DISCUSSION

A major goal of this study was to characterize the diversity and taxonomic composition of protist community in SA and ST water masses and across the frontal zones that delimits them. Although samples included in this study were collected in different seasons, the seasonal coverage was similar for STW and SAW, and thus warrants a meaningful comparison between the communities associated with these water masses throughout the year. Previous phytoplankton studies in this region of the SW Pacific had been focused mainly on the STF over the Chatham Rise region and the SA and ST waters flanking this broad frontal zone (Chang et al., 1998, Hall et al., 2001, Delizzo et al., 2009). Using microscopy, pigment, and flow cytometry methods these studies described the prevalence of larger cells and diatoms through winter and spring in the more productive waters of the STF on the rise (Bradford-Grieve et al 1997; Chang et al., 1998). ST waters on the northern flank larger were also dominated by diatoms and nanoflagellates dominated in spring but dinoflagellates in winter, while smaller cells mainly composed by haptophytes, pelagophytes and dinoflagellates dominated the eukaryotic phytoplankton in SA waters (Chang et al., 1998; Bradford-Grieve et al., 1999; Peloquin et al., 2011). Here we build on this knowledge and greater taxonomic resolution and coverage of DNA metabarcoding to provide a comprehensive characterization of the diversity and taxonomic composition of protist communities associated to these contrasting water masses and new insights about ecological preferences of major taxa from class to species and intraspecific levels.

**Alpha diversity patterns**

Species richness was higher in ST than SA waters and lowest in the STF (Fig. 3). Richness decreased latitudinally and with temperature (Fig. S4) as expected from global diversity patterns (REF) and previous reports in the region (Raes et al., 2018). The lower diversity in the more productive STF was observed in both euphotic and aphotic layers (Fig. Suppl) on average lowest in more productive STF compared to SA and ST waters (Fig. 3, Fig. S5). This water-mass related pattern was systematically observed across the different levels of nitrate and chla concentrations encompassed in this study (Fig. 6). A study conducted across the Southland Current, a coastal expression of the STF that flows along New Zealand’s South Island, did not found a significant increase in protistan diversity across this coastal frontal zone (Allen et al 2020), while a decrease in eukaryotic richness across the STF in oceanic waters east of the Chatham Rise had been reported (Raes et al. 2018). These results together with the relatively lower diversity observed in the STF suggest a negative effect of increased productivity typically associated frontal zones upon protistan communities.

Regional differences in species richness were also evident within different water masses (Fig. 3). Most notably, species richness tended to be lowest in the Campbell Plateau region in both SAW and STF for which we have no clear explanation other than speculate about the possible effect of the hydrographic and bathymetric confinement and elevated productivity of this subantarctic region (Neill et al., 2004; Gutierrez-Rodriguez; Forcén-Vazquez et al., 2021). Within the other end of species richness represented by STW, the lowest richness was observed during the Spring bloom voyage certainly due to the Lagrangian sampling strategy of this voyage, but also to the expected decrease in diversity inherent to bloom conditions (REF). One could argue that more productive waters such as the STF, Spring Bloom, and the C. Plateau exhibited lowest diversity levels. Diversity patterns associated with water masses and regions are the same when considered only the surface mixed layer (Mixed-layer depth – 0.2 C change relative to 10 m) or the twilight zone (>150 m) (Supplementary Figure 1) suggesting that whatever the reasons behind the lower diversity associated with the STF it affected the entire water column.

Despite the regional and seasonal variability surveyed within STW and SAW water masses (Fig. 1, Table 1, Fig. 2) we observed systematic differences in their taxonomic composition (Fig. 4). Such water mass specificity has been observed in the prokaryotic (Galand et al., 2009 & 2010; Agogué et al., 2009; Seymour et al., 2012; Techtmann et al., 2015) and eukaryotic component (Hamilton et al. 2008; Raes et al., 2018) of microbial communities across different oceans. Among environmental drivers, salinity (21%) rather than temperature (4%) and nitrate concentration (2%) was the physico-chemical variable that explained best the clustering of samples we obtained with the nMDS (21% of the variability). Bray-Curtis dissimilarity of surface bacterial communities across the Southland current – the coastal expression of STF in that flows along the South Island of New Zealand - was also strongly correlated with salinity (Baltar et al., 2018). These results support the view that ST and SA water masses east of New Zealand are better conceptualized as bioregions or provinces rather than habitats (sensu Martiny et al., 2006), where (phyto)plankton communities reflect oceanographic processes and history in addition to contemporary physico-chemical conditions.

Samples from the STF itself were also distinguished from those in SA and ST water masses based on their taxonomic composition, although they showed a greater overlapping (Fig. 4) reflecting the active mixing and transition role of the STF. This overlap was particularly evident between samples collected at the Bio-STF and the Bio-SAM sites located on top of the rise and its southern SA flank and between the STF flowing north of the C. Plateau and the plateau itself, which suggests a stronger connectivity of the STF with SA than ST waters. Similarly, the horizontal mixing and phytoplankton community size structure in the STF zone has been reported to be more tightly coupled across the SA than the ST (Safi et al., submitted). This contrast with the higher similarity between diatom species of the STF and STW compared to SAW reported in this region (Chang et al., 1998). At any rate, the distinct communities observed in ST and SA, and to a lesser degree in the STF, highlights the role of oceanographic features such as the STF as boundaries that influence the diversity of oceanic microbial communities in large oceanic provinces (Baltar et al., 2016; Raes et al., 2018).

Samples from different regions tended to cluster separately as well, with differences in the strength of this clustering between regions likely reflecting differences in the spatio-temporal coverage of the sampling. We interpret that the relatively looser clustering of samples within each site of the Biophysical Moorings Time-Series (Bio-STM, Bio-STF and Bio-SAM) was due to the multiple season/years span of this program. Samples from the Spring Bloom II voyage were tightly clustered in a way consistent with the Lagrangian sampling strategy adopted in this voyage (and the limited compositional changes observed during the 20-days that the subtropical bloom was tracked) while samples from the Cross-Shelf voyage cluster more loosely reflecting the more diverse environments (coastal shelf, slope and oceanic waters) and communities surveyed during this voyage.

**Phytoplankton community composition**

Our results showed the overall dominance of dinoflagellates and chlorophytes followed by diatoms, haptophytes and pelagophytes (Fig. 5). Yet consistent differences in the taxonomic composition between water masses emerged at class and species levels belonging to these groups (Figs. 6 & 7). Analysis of intraspecific diversity revealed differences in the distribution of ASVs of the same species suggesting the presence of different ecotypes in some cases (e.g. Chloroparvula pacifica; Phaeocystis antarctica) and current taxonomic gaps within certain groups that remained to be characterized (e.g. Pelagophyceae\_XXX; Dinophyceae\_XXX)(Figs. DESeq). Below we discuss the distributional patterns of major groups zooming across different taxonomic ranks in an attempt to shed some light on their ecological preferences and the level of taxonomic resolution required to link the community structure and function.

In the case of green algae for instance, the relative contribution of the two main classes found, Mamiellophyceae and Chloropicophyceae showed opposite distribution patterns (Fig. 5). Mamiellophyceae was most abundant in STW and constituted the bulk of green algae dominating these waters while its relative abundance decreased in STF to reach lowest levels in SAW (Fig. 6). Its dominance in STW was mainly driven by *Ostreococcus lucimarinus*, with *Bathycoccus prasinos* and several species of *Micromonas spp.* (Fig. 7). *O. lucimarinus* was clearly the most abundant species here and in the STF (Fig. 7) in agreement with a metabarcoding analysis conducted across the Southland Current, coastal expression of the STF in the New Zealand South Island, where O. lucimarinus was identified as the most abundant species in the ST flank with decaying concentrations with increasing distance to coast and into SA waters (Allen et al., 2020). *O. lucimarinus* emerged also among the most abundant Mamiellophyceae species in a 18S rRNA metabarcoding survey conducted on coastal waters globally (Tragin and Vaulot 2019). In addition to *O. lucimarinus,* wealsoIn SAW the relative contribution of Mamiellophyceae was substantially lower (Fig. 6) and dominated by the species identified as *Bathycoccus prasinos* and *Micromonas commoda* with very low abundance of *Ostreococcus* (Fig. 7).

The dominance of picoplanktonic Mamiellophyceae is consistent with the greater contribution of <2 um chla (80%, Fig. S4) observed in STW. It is worth noting that the highest abundance of this group was observed during the onset of the spring bloom (TAN1212, Table 1) when Mamiellophyceae accounted for 40-75% of 18S rRNA reads and diatoms contribution remained around 10% over the 3-weeks sampling (Fig. Suppl. Regions bar plots). Prasinophytes have been reported to contribute substantially to vernal blooms in temperature latitudes (Bustillos-Guzmán et al., 1996; Latasa et al., 2010; Gutierrez-Rodríguez et al., 2011; Nunes et al., 2018). Mamiellophyceae and *O. lucimarinus* were the most abundant phytoplankton class and species in the ST flank of the STF zone (TAN1516, Fig. 1, Table 1) in agreement with high prasinoxanthine and prasinophyte relative contribution reported during spring in this region (Delizzo et al., 2009). High abundance of several species of prasinophytes including Ostreococcus and Micromonas have been recently reported during the onset of the North Atlantic spring bloom from 16S rRNA amplicon sequencing analysis (Bolaños et al., 2020). The deep mixing layers (>100 m) during the Spring bloom voyage (Chiswell et al., 2020) where they dominated supports their ability to thrive under high-nutrient high-mixing conditions and highlights their important role in the development of spring blooms characteristic of temperate latitudes. Overall, our results highlight the wide ecological breadth of Mamiellophyceae and certain species like *Ostreococcus lucimarinus* which tend to dominate across a wide range of physical, chemical and trophic conditions encountered within STW.

Chloropicophyceae showed the opposite trend with the highest relative contribution associated with SAW (Fig. 5, 6). Chloropicophyceae, previously clade VII, has been recently described as a new class of picophytoplanktonic prasinophytes (Lopes dos Santos et al., 2017a). Culture representatives of Chloropicophyceae have been obtained from tropical and subtropical latitudes of the north and south Pacific (Lopes dos Santos et al., 2017a and references there in) but to our knowledge this is the first report of their presence and high abundance in subantarctic waters. The majority of Chloropicophyceae reads were assigned to a reference sequence corresponding to *Chloroparvula pacifica* and included several ASVs one of which (ASV0014) was the most protist found in SAW (Fig. 7). Chloropicophyceae is considered to be the dominant group of green algae in oligotrophic oceanic waters in contrast with the preference of Mamiellophyceae for richer coastal environments (Lopes dos Santos et al., 2017b; Tragin et al., 2018). In this study, Chloropicophyceae were most abundant in SAW which in the region east of New Zealand are considered HNLC, suggesting that the preference of this group for oligotrophic conditions reported for typically macronutrient limited waters (REF) could also encompassed iron limited HNLC systems. It is noteworthy that highest abundance of this class within SAW were observed on C. Plateau, where biological evidence of natural iron fertilization has been reported (Gutiérrez-Rodríguez et al., 2020) and in the STF north of the plateau (Fig. suppl. Regions) where *Chloropicon sieburthii* made a substantial contribution in addition to the dominant *Chloroparvula pacifica*. Moreover, an ASV belonging to this genus was also found to contribute substantially to protistan community in coastal waters of the California Current Ecosystem (Gutiérrez-Rodríguez et al., 2018), highlighting the need of further studies to better understand the ecological drivers beyond coastal-oceanic trophic gradients responsible of the water mass preferences they showed. What do we know about Chloroparvula distributions and ecology?

The relative contribution of dinoflagellates tended to be higher in SAW and the STF compared to STW (Fig. 6), consistent with previous the microscopy-based observations (Chang et al., 1998). *Heterocapsa rotundata, Karlodinium spp., and Gymnodium spp*. were most abundant photosynthetic species in SAW and STF (Fig. 5). However, ASVs affiliated to the heterotrophic *Gyrodinium* genusand particularly *G. fusiforme* were identified as the most abundant species in agreement with previous study in the Southland current where in addition to *Karlodinium* and *Gymnodinium*, *Gyrodinium helveticum* and *G. spirale* were also retrieved among the most abundant species (Allen et al., 2020). This genus is known to be an important component of microzooplankton prevailing in high latitude coastal and oceanic waters (Archer et al., 1996; Strom et al., 2001; Olson et al., 2002) being active grazers with the capability of cropping down iron stimulated diatom blooms in the HNLC waters of the subarctic Pacific (Saito et al., 2005 & 2006). While species of *Gyrodinium* were prevalent across all water masses in our study, their abundance was higher in more productive STF waters, where higher chla concentration was accompanied by increasing abundance of diatoms and larger cells, confirming their pivotal importance in pelagic foodwebs as the link between phytoplankton and mesozooplankton (Zeldis et al., 2019).

As expected, diatoms tended to be more abundant in the STF compared to STW and SAW (Fig. 5) although relatively high contribution (>30%) were observed in all water masses at times (Fig. 6). Most abundant ASVs in the STF were identified as *polar-centric-Mediophyceae* (Fig. 7) with several large diatom species including *Fragilariopsis sublineata*, *F. cylindrus*, *Chaetocerus periuvianus*, *Cylindrotheca closterium* and *Thalassiothrix longissima* were significantly more abundant in STF compared to STW (Fig. DESeq analysis). In addition to Polar-centric-Mediophyceae, other small diatoms as *Minidiscus trioculatus* and *Minutocellus polymorphus* were most abundant diatoms in agreement with the dominance of these small diatom taxa in STW of the Southland Current (Allen et al 2020). While most common genus reported in STW (and STF) by microscopy analysis (e.g. *Thallasiosira spp., Chaetoceros spp., Guinardia spp*.) (Chang et al.,1998) were also detected by DNA metabarcoding analysis, small species revealed as numerically dominant by the latter approach tended to be overlook by microscopy. Diatoms are generally conceptualized as microplankton group associated with new production and high export potential (Legendre and Le Fevre, 1995; Vidussi et al., 2001; Uitz et al., 2006). However, there are increasing evidence supporting the importance of small nano or and even pico-size diatoms in both coastal and oceanic systems (Buck et al., 2008; Lomas et al., 2009; Leblanc et al., 2018; Hernández-Ruiz et al., 2018; Arsenieff et al., 2020). Our results showing the dominance of *M. trioculatus* and *M. polymorphus* in STW particularly during the more productive conditions of Spring Bloom II and Bio-NBM spring voyages (Fig. 8 heatmap or Fig. Suppl with regional most abundance species) further support and the important role of small diatoms in open-ocean phytoplankton proliferations.

In SAW, *F. sublineata* was clearly the most abundant diatom species (Fig. 6) with ASV0036 and ASV0061 being more abundant on C. Plateau and SAF waters, respectively (Fig. Suppl. regional most abundant species bar plot). The preference of *Fragiolariopsis* species for SA has been also reported by microscopy analysis (Chang et al., 1998). DESeq analysis revealed that 9 diatom ASVs were significantly more abundant in STF compared to STW, but only 3 of them (*T. longissima*, *Minutocellus polymorphus* and *Cerataulina pelagica*) were significantly more abundant in STF relative to SA. This suggests higher connectivity between the STF and SAW in agreement with tighter coupling between mixing and phytoplankton biomass size-structure reported in the southern flank of the STF (Safi et al., submitted).

Pelagophyceae showed the opposite trend compared to diatoms and have lowest contribution observed in STF waters (Fig. 6). The contribution of this class tended to increase with depth (Regional bar-plots show this – think about other way to illustrate their depth preference) in agreement with their preference for the DCM (Le Gall et al., 2008; Latasa et al., 2016; ….) and physiological adaptation to low light and high nutrient environments (Dimier et al., 2009’ Kulk et al., 2012; Dupont et al., 2015). This vertical segregation was evident in both STW and SAW despite the different specific composition observed in each water mass. *Pelagomonas calceolata* (ASV0044) was the most abundant species in STW while unidentified pelagophyte (Pelagophyceae\_XXX.sp, ASV0081) and *Aureococcus anophagefferens* (ASV0118) were most abundant species in SAW (Fig. 7). *Pelagomonas calceolata* is a widespread species (Andersen et al., 1993; Moon-van der Staay). Whether the ubiquity of this species is bound to high genetic diversity or physiological versatility is not clear although the low intraspecific diversity observed in pelagophytes assemblages globally (Cabello et al., PhD, submitted to EM) supports the latter. In our study, several ASVs were assigned to *Pelagomonas calceolata* and while the most abundant one showed preference for STW, other less abundant ASVs were significantly more abundant in SAW (DESeq figure). Similarly, we found different water mass preference among ASVs assigned to unidentified pelagophytes (Fig. DESeq). While these observations suggest that different ASVs may reflect ecologically relevant different units (Rodriguez et al., 2005; Farrant et al., 2016) they also highlight the importance of culture isolations and species characterization to better characterize the diversity of pelagophytes assemblages.

Prymnesiophyceae were prevalent across all water masses (Fig. 5) although their relative contribution tended to be lower (7% of total 18S reads, Fig. 6) than expected for open ocean waters. The prevalence of 19-Hex pigment marker in oceanic waters and the application of quantitative methods (e.g. CHEMTAX) have shown that prymnesiophyceae represents between 20-50 % of the phytoplankton community in oceanic waters (Andersen et al., 1996; Latasa et al., 2005; Liu et al., 2009; Swan et al., 2016). Such dominance has been also depicted by improved genomic approaches which revealed the extremely high genetic and functional diversity of non-calcifying prymnesiophytes (Liu et al., 2009; Cuvelier et al., 2010). In our study, non-calcifying species, mainly assigned to *Phaeocystis* spp. and *Chrysochromulina* spp., dominated the group (Suppl. Fig. Treemaps of specific groups). The abundance and relative contribution of *Phaeocystis* spp. was lowest in STW, intermediate in STF and peaked in SAW while Chrysochromulina spp. followed the opposite trend with higher contribution associated with STW (Fig. Treemaps of specific groups). The dominance of *Phaeocystis* spp. in SAW was mainly driven by *P. antarctica* (Fig. 7) in agreement with the prominence of this group in the SO (Verity et al., 2007) and observed decreasing abundance observed from SAW towards STW of the SW Pacific region during the Autumn-Winter season (Sow et al., 2020). Strains assigned to *P. globosa* and *P. cordata* were also detected in all water masses although they tended to be more prevalent and abundant in STW compared to SAW in agreement with previous reports in the region (Sow et al., 2020).

Coccolitophores are an important component of phytoplankton communities in the SO region extending from the STF to the Polar Front known as the Great Calcite Belt (Balch et al., 2011; Chang et al., 2016; Balch et al., 2016). In our study, higher abundances of coccolitophores were observed in the STF, in agreement with previous microscopy-based studies in this region of the SW Pacific (Saavedra-Pellitero et al., 2014; Rigual-Hernández et al., 2020). The most abundant coccolitophore species we found in our study were assigned to *Gephyrocapsa oceanica*, which contribute most in the STF but emerged among the 20 most abundant species also in SAW and present in STW (Fig. 7). However, *Emiliania huxleyii* that generally dominate coccolitophore assemblages in this region (Saavedra-Pellitero et al., 2014; Chang et al 2016) and in the SO (Holligan et al., 2010; Balch et al., 2016) showed very low abundances (data not shown).

*Cryptophyceae* contribution was relatively low on average (<3%) but showed lower abundance in SAW compared to STW where they represented up to 10% of protistan reads in the euphotic zone (Fig. 6). Cryptophytes are an ubiquitous phytoplankton group with widespread distribution from coastal to ocean systems and tropical to polar latitudes (Buma et al., 1992; Piwosz et al., 2013; Nunes et al., 2018). They have been reported to form blooms in coastal embayments (Jeong et al., 2013; Johnson et al., 2013) and coastal antarctic waters favored by low salinity conditions (Moline et al., 2004; Schofield et al., 2017; Nunes et al., 2019). The higher contribution we observed in STW relative to SAW in this study argue against the direct influence of salinity at least in open-ocean waters. The genus and species composition of the group also differ substantially between ST and SA waters (Fig. S11). In STW, *Teleaulax spp*. (ASV0107) was the dominant species followed by *Plagioselmis prolonga* (ASV0102) and *Geminifera cryophile* (ASV0192), while it was virtually absent in SAW where the same strains of *P. prolonga* and *G. cryophile* became the dominant cryptophyte species, although at much lower abundances than observed in STW. Cryptophytes have been observed to response positively to iron fertilization in HNLC waters of the North Pacific Sato et al., 2009; Suzuki et al., 2009) suggesting that their lower abundance in SAW in this study could be related to iron limited conditions characteristic of the region. The contribution of cryptophytes in STW was highest during the open-ocean spring bloom (TAN1212) and in shelf-slope stations (TAN1604) consistent with their preference for richer conditions (Fuller et al., 2006; Latasa et al., 2010; Carreto et al., 2016 PO). Significant contribution of cryptophytes has been also observed in open ocean waters of the NW Mediterranean at the termination of the spring bloom (Vidussi et al., 2000) where they even dominated the surface mixed layer community in highly stratified stations (Latasa et al., submitted PO). Interestingly, the higher contribution of cryptophytes in our study occurred towards the end of the Spring bloom surveyed (TAN1212)(Fig. Suppl. Regional Bar plots), coincident with strong surface stratification and biomass accumulation (Chiswell et al., 2019) supporting the greater importance that stratification may have on the group compared to salinity itself (Latasa et al., submitted).

**Heterotrophic groups composition and vertical distribution / Aphotic community composition from biophysical moorings time series**

To be completed.

DISCUSSION STRUCTURE OPTIONS

1. **For Scientific Reports:**
2. Light discussion structured in:
3. Alpha diversity differences between water masses and patterns in relation to environmental factors.
4. Phytoplankton groups composition (class, species, intraspecies) and distributions in relation to environmental factors.
5. Heterotrophic groups composition and vertical distribution / Aphotic community composition from biophysical moorings time series
6. **For other more ecological/oceanographic journals Alternative thematic structure ( DSR, EM, NZMSS for instance)**
7. Alpha Diversity and species patterns
8. Protist groups composition in major water masses
9. Protist groups composition across major frontal zones STF and SAF
10. Intraspecific diversity – Ecotypes (collide results about different ASVs from different groups in this section)

**Group biogeographic preferences**

Study spatial-temporal coverage – Importance of regional variability

* *Implications of regional/temporal variability for interpretation of water mass preferences*
* *Discuss temporal/seasonal variability within each water mass with focus in biophysical mooring voyages that include Feb, March, May, Sept, Oct.*

Richness patterns

* *Low diversity in STF despite heterogeneity of conditions?*
* *Lower diversity in productive systems?*
* *Low diversity in C. Plateau?*
* *Rarefaction curves – diversity saturation? – sampling effort?*

One could argue that more productive waters such as the STF, Spring Bloom, and the C. Plateau exhibited lowest diversity levels. [**NOTE**: Diversity patterns associated with water masses and regions are the same when considered only he surface mixed layer (Mixed-layer depth – 0.2 C change relative to 10 m). However, differences in diversity between water masses was smoothed when only samples from the twilight zone (>150 m) where considered (Supplementary Figure 1).]

Community composition and environmental drivers

* *Class-level group distribution in relation to temperature, salinity*
* *Class-level group distribution in relation to trophic status of the system (Chla concentration or index with POC,…similar to Latasa et al., 2010)*
* *Groups distribution in relation to SFChla – e.g. diatoms pico and nano vs micro. How does the relative abundance of this group vary with community size structure – does the diatom-large phytoplankton general association sustained in our dataset (e.g. Minutucellus or Minidiscus contribution to diatoms signal does not imply an increase in community size structure)*

Here, their abundance tended to increase with temperature indicating this group preference for STW influenced waters (Figure 8 – suppl?). In STW though, they peaked in relatively cold (and deeply mixed) waters during the Spring Bloom II but decreased in warmer waters further north (Figure 8 – suppl?).

Euphotic vs aphotic – Biophysical moorings

Regional variability tax composition rel abundance of groups

Influence of regional and temporal variability on differences between water masses for discussion

* Fig. % abundance – Box-plot regional
* Species abundance – Regional
* Fig. % abundance – Env. Variables

Both alpha and beta diversity varied regionally (and temporally) within the three main water masses explored. In STW for instance, Chlorophyta dominated the community during the Spring Bloom II, driven mainly by the extremely high abundance of Ostreococcus (Mamiellophyceae) which accounted for approximately 50% of total reads (Figure treemaps – regional). The contribution of dinoflagellates during Spring Bloom, was substantial, yet minimum (<20%) compared to other STW regions (Figure Box-Plot regional). In well-stratified oligotrophic ST waters surveyed during late autumn (TAN1604), dinoflagellates were the dominant group (>40% total reads) followed by Chlorophyta (15%), with heterotrophic and mixotrophic MAST and Radiolaria increasing their contribution to similar levels (15%) (Suppl. Fig. treemaps – regional). Pelagophyceae tended to increase their contribution compared to the Spring Bloom II and Bio-NBM regions, while Prymnesiophyceae showed the opposite trend (Figure Box-Plot regional). Most abundant species,…need to re-do the plots with Bay of Plenty - Areas

The taxonomic composition of communities associated with different regions within SAW was also different. The Bio-SBM site located in the Bounty Trough south of the Chatham Rise was clearly dominated by dinoflagellates (40% reads) followed by Chlorophyta (20%) which included similar proportion of reads affiliated to Chloropicophyceae and Mamiellophyceae. Prymnesiophyceae and Pelagophyceae ranked next and accounted for a larger proportion of reads compared to diatoms (Figure regional - treemaps). The community on C. Plateau showed a larger contribution from Chlorophyta, which was dominated by Chloropicophyceae with a minor contribution from Mamiellophyceae. The contribution of MAST and diatoms increased while that of Prymnesiophyceae, Pelagophyceae, Dictyophyceae and Dinophyceae was minimum compared to SAW to the north and south of the C. Plateau. The relative contribution of diatoms increased further in colder waters of the SAF to the south together with Prymnesiophyceae they became the dominant phytoplankton/photosynthetic groups. Chloropicophyceae remained the most important group within Chlorophyta, although the contribution of green algae was lowest among SAW regions.

Similar changes at class level were also observed between the STF flowing north of the C. Plateau where Chloropicophyceae (& Prasino Clade V) were most abundant classes of green algae, and the STF over the Chatham Rise, where Mamiellophyceae became clearly the dominant class (Figure regional – treemaps).

The diversity of communities associated with the STF flowing north of the C. Plateau and over the Chatham Rise further north-east was also different (Figure – treemaps – regional – Pending to re-do the plots

STW surveyed in this study span several latitudinal degrees (33 and 41 S), seasons (spring, summer, autumn) (Table 1) and water-column conditions ranging from strongly-stratified oligotrophic waters (e.g. Cross-shelf voyage) to deep-mixing mesotrophic conditions (Spring Bloom II) and in-between conditions encounter during multiple voyages to the Bio-NBM site north of the Chatham rise (Map/Table 1).

**[5] Additional introduction/discussion points**

* Trophic index – classification similar to Latasa et al., 2010 about trophic preferences of phytoplankton chemotaxonomic groups but using taxonomic classes from DNA metaB?
* Functional groups – Taxonomic resolution with ecological significance – inferred from different distributional patterns and relation to physico-chemical drivers (T, Sal, Nutrients, …) division/class/genus/species What’s the taxonomic rank at which we see differences across water masses?
* Intraspecific variability – Differences in the spatial variability of ASVs of same species and implications for the interpretation of their functional groups and trait-based approaches. E.g. Diatoms (pigment-based) linked with accumulation of larger phytoplankton and export potential. Well, not always, if diatom increase is driven by small diatom species – Minutocellus, Minidiscus,…

NOTES on FIGURES from SN:

Figure 1 – need a diagram that shows the circulation and water masses

Suppl Figure S1 – seasonal differences across voyages? In S2?

Figure 3: need to think what these estimates of alpha diversity really mean…..

Suppl Figures S6 and S7– not sure what the Ranks refer to in terms of concentrations…..

Figure 6A and B – how are these abundances standardized? Why do they look like they do?!

FYI Cross-shelf and Spring Bloom II figures - Depth trends look interesting but need to be able to reference the samples to the depth