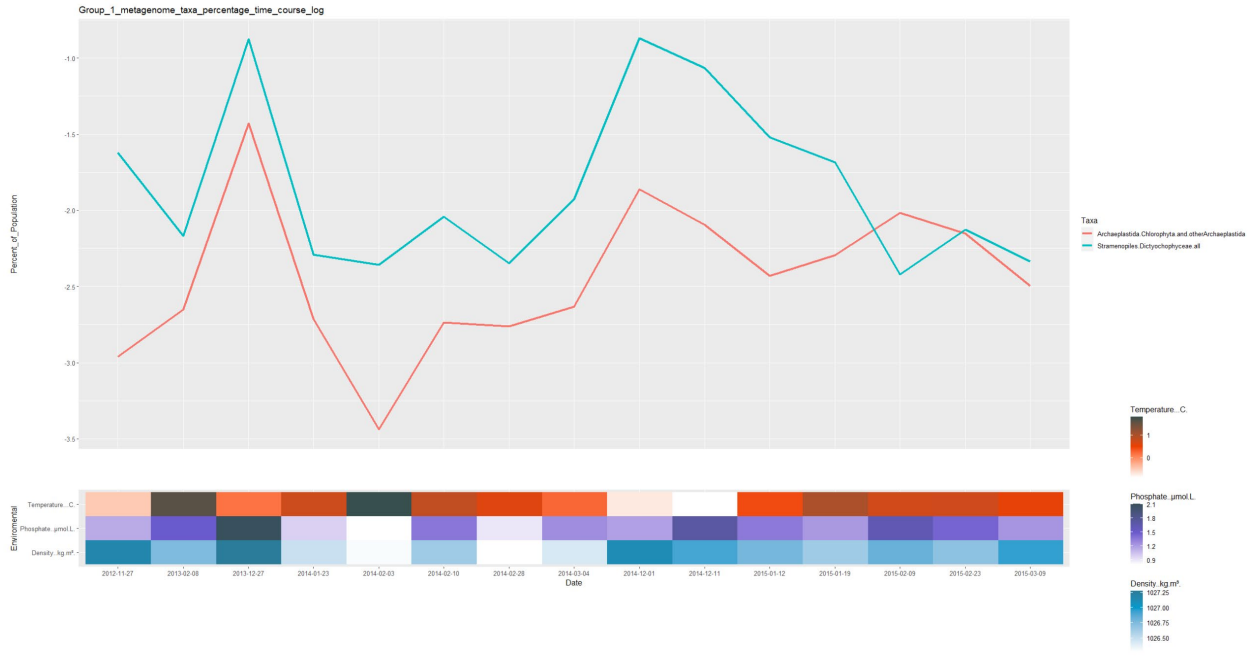


Figure 12.B

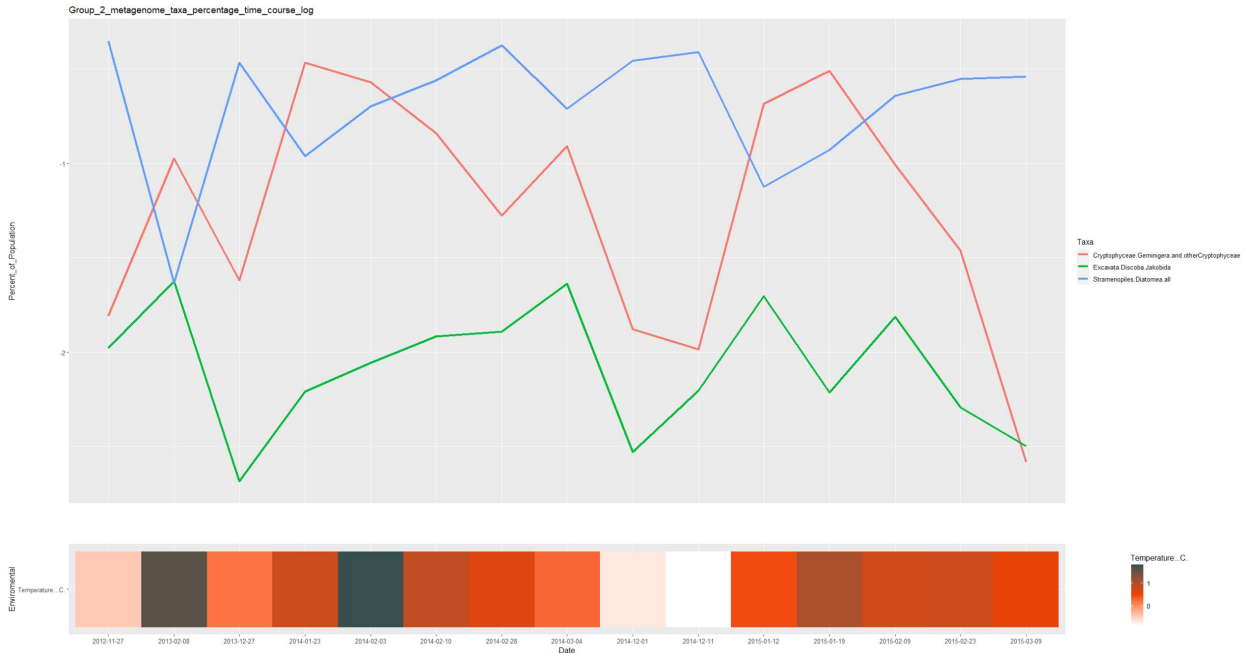


Figure 12.C

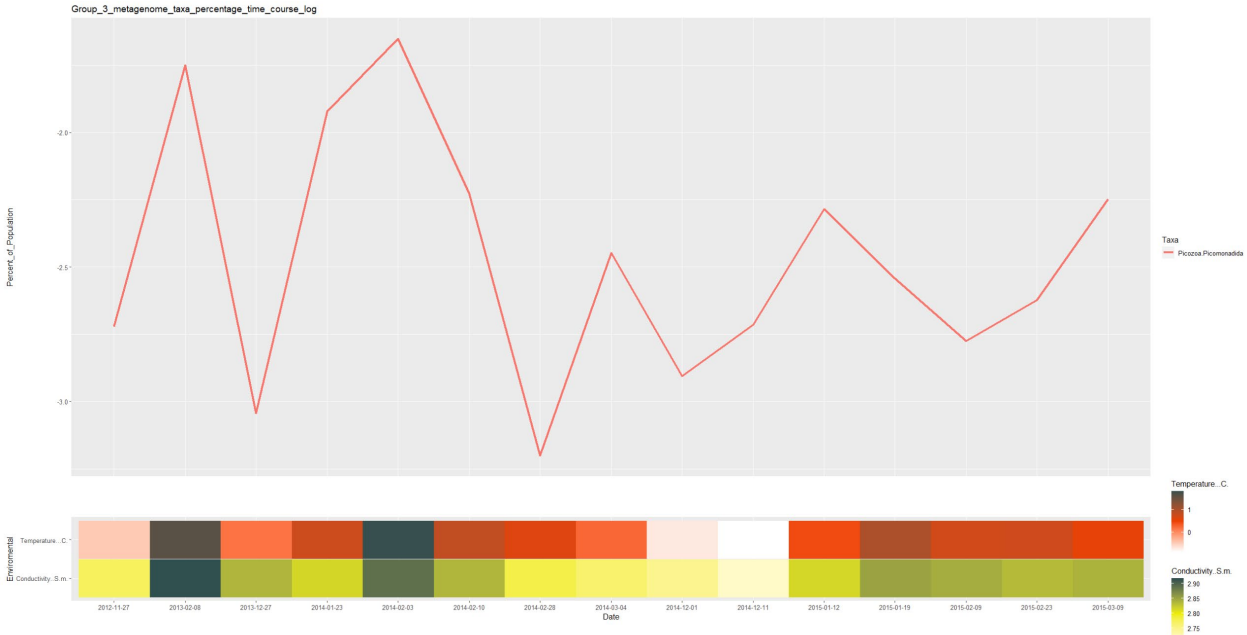


Figure 12.D

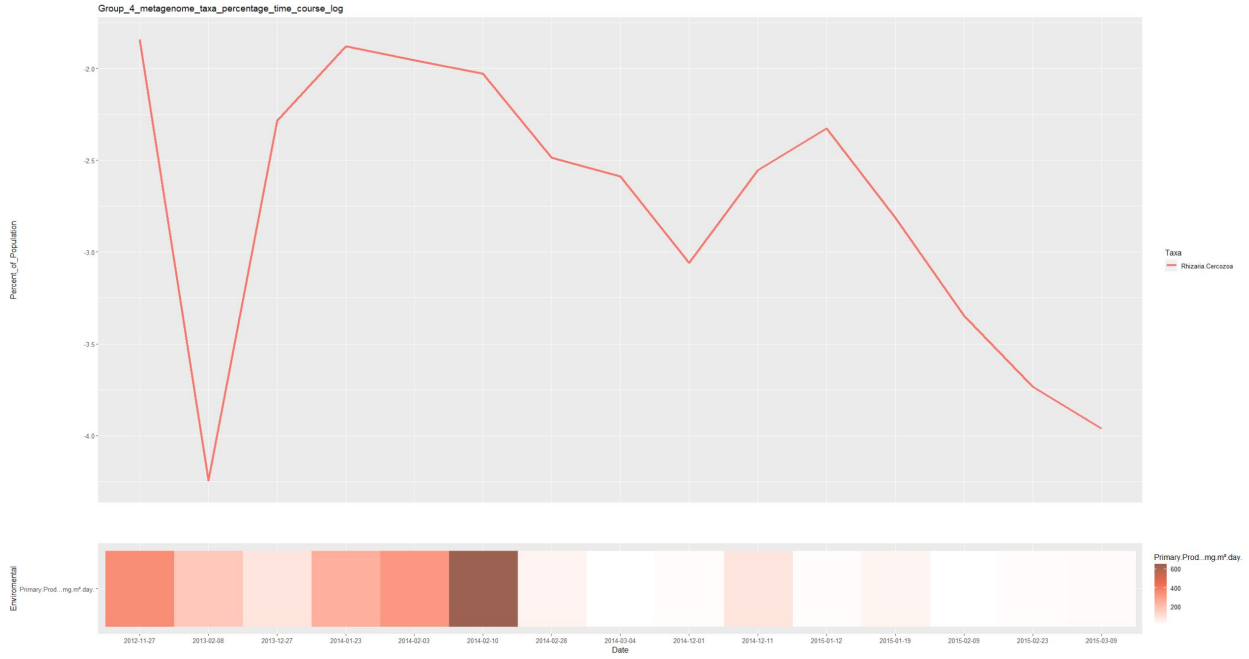


Figure 13.A

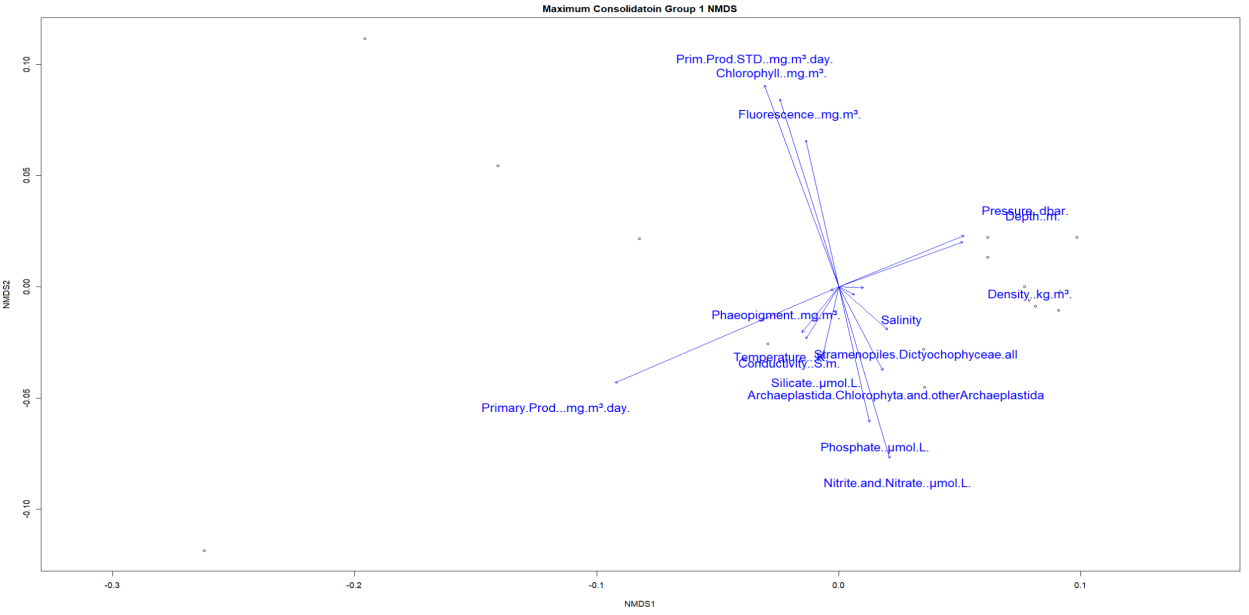


Figure 13.B

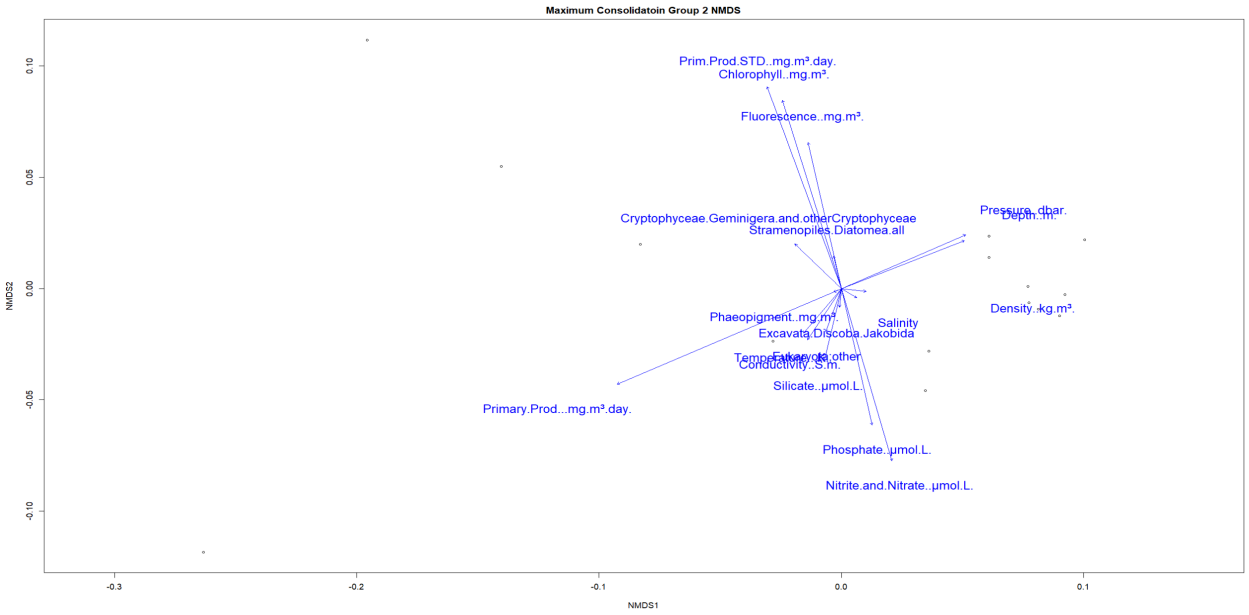


Figure 14.C

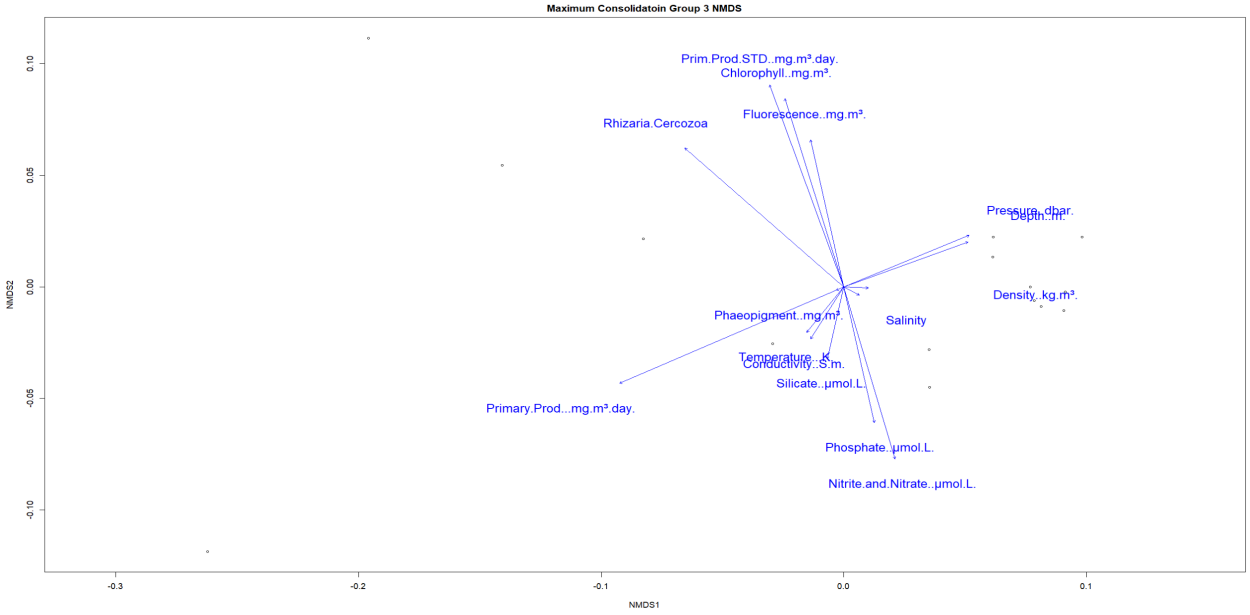
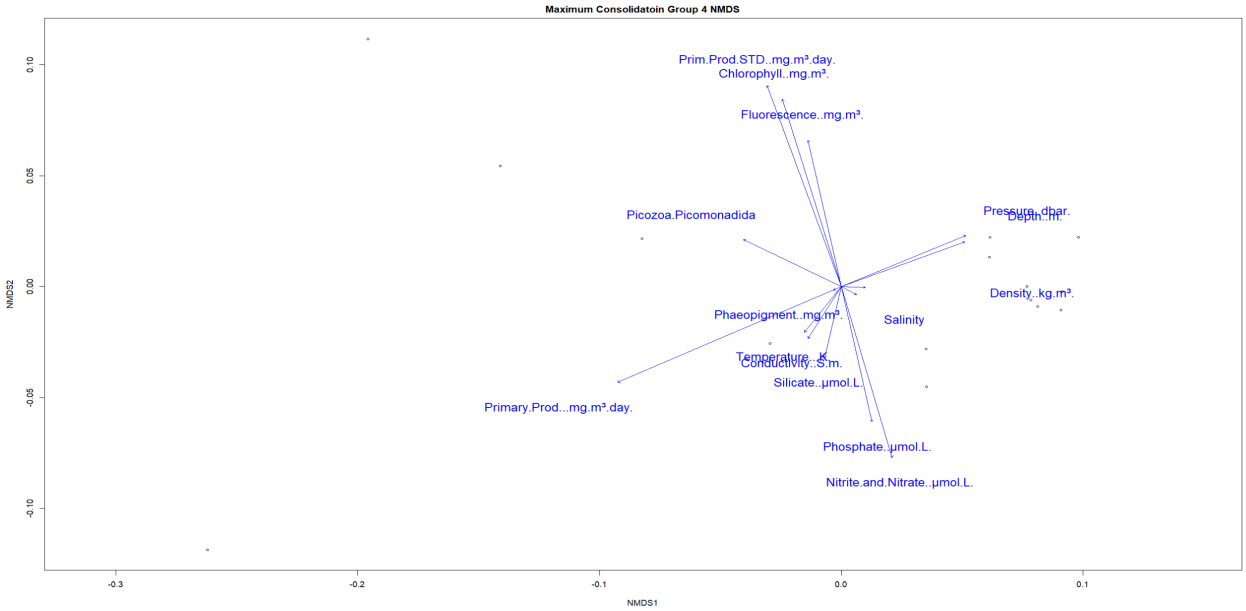


Figure 14.D


Maximum Consolidatoin Group 4 NMDS
Prim.Prod.STD..mg.m³.day.
Chlorophyll..mg.m³.
Fluorescence..mg.m³.
Picozoa.Picomonadida
Pressure..dbar.
Depth..m.
Density..kg.m³.
Phaeopigment..mg.m³.
Salinity
Temperature..K
Conductivity..S.m.
Silicate..µmol.L.
Primary.Prod...mg.m³.day.
Phosphate..µmol.L.
Nitrite.and.Nitrate..µmol.L.
NMDS1
NMDS2
0.10
0.05
0.00
-0.05
-0.10
-0.3
-0.2
-0.1
0.0
0.1