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EFFECTS OF ORGANIC MATTER ADDITION AND TEMPERATURE INCREASE ON THE MICROPLANKTON COMMUNITY (PHYTO- AND MICROZOO-)

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ΑΝΤΙΚΕΙΜΕΝΟ ΕΡΓΑΣΙΑΣ:

Effects of organic matter addition and temperature increase on the microplankton community (phyto- and microzoo-)

Τριμελής Επιτροπή Επίβλεψης και Κρίσης της Εργασίας

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Abstract

This study investigates microplankton community responses to the addition of dissolved organic matter and temperature increase in seawater. The above research question is encompassed in a broader mesocosm experiment (PROMAC: Prokaryotic maintenence respiration, activities and structural adaptation at low temperature and starvation in the sub-Arctic) whose principal aim was to study the ecological significance of substantial prokaryotic maintenance respiration in the sea, previously demonstrated in the field. To evaluate the central experimental goal and that of the present thesis, four experimental treatments were applied in triplicate during a 29-day fully factorial experiment. No manipulations were applied in Control ('C') treatment. Additionally, treatments named after Nutrients ('N'), Temperature ('T'), Temperature & Nutrients ('TN') were also established. In the 'C' and 'N' treatments, mesocosm temperature was set to 1°C, while in the 'T' and 'TN' treatments it was set to 10°C. representing summer conditions. Treatments 'C' and 'TN' are representative of winter and summer conditions respectively, while 'N' and 'T' contribute to a fully factorial experimental design without coupling to a specific seaso. Microplankton community responses to experimental manipulations was examined (thesis topic) by means of taxonomic identification and cell enumeration via inverted microscopy. Significant differences in microplankton abundance were noted among treatments. The interaction of time with imposed treatments had a significant impact on the community composition of Diatoms, Dinoflagellates and Ciliates. Abiotic drivers of such shifts in communities were also explored. It is believed that in conjunction with bacterial abundance and respiration estimations, the present study will contribute to greater understanding of how a possible alteration in bacterial growth may affect higher trophic levels.

Περίληψη

Στόχος της παρούσας πτυχιακής εργασίας είναι η μελέτη της απόκρισης της μικροπλαγκτονικής κοινότητας σε προσθήκη διαλυτής οργανικής ύλης και αύξηση της θερμοκρασίας στο θαλασσινό νερό. Το παραπάνω ερευνητικό ερώτημα εντάσσεται στο πλαίσιο ενός πειράματος μεσοκόσμων (PROMAC: Prokaryotic maintenence respiration, activities and structural adaptation at low temperature and starvation in the sub-Arctic) .Ειδικότερα, σημαντικές μεταβολικές διεργασίες των βακτηρίων (π.χ. ειδικοί ρυθμοί ανάπτυξης) τροποποιήθηκαν και μετέπειτα μελετήθηκαν σε ένα πλήρως παραγοντικό πείραμα (fully factorial experiment) που διήρκησε τέσσερις εβδομάδες (οκτώ πειραματικές ημέρες). Το πείραμα περιλάμβανε συνολικά δώδεκα μεσοκόσμους, με τέσσερις πειραματικές συνθήκες σε τρεις επαναλήψεις η καθεμία: συνθήκες ελέγχου (Control, "C"), συνθήκες αυξημένης προσθήκης διαλυτής οργανικής ύλης (Nutrients, "N"), συνθήκες αυξημένης θερμοκρασίας κατά 10°C (Temperature, "T") και τέλος, συνθήκες συνδυασμού προσθήκης θρεπτικών και αύξησης της θερμοκρασίας (Nutrients, Temperature,

“TN”). Για το προτεινόμενο ερευνητικό ερώτημα στην παρούσα μελέτη πραγματοποιήθηκε η ταξινομική αναγνώριση και καταμέτρηση των μικρο- πλαγκτονικών (φυτο- και μικροζωο-) οργανισμών στους διαφορετικούς μεσοκόσμους με χρήση ανάστροφης μικροσκοπίας. Σε συνδυασμό με τις μετρήσεις της βακτηριακής αφθονίας και αναπνοής, αυτή η μελέτη θα συνεισφέρει στην κατανόηση της απόκρισης της μικροπλαγκτονικής κοινότητας κάτω από ελεγχόμενες συνθήκες, και πιο συγκεκριμένα μετά από αλλαγές στη λειτουργία της βακτηριακής κοινότητας.

Contents

1. Introduction

1.1. An introduction to the planktonic microbial food web

Marine microorganisms, including prokaryotes (bacteria and archaea), eukaryotic phyto- and zooplankton and viruses are linked together through trophic interactions, forming an elaborate network known as the planktonic microbial food web(Sherr and Sherr, 1991; Herndl et al., 2008; Trombetta et al., 2020) . Planktonic marine microbes fall into several size fractions, including femto- (0.02-0.2 μm), pico- (0.2-2 μm), nano- (2-20 μm), micro- (20-200 μm) plankton (Sieburth et al., 1978). In addition to variation in size, members of this food web display diversity with regard to trophic type, i.e. autotrophy, heterotrophy, mixotrophy and, when speaking for viruses, more frequently lytic infection and lysis of all above-mentioned microbial groups (Sherr and Sherr, 1991; Mostajir et al., 2015) .

Trophic interactions linking planktonic microbes include predation, commensalism or symbiosis, competition for nutrients and parasitism (Azam et al., 1983; Lima-Mendez et al., 2015; Trombetta et al., 2020). With the exception of parasitism, all trophic relationships mentioned above are represented within the concept of the "microbial loop". Briefly, dissolved organic matter (DOM) produced by phytoplankton is capitalised by heterotrophic bacteria which are preyed on mostly by heterotrophic flagellates but also by other eukaryotes to a lesser extent, like ciliates. In turn, predators of bacteria act as prey for microzooplankton(Azam et al., 1983). Particulate organic carbon (POC) that has been produced through sloppy grazing, cell death and decomposition or other processes may also be capitalised by heterotrophic bacteria and thus, re-introduced in the food web. A representation of the pelagic food web, including the microbial loop, is presented in Fig 1. The "microbial food web" complements the "traditional" food chain in which diatoms can be described as the main phytoplankton group and are consumed by copepods, with the latter being the pray of fish and cetaceans (Legendre and Rassoulzadegan, 1995; Pernthaler, 2005).

The significance of the microbial food web with regard to energy and nutrient pathways in the pelagic environment has been highlighted due to the recognition of the great microbial abundance with significant growth rates in the oceans (Azam et al., 1983;Pomeroy, 1974). Indeed, the planktonic microbial food web is a modulator of energy and nutrient transfer within the pelagic food web; depending on microbial functioning, on one hand, energy and nutrients may be transferred higher up in the food chain or on the other hand, may be removed to the dissolved fraction of the water column(Azam et al., 1983; Baretta-Bekker et al., 1995; Trombetta et al., 2020). For instance, the pool of available DOC is altered by the viral shunt, whereby DOC is produced through the infection and lysis of

bacteria (Wilhelm and Suttle, 1999). Additionally, micro-zooplankton, in conjunction with heterotrophic flagellates contribute to nutrient remineralisation via grazing activity (McManus and Santoferrara,2013) .

Overall, the structure and functions of the planktonic microbial food web are governed by bottom-up (nutrient availability) and top-down (predation) control mechanisms(Mostajir et al., 2015; Andersson et al., 2017). Although there is evidence suggesting that the significance of top-down processes has been underrated, predation remains a dominant selection pressure on marine populations(Tiselius and Møller, 2017). Certainly, incorporation of viruses within modelling efforts of the pelagic food web would increase their predictive capacity (Legendre and Rivkin, 2008) and consequently our understanding of the food web functioning.

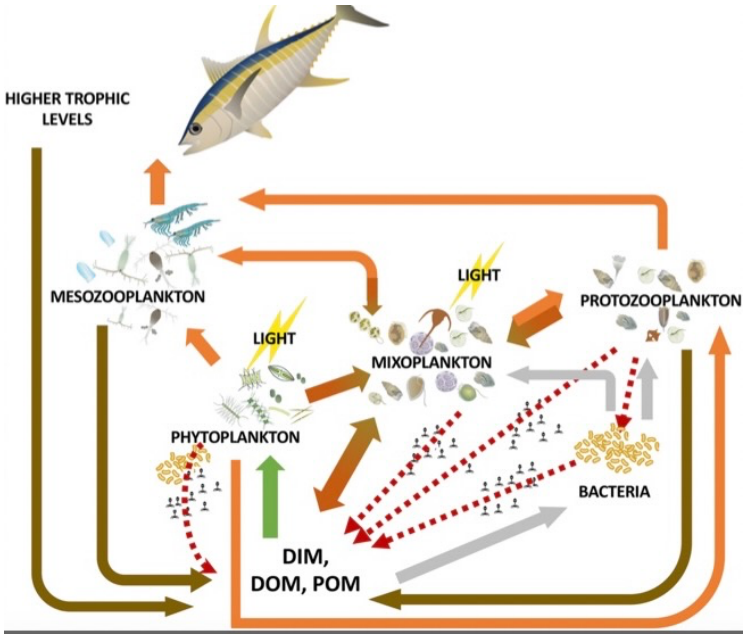

Figure 1. Representation of the pelagic food web including the microbial loop. Figure adapted from: Gilbert and Mitra, (2022).

1.2.The roles of microplankton

Planktonic organisms within the size fraction of 20-200 μm are collectively known as microplankton(Sieburth et al., 1978). Microplankton are a diverse group of organisms, primarily represented by diatoms (silicifiers), ciliates and dinoflagellates (Quere et al., 2005; Romano and Pitta, 2021), which dominate in waters of higher trophic status (eutrophic) in terms of biomass (Uitz et al., 2006). In mesotrophic and oligotrophic waters, nanoplankton and picoplankton biomass respectively is predominant (Fuhrman et al., 1989; Uitz et al., 2006). Traditionally divided into phytoplankton and micro- or proto- zooplankton(Flynn et al., 2013; Mitra et al., 2014), microplankton trophic strategies range from strict autotrophy to mixotrophy and heterotrophy. Mixotrophy is currently increasingly

recognized as a predominant trophic strategy among microplankton(Mitra et al., 2016). Each of the groups presented above, may represent a "functional type" (i.e., phytoplankton silicifiers, protozooplankton) influencing significant ecosystem processes(Quere et al., 2005).

Diatoms, silicifying phytoplankton ranging between a few μm to 2 mm when in chains, predominate in systems of higher productivity with adequate light availability(Quere et al., 2005; Sarthou et al., 2005; Armbrust, 2009; Leblanc et al., 2012). The global estimate of diatom biomass is 0.11Pg C, whilst diatom abundance around the globe falls between 1 and $6.95*10^7$ cells L^{-1}(Quere et al., 2005; Uitz et al., 2006; Leblanc et al., 2012). Diatoms are responsible for approximately 40 to 45% of annual primary production in the global ocean and account for 20% of breathing oxygen (Mann, 1999; Quere et al., 2005; Leblanc et al., 2012;). It is therefore obvious that diatoms have a significant influence on the carbon (C) cycle, and the cycling of other elements, particularly silica (Si) (Yool and Tyrrell, 2003; Sarthou et al., 2005;). In addition, diatoms are significant C exporters to the ocean's interior, with their dimensions and ability to attract/collide to other particles contributing to this fast sinking(Boyd and Newton, 1999; Yool and Tyrrell, 2003; Quere et al., 2005; Sarthou et al., 2005; Cermeño et al., 2006). In temperate coastal waters, especially, aggregated diatoms and sinking blooms transport nutrients as well as carbon to the seabed (Thornton, 2002). Moreover, diatoms are important elements of marine snow, diverse particle aggregates >500 μm long, whose sinking contributes to the export of organic matter (Alldredge and Silver, 1988; Azam and Malfatti, 2007).

Ciliates are essentially cosmopolitan planktonic heterotrophic and mixotrophic organisms falling within the nano- and micro- size classes (Pierce and Turner, 1992; Pitta et al., 2001). Ciliates are predators of pico- and nano-plankton, whilst acting as prey for larger zooplankton such as copepods and metazoans (Setälä and Kivi, 2003; Pomeroy et al., 2007). Consequently, ciliates act as a bridge between the microbial food web and higher trophic levels, representing a critical component of the pelagic food web, especially in oligotrophic waters(Pierce and Turner, 1992; Pitta et al., 2001; Romano and Pitta, 2021). It is noteworthy that mixotrophy in ciliates may be widespread, for example during summer months, with mixotrophic ciliates representing circa 20% of integrated to 200m depth ciliate abundance and biomass in the Mediterranean in June and >60% of ciliate abundance and biomass in the mixed layer of the Eastern Bering Sea(Pitta et al., 2001; Stoecker et al.,2014). Photoautotrophy of ciliates has also been reported in the past, in the case of *Mesodinium rubrum* (Stoecker et al., 1989; Setälä and Kivi, 2003). Overall evidence suggests that *M. rubrum* in combination with mixotrophic ciliates may contribute significantly to photosynthesis at low algal biomass in addition to acting as prey for larger plankton(Stoecker et al., 1989).

Dinoflagellates are cosmopolitan flagellated protists of multiple size fractions (pico- to meso-), and trophic strategies ranging from autotrophy to heterotrophy and mixotrophy (Adl et al., 2005; Jeong et al., 2010; Hansen,

2011; le Bescot et al., 2016). Dinoflagellates are often dominant in plankton assemblages(Jeong et al., 2010; Murray et al., 2016), identifiable as culprits of Harmful Algal Blooms (HABs) usually at warm water temperatures (Gomez, 2012; Murray et al., 2016). They represent a significant component of microplankton in distinct ecosystems(Hansen, 1991; Sherr and Sherr, 2007; Stoecker et al., 2014) including the poles (Sherr and Sherr, 1994) and the Eastern Mediterranean(Romano and Pitta, 2021). In the coastal ocean, dinoflagellates appear to be abundant simultaneously with larger phytoplankton(Hansen, 1991). Evidence suggests that heterotrophic dinoflagellates exert significant grazing pressure on diatoms and are prominent herbivores(Levinsen et al., 2002; Sherr and Sherr, 2007). The significance of symbiotic mixotrophic dinoflagellates as predators is poorly understood (Hansen, 2011). Additionally, dinoflagellates can prey on organisms on the lower end of the size spectrum (picoplankton), thus bridging bacteria to higher trophic levels through the food web(Sherr and Sherr, 1994; Jeong et al., 2010)., similarly to ciliates. Within the food web, dinoflagellates appear to compete with ciliates for food(Hansen, 1991; Jeong et al., 2010). Due to diverse trophic strategies, they carry out diverse critical roles within the food web and elemental cycles(Levinsen et al., 2002; Gomez, 2012; Jeong et al., 2010; Murray et al., 2016). However, it is evident that their importance within the food web may have been overlooked. To understand the impact of dinoflagellates on marine food webs and biogeochemical cycles it is suggested that this should be examined on a size fraction basis(le Bescot et al., 2016).

As seen above, microplankton carry out key ecosystem roles(Quere et al., 2005; Sarthou et al., 2005; Jeong et al., 2010; Romano and Pitta, 2021) and therefore understanding the factors controlling their community structure (abundance, biomass, diversity) is essential especially when faced with challenges such as climate change(Winder and Sommer, 2012). A large proportion of studies concerning microplankton variability are divided into phytoplankton and microzooplankton investigations (Calbet and Landry, 2004; Irigoien et al., 2004; Marãnón et al., 2014; Stoecker et al., 2014), although this may be a false dichotomy(Flynn et al., 2013). Regardless, it is evident that shifts in both categories are regulated by abiotic and biotic parameters(Cermeño et al., 2006; Marañón et al., 2000; 2014; Stoecker et al., 2014) that are described below.

Nutrient limitation (Irigoien et al., 2004; Litchman and Klausmeier, 2008; Marañón, 2005; 2014), including Fe limitation in oceanic locations(Chavez et al., 1991; Boyd et al., 2000), and with few exceptions light(Andersson and Hagstrom, 1994; Marañón et al., 2000; Cermeño et al., 2006;), appear to be key abiotic parameters regulating phytoplankton distribution and growth rates. Equally, temperature may control phytoplankton succession in temperate seas (Andersson and Hagstrom, 1994) but not growth and production rates in the ocean(Marãnón et al., 2014). On the other hand, multiple studies suggest that grazing and sinking are critical biotic components controlling phytoplankton communities in terms of both abundance and diversity(Calbet and Landry, 2004; Sarthou et al., 2005; Litchman and Klausmeier, 2008). Cell lysis may also contribute to phytoplankton loss(Sarthou et al., 2005). Similarly to phytoplankton, grazing appears to be a key regulatory parameter of microzooplankton community structure(Levinsen et al., 2002; Pomeroy et al., 2007; Stoecker et al., 2014). Abiotic parameters such as

nutrient availability(Pitta et al., 2001; Levinsen et al., 2002), possibly controlled by mixing and stratification(Stoecker et al., 2014), and likely temperature (Romano and Pitta, 2021) control microzooplankton assemblages.

Throughout this section, it has become evident that microplankton members often act as a bridge with higher trophic levels within the pelagic food web (Pomeroy et al., 2007). The microbial loop is a representative example of the relationships between microplankton and bacteria, which consist of commensalism, symbiosis, parasitism, competition for resources and predation. At this point, it should be noted that coupling of bacteria to primary producers is not necessarily strict, and the variability of this relationship is highly dependent on ecosystems(Morán et al., 2002; Azam and Malfatti, 2007;). Moreover, predation may well be one of the earliest eukaryotic- prokaryotic interactions that have been recognized(Cavalier-Smith, 2002). Nonetheless bacteria have been known to form symbiotic relationships with micro-phytoplankton in particular (Armbrust, 2009; Amin et al., 2015; Durham et al., 2015).Furthermore, protist mixotrophy may lead to a microplankton-bacteria relationship akin to “farming”(Mitra et al., 2014) whereby mixotrophs supply bacteria with DOM enabling their growth and bacteria supply mixotrophs with inorganic nutrients through grazing. Overall, microbial interactions in the ocean are highly complex and gaining insight into microscale processes should increase our understanding into biogeochemical paths(Azam and Malfatti, 2007).

Given that bacterial-microplankton interactions influence ecosystem processes(Azam et al., 1983), understanding the influence of bacterial growth alterations on the ecosystem (and thereby on microplankton too) should increase our insight into future ocean functioning. Nonetheless, available bibliography is centered on the parameters controlling bacterial growth predominantly within the context of climate change (Huete-Stauffer et al., 2015; Morán et al., 2017). However, it can be surmised that ocean warming will possibly enhance both bacterial standing stocks and grazing, possibly placing microbes at the center of the marine carbon cycle(Sarmento et al., 2010). Therefore, it could be argued that knowledge of regulatory mechanisms behind bacterial growth will enable us to better understand the interactions between bacterial and microplankton growth, and the marine ecosystem as a whole.

1.3. The microbial plankton of the Baltic Sea

The Baltic Sea is one of the vastest bodies of brackish water on the globe, situated between 53°55’N and 65°48’N (Snoeijs-Leijonmalm and Andren, 2017). It is a semi-enclosed sea, linked to the Atlantic and North Sea through the Skagerrak, which is considered its entrance, and is composed of the Gulf of Finland, the Gulf of Bothnia and the Baltic Proper (HELCOM, 1993; Wasmund and Uhlig, 2003; Carstensen and Heiskanen, 2007). The brackish nature of the Baltic Sea is primarily due to receiving water from over 200 rivers and limited water exchange with the North Sea (Snoeijs-Leijonmalm and Andren, 2017). Due to its northern latitude, seasonality is highly pronounced in the

region (Kaikkonen et al., 2020). Additionally, the vast geographic gradient of this sea produces significant differences in abiotic parameters such as salinity, dissolved organic carbon concentration, ice cover, light regime affecting species distribution (Granskog et al., 2006; Snoeijs-Leijonmalm and Andren, 2017; Olofsson et al., 2020).

Salinity in particular appears to exert significant influence on the spatial distribution of microbial plankton including bacteria and phytoplankton, in the different Baltic Sea regions(Andersson et al., 1996; Olofsson et al., 2020; Camarena-Gómez et al., 2021;). Whilst examining seasonal variation in microplankton community structure across varying salinities in the region, Olofsson et al. (2020) observed that peak abundance of phytoplankton was reached earlier in the year (winter-spring months) in higher salinity stations and that lower salinity stations displayed higher interannual variability in phytoplankton abundance. Nonetheless, seasonality in the microbial plankton community of the Baltic Sea, including the appearance and termination of the spring bloom, appears to be largely regulated by temperature variation and nutrient limitation (Kivi et al., 1993; Andersson et al., 2010; Jurgensone et al., 2011; Kaikkonen et al., 2020; Alegria Zufia et al., 2021).

The initiation of the spring bloom varies across the different basins of the Baltic Sea(Kahru and Nommann, 1990). Briefly, spring bloom appearance in the different basins occurs between March in the southern Baltic Sea and May in the Gulf of Bothnia(Camarena-Gómez et al., 2021). The spring bloom across all basins is terminated due to depletion of available nutrients, (Hagström et al., 2001), a topic that is discussed later in this section . Across the Baltic Sea, spring blooms are primarily composed of diatoms, dinoflagellates, or a combination of the two whilst ciliates may also be present (Camarena-Gómez et al., 2021). It is noteworthy, that during the last decades, a shift from diatom to dinoflagellate dominated spring blooms has been observed in the Baltic Sea (Wasmund and Uhlig, 2003; Jurgensone et al., 2011; Spilling et al., 2018; Hjerne et al., 2019) . There is evidence suggesting that diatoms are predominant following cold winters, with vast ice coverage whilst dinoflagellates dominate blooms after mild winters (Jurgensone et al., 2011; Hjerne et al., 2019; Spilling et al., 2018) .

Representative diatoms of the Baltic Sea regions include the centric diatom*s Thalassiosira baltica, Thalassiosira levanderi, Chaetoceros wighamii, Chaetoceros sp., Skeletonema marinoi, Melosira arctica* and the pennate diatoms *Pauliella taeniata (also identified as Navicula* sp*.* (Eriksson et al., 1977; Andersson et al., 1996; Enberg et al., 2018; Hjerne et al., 2019). The chain-forming dinoflagellate *Peridiniella catenata* is ubiquitous across the region, whilst elliptical medium sized dinoflagellates are common in the northern Baltic Sea(Andersson et al., 1996; Spilling et al., 2018; Camarena-Gómez et al., 2021). The most commonly encountered ciliate during and after the spring bloom is the mixotrophic *Mesodinium rubrum* and oligotrichs such as strombilidiids and strombidiids are found in the summer (Eriksson et al., 1977; Setälä and Kivi, 2003; Mironova et al., 2012). In late summer, the microplankton community composed of the abovementioned taxa gives away to a cyanobacterial bloom mostly consisting of *Aphanizomenon* sp*.* and *Nodularia spumigena* (Andersson et al., 1996; Jurgensone et al., 2011; Spilling et al., 2018).

Secondary peaks of diatoms have also been reported during autumn (Andersson et al., 1996; Hagström et al., 2001; Jurgensone et al., 2011).

One of the low productivity areas in the Baltic Sea, with only four predominant microplankton species (constituting >25% of total biomass) during the spring bloom, is the Bothnian Bay, a subregion of the Gulf of Bothnia (Andersson et al., 1996; Snoeijs-Leijonmalm and Andren, 2017). Overall, the Gulf of Bothnia is considered oligotrophic whilst the remaining basins are mesotrophic and possibly eutrophic along the coast (Nixon, 1995; Snoeijs-Leijonmalm and Andren, 2017).Additionally, the Gulf of Bothnia is predominantly phosphorus limited, whilst other regions in the Baltic Sea are predominantly nitrogen limited and possibly silicate limited in the case of the Gulf of Riga (Andersson et al., 1996; Hagström et al., 2001; Tamminen and Andersen, 2007; Jurgensone et al., 2011). Remarkably, the Gulf of Bothnia is net heterotrophic, with pelagic respiration theoretically exceeding photosynthetic carbon fixation by 60% in the Bothnian Bay and 30% in the Bothnian Sea (Algesten et al., 2004; Sandberg et al., 2004). A likely source of carbon for bacteria is Terrigenous Dissolved Organic Carbon (TDOC) (Sandberg et al., 2004). The dominance of bacteria with regard to respiration in the Gulf of Bothnia (Sandberg et al., 2004) is significant. A large proportion of respiration is driven by riverine DOC and maintenance respiration of bacteria in the Öre Estuary (northern Bothnian Sea, Vikström and Wikner (2019), Vikström et al. 2020).

Overall, the Baltic Sea is a sub-Arctic estuary with vast climatic variation and a pronounced salinity gradient. This is influential on the spatial distribution of species. Higher productivity in all basins of the Baltic Sea occurs during spring whilst secondary peaks during autumn may occur. Shifts in the species composition of the spring bloom are occurring in the Baltic Sea, a process for which climate change has been implicated. Finally, oligotrophy is a feature of the northern Baltic Sea where heterotrophy seems to be more pronounced.

1.4. The value of mesocosm experiments

Often, in the aquatic environment, the study of ecological phenomena in the field is costly and does not yield results of high statistical power due to natural spatiotemporal variability (Båmstedt and Larsson, 2018) as well as methodological variability. Reducing the volume of water, and duration of time over which these phenomena are studied by conducting laboratory experiments, may increase scientific understanding of ecological functioning (Stewart et al., 2013). However, microcosm experiments in particular are a poor reflection of the intricacy observed in nature(Stewart et al., 2013; Båmstedt and Larsson, 2018). By bridging the gap between natural conditions and the laboratory, mesocosms are an invaluable tool to ecologists(Petersen et al., 2009; Riebesell et al., 2010). The prevalence of mesocosm experiments in ecological research is increasing steadily due to their ability to capture ecosystem dynamics on a high organisational level and geochemical processes(Petersen et al., 2009; Riebesell et al., 2013; Stewart et al., 2013; Petersen and Kemp, 2019). Additionally, mesocosm experiments are characterised by a high degree of control, replication and repeatability (Riebesell et al.,2010; Petersen and Kemp, 2019).

One of the main ecological challenges addressed through mesocosm science is the consequences of climate change on marine and freshwater ecosystems(Stewart et al., 2013; Båmstedt and Wikner, 2016; Celussi et al., 2017; Mustaffa et al., 2020; Hébert et al., 2022) . One such example is the investigation of the effects of increased organic matter availability due to climate change on plankton communities in the Baltic Sea(Båmstedt and Wikner, 2016; Mustaffa et al., 2020). In oligotrophic regions of the Mediterranean, Celussi et al. (2017) employed offshore anchored mesocosms to determine the impact of ocean acidification on prokaryotic communities. As climate change is a global phenomenon, Hébert et al. (2022) demonstrated detrimental effects of freshwater salinisation on biodiversity in a coordinated mesocosm experiment across Europe and North America.

In addition to climate change, mesocosms have been used to address highly relevant challenges such as pollution, multiple stressors and ecological questions of high relevance(Pitta et al., 2016; Tsiola et al., 2018; Magiopoulos et al., 2020; Juvigny-Khenafou et al., 2021). For instance, in the eastern Mediterranean, Magiopoulos et al. (2020) demonstrated substantial detrimental effects of in situ oil burning on plankton communities using land based mesocosms. Similarly, Tsiola et al. (2018) noted the impacts of increasingly relevant silver engineered nanoparticle pollution on microbial plankton. In the freshwater environment, mesocosms in streams were used to investigate ecosystem service losses in benthic macroinvertebrates as a result of multiple pressures(Juvigny-Khenafou et al., 2021). Ecological questions addressed through mesocosm experiments include phosphorus cycling dynamics in the ultra-oligotrophic eastern Mediterranean and food web dynamics(Pitta et al., 2016; Degerman et al., 2018).

The above examples demonstrate the multidisciplinary character of mesocosm experiments and their ability to foster multinational cooperation(Riebesell et al., 2010). Finally, it is evident that mesocosm experiments are an effective tool in addressing current and future challenges in aquatic research.

1.5. Study rationale and aims

In the Baltic Sea, productive periods (spring bloom) are dominated by microplankton such as diatoms and dinoflagellates (Camarena-Gómez et al., 2021). The northern Baltic Sea is an oligotrophic, net-heterotrophic area(Algesten et al., 2004; Snoeijs-Leijonmalm and Andren, 2017), where respiration exceeds carbon fixation (Sandberg et al., 2004). Bacterial respiration represents the conversion of DOC into CO_2 and accounts for a significant proportion of community respiration (del Giorgio et al., 1997; Martínez-García et al., 2013). A fraction of this respiration is a result of mechanisms allocated to cell survival (maintenance energy) (Pirt, 1982) . Maintenance energy of bacteria shows inverse pattern along the trophic gradient, being higher in oligotrophic areas (del Giorgio and Cole, 1998). In the northwestern Bothnian Sea, maintenance respiration was a significant fraction of yearly bacterial respiration (Vikström and Wikner, 2019).

The significance of maintenance respiration was validated in a mesocosm experiment (PROMAC: Prokaryotic maintenance respiration, activities and structural adaptation at low temperature and starvation in the sub-Arctic), by manipulating bacterial specific growth rate through temperature and substrate manipulations. Within PROMAC,

the present thesis aimed to examine the influence of temperature and substrate manipulations on the higher trophic levels, and specifically on microplankton abundance and community structure. This was achieved through collection and analysis of water samples with the use of inverted microscopy. Associated measurements (temperature, salinity, and concentrations of nutrients and chlorophyll) were also collected for identifying explanatory variables.

In today's world, in view of physicochemical alterations, slight changes at the foundations of the food web may be magnified via food chains (Sarmento et al., 2010). Since microplankton is significant in its interactions within the planktonic microbial food wed, it is therefore important that it is studied in a changing ocean.

2. Materials and Methods

2.1. Experimental design

The mesocosm experiment took place at the Mesocosm Facility, Umeå Marine Sciences Centre (MF-UMSC), between March and April 2020 (05/3 – 03/4). MF-UMSC is located on the Swedish east coast, specifically the Gulf of Bothnia (63°33'52.8"N 19°49'53.2"E) (https://www.umu.se/en/research/infrastructure/mesocosm-facility/). Ice-covered brackish water was directly retrieved 800 m offshore the facility at a maximum depth of 5 m using standard polyethylene water pipes. Initially filtered through 2 mm pore size filters operating under the Bernoulli principle (Bernoullifilter, Sweden), water was then evenly distributed in 12 indoor insulated polyethylene mesocosms. Individual mesocosm dimensions are 4.86 m high with a diameter of 0.73 m, with an approximate total volume of 2 m^3 of water. Tank temperature was regulated by sensors (Honeywell AB, Sweden) while the light source was set to a 12-hour light – 12-hour dark cycle, mimicking the end of April daylight spectrum (Valoya R-258, Light DNA). Mixing within the mesocosm is achieved through convective stirring, 4-hour turnover in the present experiment, generated by the temperature control system (Båmstedt and Larsson, 2018).

Image 1. Indoor Mesocosms at the Umeå Marine Sciences Center (UMSC). Source: https://www.umu.se/en/research/infrastructure/mesocosm-facility/. Image by Markus Nordin.

For the evaluation of the central experimental goal and that of the present thesis, four experimental treatments were applied in triplicate during a 29 day (8 Sampling Days) fully factorial experiment. Control ('C') treatment was representative of winter conditions and no manipulations were applied. Labile dissolved organic matter (DOM) in the form of yeast extract (59 µmol C L^{-1} final concentration) was added to the Nutrients ('N') and Temperature and Nutrients ('TN') treatments on Sampling Day 0. Yeast extract additions continued throughout the course of the

experiment after each main sampling (8 additions). Temperature of the collected water (1 m depth) was 0°C, and that of the treatments 'C' and was set to 1°C thus representing winter conditions. A Temperature ('T') treatment was also established. In the 'TN' treatments, mesocosm temperature was set to 10°C and nutrients were added thus representing summer conditions. Additional treatments were nutrient addition at 1 ° C ('N') and temperature at 10°C ('T') creating a full orthogonal design. Sampling events, eight in total, occurred in the mornings (between 08:00 and 10:00) two times per week, specifically Mondays and Thursdays. A total of 25 L were sampled per mesocosm per event, using acid washed (1 mol dm^3 HCl) polycarbonate bottles of the same volume from a hose situated 1.55 m from the bottom of each mesocosm. Samples were subsequently transported to temperature-controlled rooms set to 1 °C ('C' and 'N' treatment samples) and 10 °C ('T' and 'TN' treatment samples). The removed volume was subsequently replaced the following day with 1 µm filtered water to maintain constant water levels in the tanks and prevent further entry of organisms. Sampling preceded yeast extract addition when the latter occurred.

2.2. Estimation of micro-plankton abundance and community composition

To study the microplankton community, 100 mL of water were obtained from each of the larger 25 L bottles on sampling days. This took place in the morning immediately after sample transportation to the temperature-controlled rooms. Sub-sampling bottles were rinsed once with mesocosm water prior to water collection. Shortly thereafter, sub-samples were equally divided in replicated 50 mL centrifuge tubes and fixed with acid Lugol's solution (elemental Iodine, Potassium iodide and acetic acid solution), at 1% final concentration. The replicates were carefully stored in a refrigerator (4°C) until further analysis in the lab at the Hellenic Centre for Marine Research (https://www.hcmr.gr/en/).

Within eighteen months of collection, 25 mL of each single replicate was placed in sedimentation chambers for 24 hours (Utermöhl, 1931). Cells were examined using an Olympus IX-70 inverted microscope (magnification x 150) equipped with a camera for image capturing (SPOT Basic, Version 5.1.3., SPOT™ Imaging Solutions). Diatoms, dinoflagellates, ciliates, ochrophyta, chlorophyta and other taxa were counted and identified to family and subsequently genus level when possible.

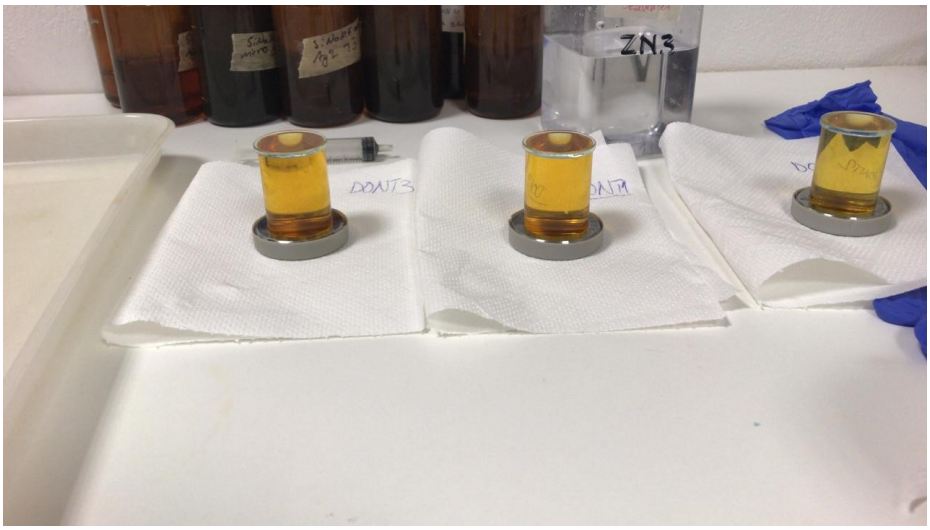

Image 1. Sedimentation chambers (Utermöhl, 1931), **Hydro-Bios Apparatebau GmbH.**

2.3. Estimation of relevant abiotic parameters

Relevant abiotic parameters were estimated in situ or through the collection of water samples from mesocosms two times per week during sampling events. The concentration of O_2 in μM, CDOM (Coloured Dissolved Organic Matter) in μgL^{-1}, temperature and salinity were measured in situ with a CTD (Conductivity, Temperature, Depth) instrument (Seabird SBE 19 plus, Seabird Electronics, Washington, USA). Water samples for the determination of Total Dissolved Nitrogen (TDN) and Total Dissolved Phosphorus (TDP) were collected and stored in the dark at 4°C prior to analysis. TDN and TDP concentrations were determined using a four-channel auto analyser (Quattro marine; Bran and Luebbe®, Sweden) according to standard protocols of the Umeå Marine Sciences Centre and Grasshoff et al., (1999). Chlorophyll a (Chl a) concentration was determined fluorometrically, following ethanol extraction, as per Reigstad et al., (2002) with a Perkin Elmer LS 30 Luminescence spectrometer.

2.4. Statistics

Differences between treatments (C, N, T, TN) during the course of the experiment in examined variables were tested using repeated measures analysis of variance (RM ANOVA). One-way analysis of variance (one-way ANOVA) was used to test for differences between treatments in specific variables on particular experimental days. To examine statistically significant differences between treatments, post hoc Tukey tests (Tukey HSD) were applied. Prior to running the analyses assumptions of sphericity (Mauchly's test) and homogeneity of variance (Levene's test) were verified for RM ANOVA and one-way ANOVA respectively. RM and one-way ANOVA were carried out with IBM SPSS Statistics (v.23) (IBM Corp., USA). Data was introduced as total sum of each examined variable per mesocosm.

The effects of experimental factors 'time' and 'treatment' including the interaction of the two factors on micro-plankton taxa community composition were tested using permutational multivariate analysis of variance

(PERMANOVA) with two factors. Biotic data (abundance) was square root transformed, and a resemblance matrix was constructed using Bray-Curtis similarity. The null hypothesis, no differences in community composition throughout the experiment, was tested using 999 permutations. Within major taxonomic units, Principal Coordinate analysis was applied, to discern variation in community composition based on applied treatments among samples. Within major taxonomic units, a two-way analysis ('time', 'treatment') of similarity percentages and taxon contribution (SIMPER) was applied on sample data. Diversity indices, Shannon diversity index and Pielou's evenness, were calculated per sample for each major taxonomic unit (Pielou 1966; Shannon and Weaver, 1948).

Abiotic drivers of micro-plankton community composition were determined through distance linear models, with results plotted as a distance-based redundancy analysis (dbRDA). Abiotic data were normalised, and a similarity matrix based on Euclidian distance was constructed. Community composition data including abiotic drivers were analysed using PRIMER 6 (v 6.1.16) with PERMANOVA + software (v. 1.0.6) (PRIMER-E Ltd, Plymouth Marine Laboratory, Natural Environmental Research Council, UK) (Anderson et al., 2008; Clarke and Gorley, 2006).

3. Results

3.1. Brief description of micro-plankton community in the mesocosms

The microplankton community in the mesocosms, as identified through microscopy, is primarily comprised of diatoms, dinoflagellates and ciliates. Representative genera were identified in each of these taxa and were present in all samples in proportionately high abundances. Briefly, in diatoms these include the genera *Skeletonema* and *Chaetoceros*, in dinoflagellates the order *Gymnodiniales* and the genus *Peridiniella* and in ciliates the genera *Strombidium*, *Strobilidium, Mesodinium* and *Lohmaniella*. Other notable taxa include the phyla *Ochrophyta* and *Chlorophyta*, primarily represented by the genera *Dynobryon* and *Monoraphidium*. Further taxa were identified at considerably lower abundances and are referred to as 'Other taxa' herein. Within this section, significant differences in total abundance and community composition of the taxa mentioned above are explored in detail. Additionally, abiotic drivers on taxa community are identified.

3.2. Diatoms

3.2.1. Abundance of diatom community

Under increased temperature (T, TN), diatom abundance remained low.

Under sole nutrient enrichment (N), diatom abundance increased mildly towards the end of the experiment.

Significant differences in total diatom abundance were noted between controls (C) and all other treatments (RM ANOVA, $F(3) = 16.7$). Specifically, total diatom abundance was higher in C compared to all other treatments (Tukey HSD test, $p < 0.05$). As seen in Fig. 2, diatom abundance appeared to follow a similar pattern across all treatments up to D4, remaining at levels between 26560 cells L^{-1} and 322907 cells L^{-1}. From D5, diatom abundance at C continued to increase until the end of the experiment, reaching approximately 8 times higher abundances compared to the beginning. Diatom abundance remained relatively stable in N until D8, whilst experiencing a peak and crash after D6 in T and TN falling to 24242 cellsL^{-1} and 104061 cellsL^{-1}, respectively.

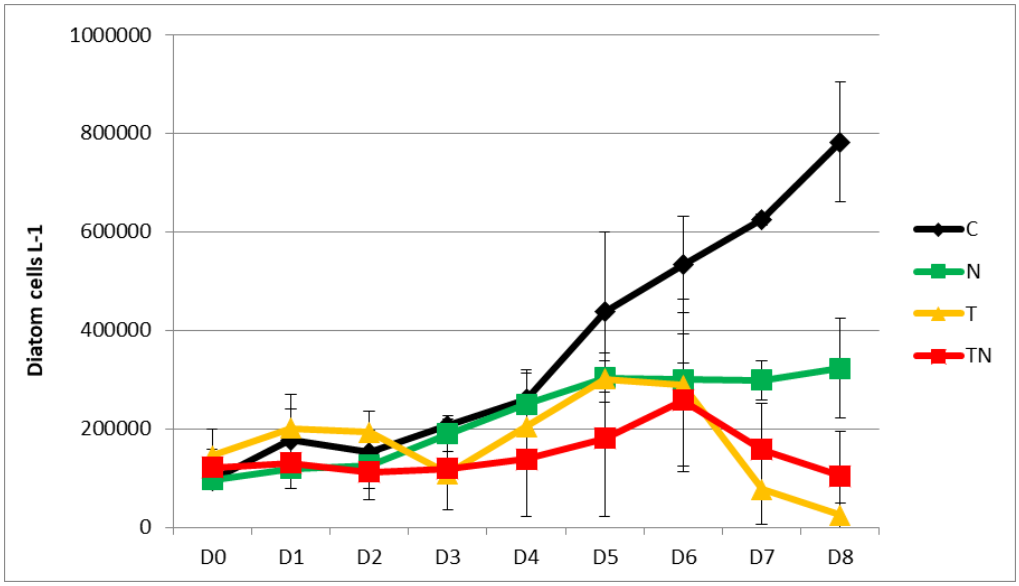

Figure 2. Mean diatom abundance in all treatments across all days of the experiment. Data is based on triplicate counts (three mesocosms per treatment). C corresponds to controls, N to nutrient addition in the mesocosms, T to mesocosms with temperature set to 10°C and TN to mesocosms that received nutrients whilst having their temperature set to 10°C.

3.2.2. Community composition of diatoms

Increased temperature triggered most of the differences in diatom community composition, while nutrient enrichment was not adequate to trigger large differences.

Diatom evenness did not follow a significantly different pattern between controls and treatments (data not shown, RM ANOVA $F(3) = 2.8$, $p < 0.05$). Shannon- Wiener diversity index values for the diatom community were significantly different between controls and T, and between N and treatments T and TN (data not shown, RM ANOVA $F(3) = 7.6$, $p<0.05$). According to PERMANOVA results, the interaction of the experimental factors 'time' and 'treatment' resulted in significant differences in diatom community composition patterns (PERMANOVA, pseudo-$F=2.79$, $p < 0.05$). The highest degree of diatom community dissimilarity was observed between controls (C) and T (29.14, SIMPER) with *Skeletonema* contributing to this by almost 44%, whilst the lowest degree of dissimilarity was recorded between controls (C) and N (14.65, SIMPER), with *Skeletonema* accounting for 39.60 % of the differences.

As seen in Fig. 3 the genera *Skeletonema* sp. and *Chaetoceros* sp. cumulatively accounted for approximately 90% of the relative diatom abundance across all treatments and days. Overall, *Chaetoceros* sp. did not exceed 30% of relative diatom abundance with the exception of two occasions. Specifically, on D3, treatment T, *Chaetoceros* sp. relative abundance exceeded 40% and on D8, treatment TN, was approximately 40%. Despite the dominance of the two genera, *Skeletonema* sp. *and Chaetoceros* sp.*,* with regard to relative abundance, a total of 31 diatom genera were identified in the samples.

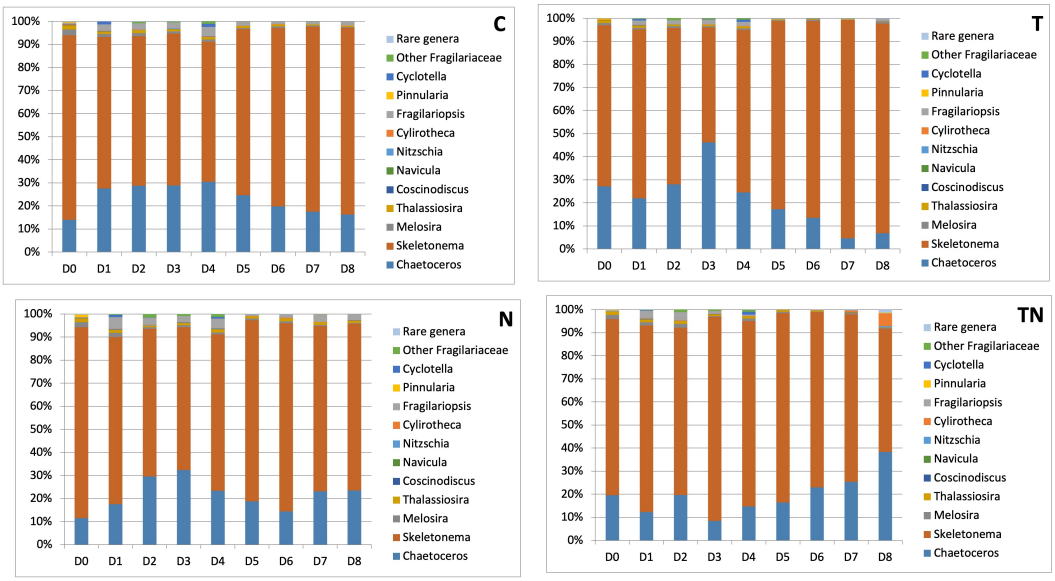

Figure 3. Diatom community composition. Relative abundance of diatom genera over the course of the experiment in all treatments. Rare genera include: *Pseudo-Nitzschia, Grammatophora, Fragilaria, Leptocylindrus, Synedra, Thalassionema, Stauroneis, Amphora, Gomphonemopsis, Licmophora, Peronia, Achnanthes, Aulacoseira, Ctenophora, Actinocyclus, Amphipleura, Stephanodiscus, Rossithium, Amphiprora, Tabularia*. Data is based on triplicate counts (three mesocosms per treatment). C corresponds to controls, N to nutrient addition in the mesocosms, T to mesocosms with temperature set to 10°C and TN to mesocosms that received nutrients whilst having temperature set to 10°C.

3.2.3. Abiotic drivers of diatom community

Diatom community composition patterns were significantly associated with O_2 concentration, Chl a, TDN, TDP concentration (R^2 = 0.32, pseudo-F = 4.36, p< 0.05). Temperature was a significant driver in community composition when considered separately from other parameters (pseudo-F = 18.12, p<0.05) but did not contribute significantly to the model. Nonetheless, the best model solution (selection criteria: R^2, selection procedure: forward) included all parameters mentioned above (R^2 = 0.37). According to this, all 5 parameters cumulatively accounted for 36.64% in diatom community variation (Fig. 4).

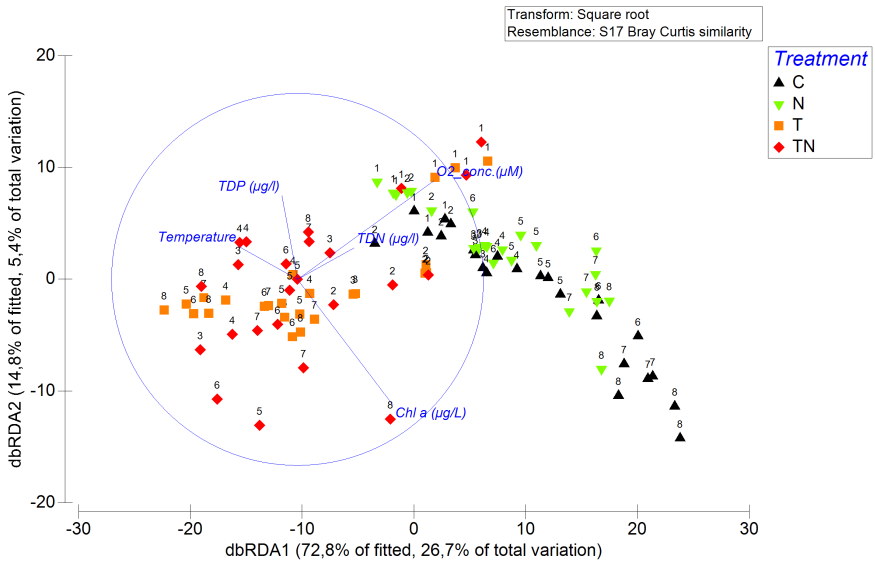

Figure 4. Distance based linear mode for diatom communities. Results are presented as a distance-based redundancy analysis (dbRDA) plot. All parameters contributing to the linear model are shown, specifically Temperature, Salinity, TDP; Total Dissolved Phosphorus (μgL^{-1}), O2 concentration (μM), TDN; Total Dissolved Nitrogen (μgL^{-1}); CDOM; Coloured Dissolved Organic Matter (μgL^{-1}), Chl a; Chlorophyll a (μgL^{-1}). Positive/negative association of parameters and relative influence on community composition denoted through vector length and direction respectively. Community similarity is based on Bray-Curtis index of square root transformed abundance data. Control samples in black, N samples in green, T in orange and TN in red. Numbers above symbols denote experimental day.

3.3.Dinoflagellates

3.3.1 Abundance of dinoflagellate community

Under increased temperature (T, TN), dinoflagellate abundance peaked mildly and shortly in the middle of the experiment

Under sole nutrient enrichment (N), dinoflagellate abundance increased but did not differ from control mesocosms.

Significant differences in total dinoflagellate abundance were noted between controls (C) and T (RM ANOVA, $F(3) = 12.49$). Specifically, dinoflagellate abundance was higher in C compared to T (Tukey HSD test, $p < 0.05$). Likewise, total dinoflagellate abundance in C was significantly different from TN with higher abundance in C (Tukey HSD test, $p < 0.05$). Additionally, total dinoflagellate abundance differed significantly between N and treatments T and TN individually (RM ANOVA, $F(3) = 12.49$).

As seen in Fig. 5, dinoflagellate abundance appeared to follow two patterns (treatments C, N and treatments T, TN) during the experiment. On D0, dinoflagellate abundance in C was approximately 2 times higher than all other treatments, declining on D1 up to D3, remaining at levels between 5840 cellsL^{-1} and 13520 cellsL^{-1}. From D4, dinoflagellate abundance almost doubled at C and quadrupled at N compared to D3, before reaching a plateau up to D6 in both treatments. On D6 abundance of dinoflagellates in C experienced a decline, falling to 14631 cellsL^{-1} on D8 and crashed in N reaching 10827cells L^{-1} on D8. In T, after a first decrease, the peak in dinoflagellate abundance was observed at D4 followed by a crash, falling to 2413 cells L^{-1} on D8. In TN, this peak occurred on D5 followed by a crash until D7 and a recovery to 11640 cells L^{-1} on D8.

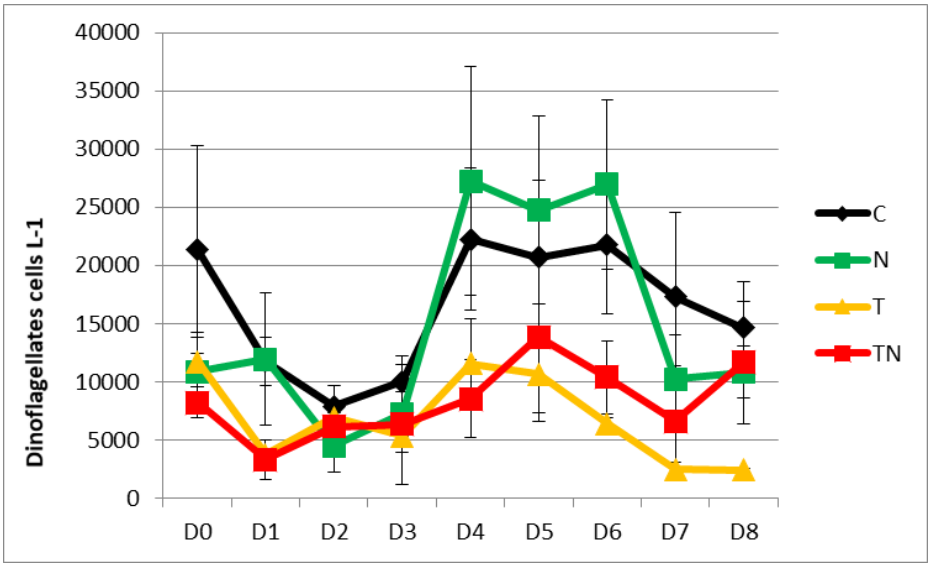

Figure 5. Mean dinoflagellate abundance in all treatments across all days of the experiment. Data is based on triplicate counts (three mesocosms per treatment). C corresponds to controls, N to nutrient addition in the mesocosms, T to mesocosms with temperature set to 10 °C and TN to mesocosms that received nutrients whilst having their temperature set to 10°C.

3.3.2. Community composition of dinoflagellate community

Increased temperature triggered most of the differences in dinoflagellate community composition

Dinoflagellate community evenness presented significantly different patterns between treatments (data not shown, RM ANOVA, (F3) = 17.1, $p<0.05$). The diversity index (Shannon) of the dinoflagellate community did not show significantly different patterns between treatments (data not shown, RM ANOVA, F (3) = 0.9, $p>0.05$). The significant effect of the interaction between experimental factors 'time' and 'treatment' on dinoflagellate community composition is confirmed by PERMANOVA results (pseudo-F = 2.9, $p<0.05$). According to Principal Coordinates analysis, temperature setting at 10°C in mesocosms (treatments T and TN) accounts for 35.5% of total variation between samples in the dinoflagellate community.

The highest degree of dinoflagellate community dissimilarity was observed between treatments N and T (35.7 average dissimilarity, SIMPER), whilst the lowest degree of dinoflagellate community dissimilarity was observed between treatments C and N (24.6 average dissimilarity, SIMPER). On both occasions, the two primary genera contributing to the dissimilarities include unidentified genera of the order *Gymnodiniales* (size class 1, <20 µm) and the genus *Peridiniella* (40.7% and 35.8 % cumulative contribution towards dissimilarity respectively).

As seen in Fig. 7 the taxa *Gymnodiniales* (size class 1, <20 µm and size class 2, >20 µm) and *Peridiniella* cumulatively exceeded 70% of relative dinoflagellate abundance in all treatments and experimental days. In C, the two taxa exceeded 90% of relative dinoflagellate abundance on all experimental days excluding D1. On D1, the two taxa contributed to dinoflagellate relative abundance by 85%. The taxa *Gymnodiniales* (size class 1, <20 µm and size class 2, >20 µm) and *Peridiniella* represented more than 90% of dinoflagellate abundance in treatments N, T and TN

on D0, D2, D3. In treatments N and TN this was also observed on D5. Additionally, the two taxa exceeded 90% of relative abundance in N on D6 and in TN on D8. Most notably, in treatments T, N and TN, especially in T there was an increase in rare taxa contribution to relative abundance from D5 onwards until the end of the experiment. The two most abundant taxa classed as rare in this study due to low abundances include the genera *Gyrodinium* sp. and *Peridinium* sp.. An increase in *Peridiniella* sp.,contribution was seen from the beginning towards the end of the experiment in C and N, while in T and TN this increase was observed towards the middle of the experiment. Rare taxa contribution is increased towards the end of the experiment in T and TN mostly, reaching 10% in the final two days of the experiment. In Fig.7 only members of the order *Gymodiniales* of uncertain taxonomy are included in this taxon, hence *Gymnodinium* sp. are presented separately.

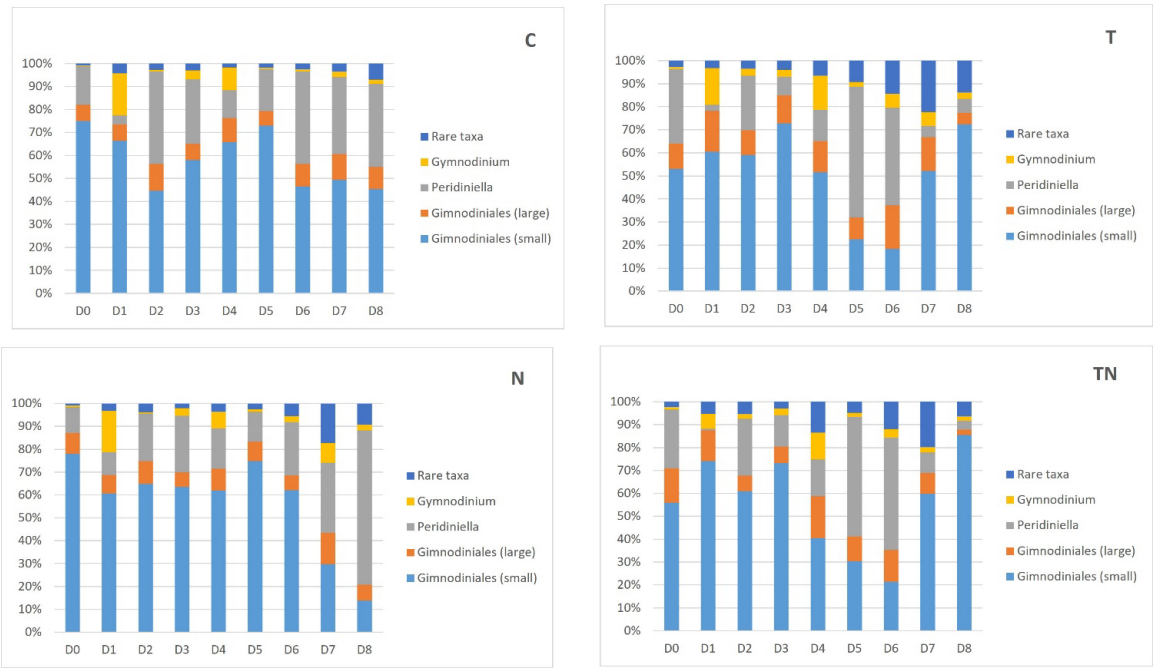

Figure 7. Community composition of dinoflagellates. Relative abundance of dinoflagellate genera over the course of the experiment in all treatments. Genera: *Gymnodinium, Peridiniella*. Order*: Gymnodinialles, Rare taxa: Gyrodinium, Karenia, Gonyaulax, Peridinium, Protoperidinium, Aureodinium, Cochlodinium, Ceratium, Protoceratium, Oxytoxum, Prorocentrum, Heterocapsa, Dinocyst, Karlodinium, Dinophysis, Scrippsiella, Amphidinium, Woloszynskia, Alexandrium, Amphidiniopsis, Peridiniopsis, Phalacroma, Preperidinium, Akashiwo, Apocalathium, Dicroerisma, Pentapharsodinium, Micracanthodinium, Sclerodinium, Diplopsalis, Achradina, Podolampas, Amyloodinium, Pyrocystis, Actiniscus, Other dinoflagellates.* Data is based on triplicate counts (three mesocosms per treatment). C corresponds to controls, N to nutrient addition in the mesocosms, T to mesocosms with temperature set to 10°C and TN to mesocosms that received nutrients whilst having their temperature set to10°C.

3.3.3. Abiotic drivers of dinoflagellate community

Dinoflagellate community composition patterns were significantly associated with Temperature, Chl a and O_2 concentration (R^2 =0.18, pseudo-F = 5.05, p<0.05). Total Dissolved Nitrogen (TDN) concentration was significant as an individual driver of variation (pseudo-F = 2.6, p<0.05) but did not contribute significantly to the model. Total Dissolved Phosphorus (TDP) concentration was not significantly associated with dinoflagellate community

composition patterns (pseudo-F = 1.6, p>0.05). However, all five abovementioned parameters were included in best distant linear model solution (selection criteria: R^2, selection procedure: forward, R^2= 0.24). According to model results, 23.6% of total variation between samples in the dinoflagellate community can be explained by the cumulative effect of the five model parameters (Fig. 8). Additionally, samples form two distinct clusters on the basis of temperature (treatments T, TN and treatments C, N) and dinoflagellate community structure was also explained by nutrient availability.

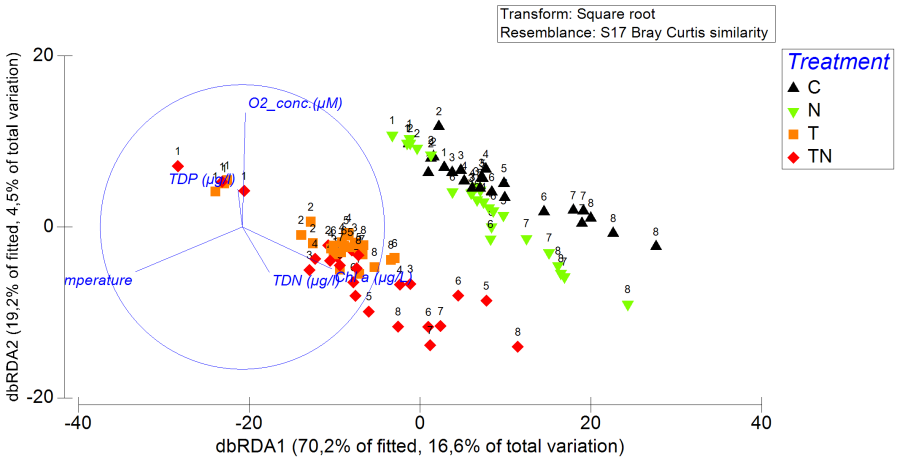

Figure 8. Distance based linear mode for dinoflagellate communities. Results presented as a distance-based redundancy analysis (dbRDA) plot. All parameters contributing to the linear model are shown, specifically Temperature, Salinity, O2 concentration (µM), Chl a; Chlorophyll a (µgL^{-1}). Positive/negative association of parameters and relative influence on community composition denoted through vector length and direction respectively. Community similarity based on Bray-Curtis index of square root transformed abundance data. Control samples in black, N samples in green, T in orange and TN in red. Numbers above symbols denote experimental day.

3.4.Ciliates

3.4.1. Abundance of ciliate community

Under increased temperature (T, TN), ciliate abundance increased in the middle of the experiment but crashed rapidly

Under sole nutrient enrichment (N), ciliates thrived in abundance until the end of the experiment

Significant differences in total ciliate abundance were noted between controls (C) and N (RM ANOVA, F (3) =4.6, p<0.05) and between N and TN (One-way ANOVA, F (3) = 135.5). Specifically, total ciliate abundance was higher in N compared to C, T and TN (Tukey HSD test, p < 0.05).). On D5, significant differences in ciliate abundance were observed between controls and T (One-way ANOVA, F (3) = 4.2). In detail, total ciliate abundance was lower in C

compared to T on that day (Tukey HSD test, $p < 0.05$). On D8, total ciliate abundance was significantly different in controls compared to all other treatments (One-way ANOVA, $F(3) = 135.5$).

As seen in Fig. 9, ciliate abundance appeared to follow a similar pattern across all treatments up to D2, remaining at levels between 133 cells L^{-1} and 2840 cellsL^{-1}. From D3, ciliate abundance at N continued to increase until the end of the experiment, reaching approximately 17 times higher abundances compared to the beginning. Ciliate abundance in C constantly increased until D8, whilst experiencing a peak and crash in treatments TN and T respectively with a time lag of one experimental day. To be specific, in TN, the peak in ciliate abundance was observed at D4 followed by a crash immediately after, falling to 2173 cells L^{-1}. In T, this peak occurred at D5 followed by a crash immediately after, falling to 453 cells L^{-1}. The decline in the T and TN treatments match with a higher abundance of mesozooplankton biomass in these treatments than the C or N treatments, as determined at D8 (data not shown).

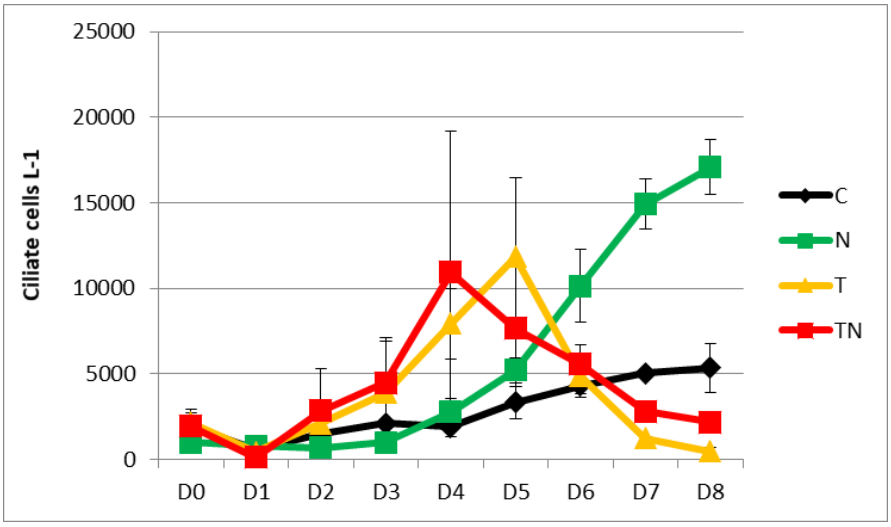

Figure 9 Mean ciliate abundance in all treatments across all days of the experiment. Data is based on triplicate counts (three mesocosms per treatment). C corresponds to controls, N to nutrient addition in the mesocosms, T to mesocosms with temperature set to 10°C and TN to mesocosms that received nutrients whilst having their temperature set to 10 °C.

3.4.2. Community composition of ciliate community

Increased temperature triggered most of the differences in ciliate community composition, but also nutrient enrichment led to high dissimilarity between mesocosms

Ciliate evenness and Shannon diversity indices did not present significantly different patterns between controls and treatments (data not shown, RM ANOVA $F(3) = 3.1$, $p>0.05$ and RM ANOVA $F(3) = 3.8$, $p> 0.05$, respectively).

The significant effect of the interaction between experimental factors 'time' and 'treatment' on ciliate community patterns was confirmed by PERMANOVA results (pseudo-$F = 2.8$, $p< 0.05$). According to Principal Coordinates analysis, 57.2 % of total variation (cumulative for 2 axes) between samples in the ciliate community was explained

by temperature setting at 10°C in mesocosms (treatments T and TN). The highest degree of ciliate community dissimilarity was observed between treatments N and T (47.5 average dissimilarity, SIMPER) with the genera *Strombidium* and *Lohmaniella* cumulatively contributing towards this by approximately 44%. The lowest degree of ciliate community dissimilarity was observed between treatments C and N (37.8 average dissimilarity, SIMPER) with the genera *Strombidium* and *Strobilidium* cumulatively contributing towards this by approximately 38%.

As seen in Fig 11, above 60% of relative ciliate abundance in treatments T, N and TN for all days can be accounted for by 4 genera (*Lohmaniella, Mesodinium, Strombidium, Strobilidium*). Except for D1 and D6, the same is true of relative ciliate abundance in C. On D1 and D6 the cumulative contribution of the four genera to ciliate relative abundance is approximately 55% and 60% respectively. Irrespective of treatment and experimental day, the four genera did not contribute equally to the abovementioned sum. For instance, in C on D2 and D3 *Strobilidium* accounted for the greater part of the sum. In T on D5 and D6 *Lohmaniella* accounted for this. *Stombidium* accounted for the greater part of the sum on multiple experimental days in N including D6, D7 and D8. In TN, the greater part of the sum was mostly accounted for by *Lohmaniella* (D2, D3, D4, D5). In treatments T and TN on D5 and D6 respectively *Lohmaniella, Mesodinium, Strombidium* and *Strobilidium* cumulatively accounted for approximately 90% of relative ciliate abundance. In C and N, the highest cumulative contribution of these genera occurred on D2 and D1 respectively and in both cases amounted to approximately 85%. Other noteworthy taxa included the family *Tintinnidae* whose contribution to ciliate relative abundance consistently exceeded 10% in C and N on all experimental days except for D1 in N (approx. 5%). In treatments T and TN *Tintinnidae* contribution is lower, whilst the contribution of unidentified genera reached a maximum of approximately 10% on D0, D1, D6-D8 (T) and D0,D5-D8 (TN). Additionally, a rise in *Strombidinopsis* contribution was observed exclusively in N from D5 until the end of the experiment.

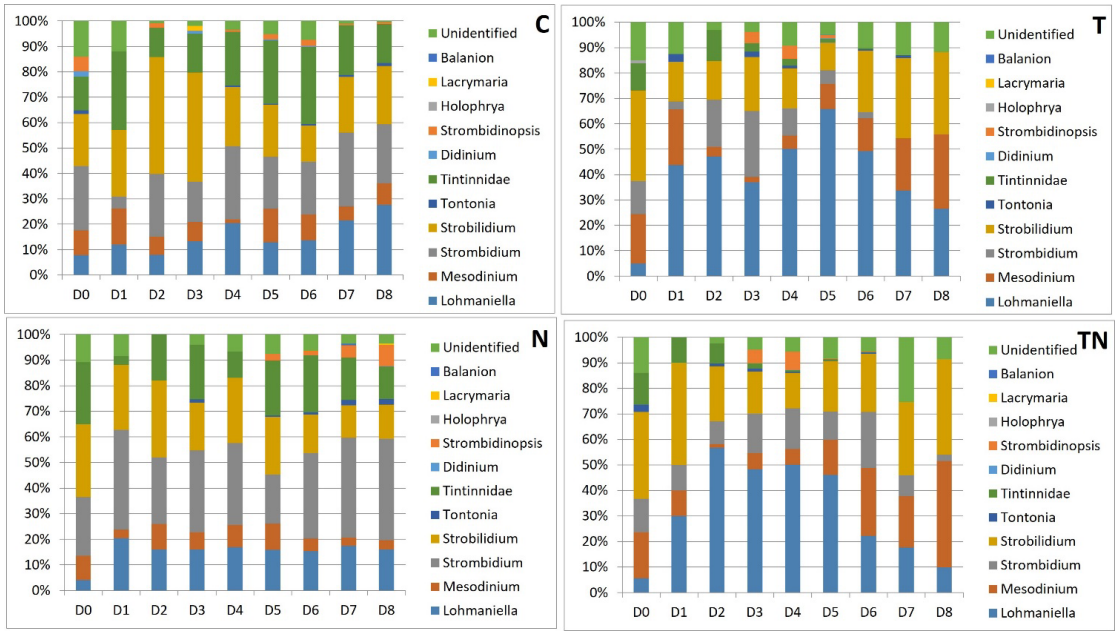

Figure 11. Community composition of ciliates. Relative abundance of ciliate taxa over the course of the experiment in all treatments. C corresponds to controls, N to nutrient addition in the mesocosms, T to mesocosms with temperature set to 10°C and TN to mesocosms that received nutrients whilst having their temperature set to10°C. Data is based on triplicate counts (three mesocosms per treatment). Genera: *Balanion, Lacrymaria, Holophrya, Stombidinopsis, Didinium, Tontonia, Strobilidium, Strombidium, Unidintified, Family: Tintinnidae*

3.4.3. Abiotic drivers of ciliate community

Ciliate community composition patterns were significantly associated with O_2 concentration, Chl a concentration and temperature, hence all three parameters were included in the best distant linear model solution (selection criteria: R^2, selection procedure: forward, R^2= 0.23). According to model results 22.51% of total variation between samples in the ciliate community was explained for by the cumulative effect of the three model parameters (Fig.12). As seen in Fig. 12, samples form two distinct clusters on the basis of temperature setting at 10°C in mesocosms (treatments T, TN and treatments C, N).

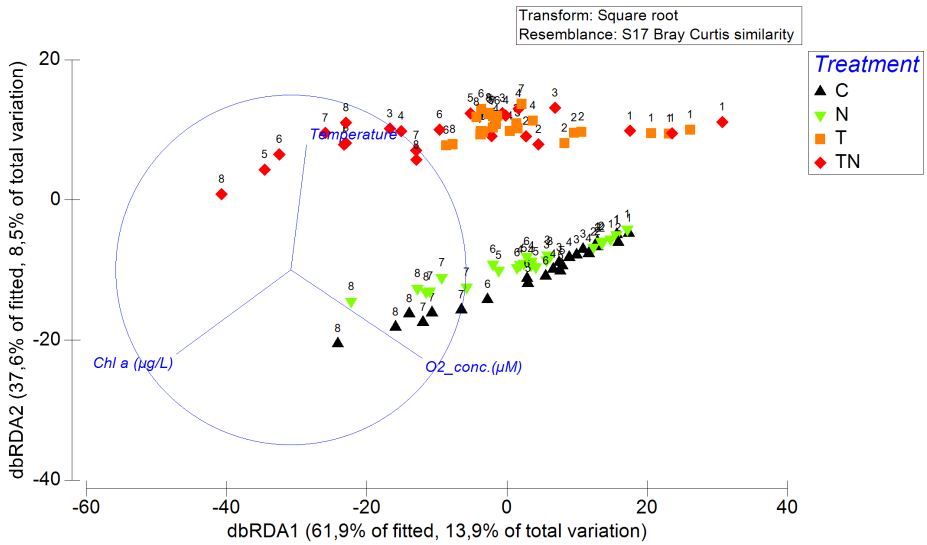

Figure 12. Distance based linear mode for ciliate communities. Results presented as a distance-based redundancy analysis (dbRDA) plot. All parameters contributing to the linear model are shown, specifically Temperature, Salinity, O_2 concentration (µM), Chl a; Chlorophyll a (µgL^{-1}). Positive/negative association of parameters and relative influence on community composition denoted through vector length and direction respectively. Community similarity based on Bray-Curtis index of square root transformed abundance data. Control samples in black, N samples in green, T in orange and TN in red. Numbers above symbols denote experimental day.

3.5.Ochrophyta

3.5.1. Ochrophyta abundance

No significant differences in total ochrophyta abundance were noted between controls (C) and all other treatments (RM ANOVA, F (3) = 9.7) presumably due to the large variation between C replicates on D8. Significant differences in total ochrophyta abundance were observed between N and T (RM ANOVA, F (3) = 9.7). Specifically, total ochrophyta abundance was higher in N compared to T (Tukey HSD test, $p < 0.05$). Additionally, ochrophyta abundance in N differed significantly from that in TN, being higher in the former (Tukey HSD test, $p < 0.05$).

As seen in Fig 13, ochrophyta abundance appeared to follow a similar pattern across all treatments up to D4, similarly to diatoms, remaining at levels between 40 cellsL^{-1} and 2120 cellsL^{-1}. From D5, ochrophyta abundance at treatments C and N continued to rise until the end of the experiment reaching approximately 30 times higher abundances compared to the beginning for C and approximately 50 times higher abundances compared to the beginning for N. Ochrophyta abundance in T and TN increased to a lesser extent compared to the two other treatments until the end of the experiment.

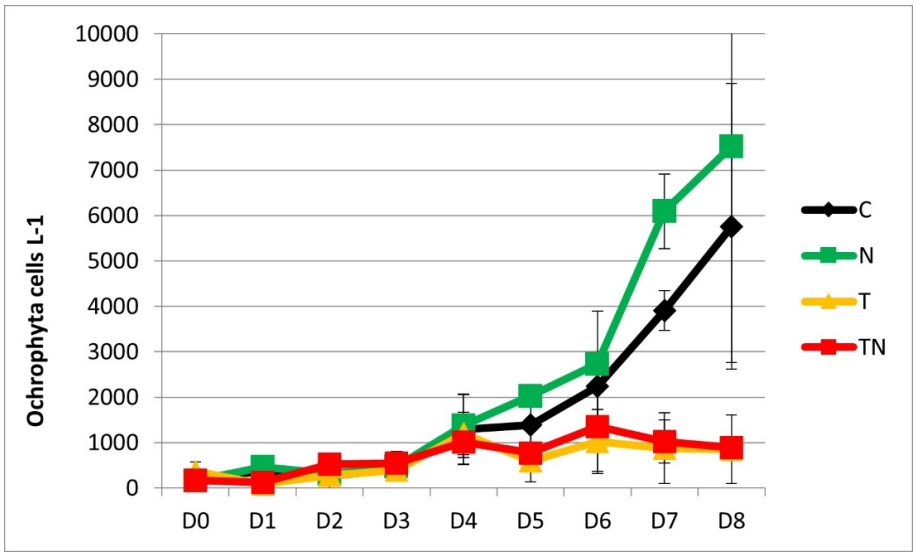

Figure 13. Mean ochrophyta abundance in all treatments across all days of the experiment.

3.5.2. Community composition of ochrophyta community

Significant effects on ochrophyta community composition due to interaction of experimental factors 'time' and 'treatment' are observed (PERMANOVA, Pseudo-F = 1.8, $p < 0.05$). As seen in Fig 14, the genus *Dinobryon* consistently composed more than 90% of relative ochrophyta abundance in C. The same is true in N treatment, with a single exception, D3. On D3 in N the genus *Chrysococcus* and rare ochrophyta genera approximately equally contributed to 20% of ochrophyta relative abundance. In both T and TN, the contribution of *Dinobryon* to ochrophyta relative abundance exceeded 90% on D0. In T this is also observed on D7 and D8 whilst in TN it is also

noted on D1. On D1 through to D6 in T and D2 through to D8 in TN the contribution of other genera to ochrophyta relative abundance ranged between 10% (D2, D6, both treatments) and 45% (D4, both treatments).

The contribution of the genera *Gonyostomum, Chromulina, Chrysococcus* and rare genera to this range was different between the treatments T and TN. Specifically on D2, *Gonyostomum* and *Chromulina* equally contributed to remaining relative abundance in T, whilst in TN relative abundance of the two genera was lower compared to T and *Chrysococcus* was present. On D6 in T, *Chrysococcus* and *Chromulina* were present, and the relative abundance of rare genera was lower compared to TN where the two genera were absent. Moreover, on D4, the contribution of rare genera and *Chromulina* respectively to remaining relative abundance was similar in both treatments, however the contribution of *Gonyostomum* was higher TN, whilst that of *Chrysococcus* in higher in T.

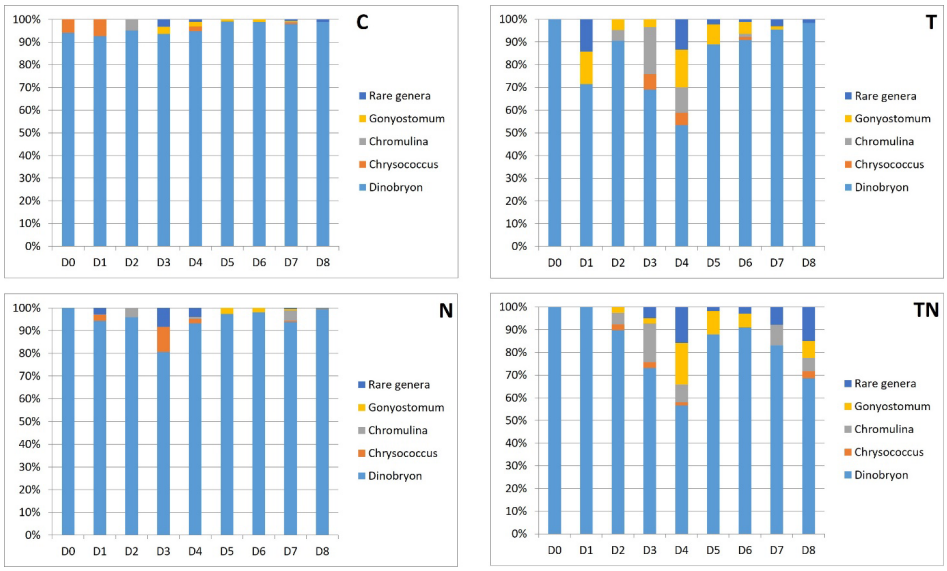

Figure 14. Community composition of ochrophyta. Relative abundance ochrophyta taxa over the course of the experiment in all treatments. C corresponds to controls, N to nutrient addition in the mesocosms, T to mesocosms with temperature set to 10°C and TN to mesocosms that received nutrients whilst having their temperature set to 10°C. Data is based on triplicate counts (three mesocosms per treatment). Genera*: Dinobryon, Chrysococcus, Chromulina, Gonyostomum.and* Rare genera.

3.5.3. Abiotic drivers of ochrophyta community

Winter conditions (C,N samples) are not clearly differentiated from summer conditions (T,TN samples)

Ochrophyta community composition patterns were significantly associated with Chl a concentration, temperature, O_2 concentration and salinity (R^2 = 0.28, pseudo-F = 3.63, p< 0.05). TDN and CDOM concentrations were significant drivers of ochrophyta community composition patterns individually (pseudo-F = 6.08, p<0.05, pseudo-F = 6.91, p<0.05 and respectively) but did not contribute significantly to the linear distance model. TDP concentration was not a significant driver of variation in the ochrophyta community. However, all seven abovementioned parameters were included in the best distant linear model solution (selection criteria : $R^{2,}$ selection procedure : forward, R^2=

0.34). According to the model outcome 34.38% of total variation between samples in the ochrophyta community could be explained by the cumulative effect of the seven model parameters (Fig. 15).

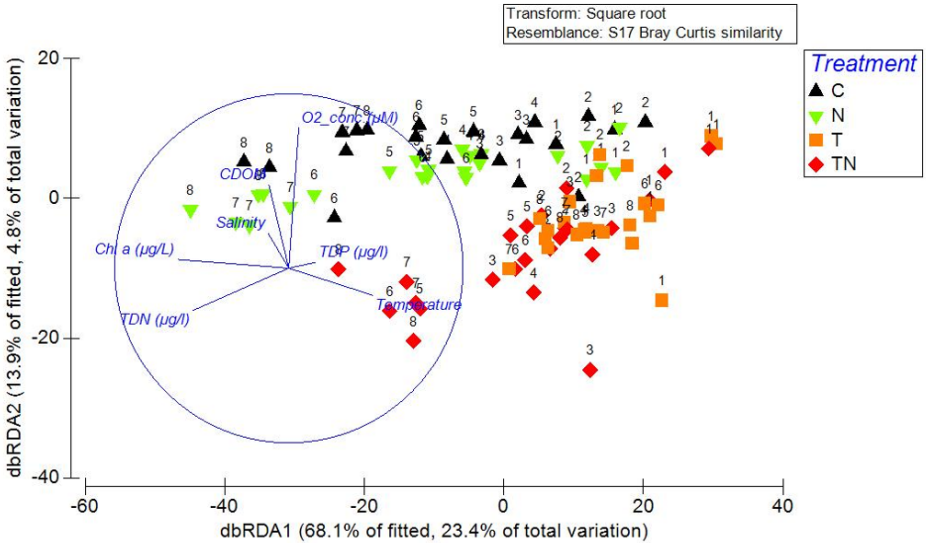

Figure 15. Distance based linear mode for ochrophyta communities. Results presented as a distance-based redundancy analysis (dbRDA) plot. All parameters contributing to the linear model are shown, specifically Temperature, Salinity, TDP; Total Dissolved Phosphorus (µgL^{-1}), O2 concentration (µM), TDN; Total Dissolved Nitrogen (µgL^{-1}) ; CDOM; Coloured Dissolved Organic Matter (µgL^{-1}),Chl a; Chlorophyll a (µgL^{-1}). Positive/negative association of parameters and relative influence on community composition denoted through vector length and direction respectively. Community similarity based on Bray-Curtis index of square root transformed abundance data. Control samples in black, N samples in green, T in orange and TN in red. Numbers above symbols denote experimental day.

3.6. Chlorophyta

3.6.1. Abundance of chlorophyta community

Temperature and nutrient manipulations did not affect chlorophyta abundance

No significant differences in total chlorophyta abundance were noted between controls (C) and all other treatments (RM ANOVA, F (3) =0.3). Nonetheless, chlorophyta abundance on D2 was significantly different between C and T and between N and T (One-way ANOVA, F (3) = 8.7). Chlorophyta abundance in C and in N was lower compared to T (Tukey HSD test, $p < 0.05$). As seen in Fig. 16 chlorophyta abundance in C peaked on D5 and thereafter remained relatively stable until the end of the experiment. In N chlorophyta abundance rose steadily after D1, peaking on D6 and subsequently crashed until D8, falling to 45111 cellsL^{-1}. Chlorophyta abundance in treatments T and TN followed a similar pattern of two consecutive peaks and crashes, terminating in a peak on D8. The initial peak in chlorophyta abundance in T occurred on D2 followed by a relatively steady decline up to D5. Chlorophyta abundance in TN initially peaked on D3, one experimental day later than in T. Both treatments T and TN experienced their second peak in chlorophyta abundance at D6, crashing up to D7 and recovering on D8.

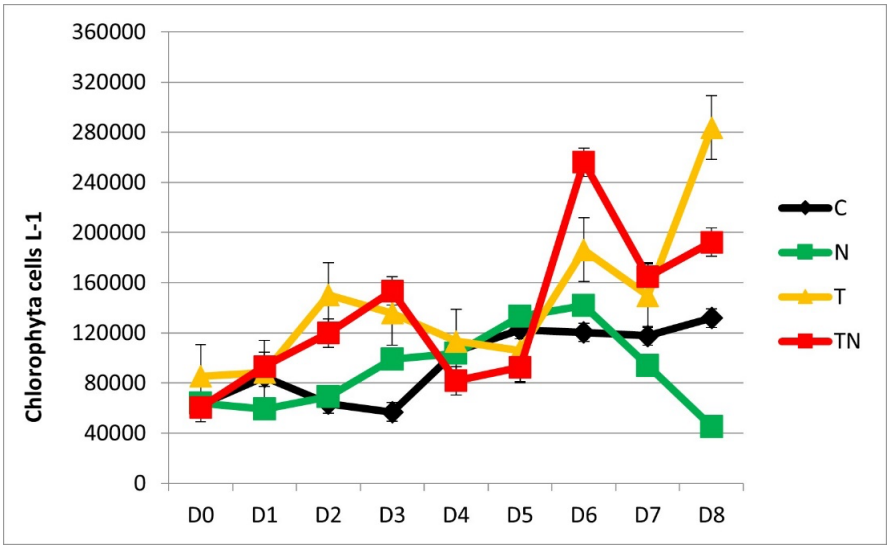

Figure 16. Mean chlorophyta abundance in all treatments across all days of the experiment.

3.6.2. Community composition of chlorophyta community

A single genus largely composed the chlorophyta community during the experiment.

Chlorophyta community evenness and Shannon diversity indices did not present significantly different patterns between controls and treatments (RM ANOVA F (3) = 0.7 and RM ANOVA F (3) = 0.9, respectively). No significant effect on chlorophyta community composition due to the interaction of the experimental factors 'time' and 'treatment' was observed (PERMANOVA, Pseudo-F = 1, $p>0.05$). However, the experimental factor 'treatment' appeared to have a significant effect on community composition (PERMANOVA, Pseudo-F = 4.7,$p< 0.05$). Most notably, a single genus, *Monoraphidium*, accounted for more than 98% of chlorophyta relative abundance in all treatments and days. Other notable genera, include *Scenedesmus, Desmodesmus* and *Oocystis* as their cumulative contribution to chlorophyta exceeded 1% on D1 in treatments C,N and TN. On D1 in T the three genera contributed approximately 0.4% of chlorophyta relative abundance. When all experimental days are considered, the cumulative contribution of the three genera did not exceed 0.5% of relative chlorophyta abundance with few exceptions per treatment. These include D1, D4 in treatments C and TN, D2 and D4 in T, D1 in N. Of the three genera, Oocystis contributes the least (<0.2%) to this sum on all occasions.

3.6.3. Abiotic drivers of chlorophyta community

Winter conditions (C, N samples) are not clearly differentiated from summer conditions (T,TN samples)

Chlorophyta community composition patterns were significantly associated with O_2 concentration, Chl a, TDP concentration (R^2 = 0.28, pseudo-F =7.26, p< 0.05). Temperature, CDOM concentration and TDN concentration were significant drivers of chlorophyta community composition patterns individually (pseudo-F = 5.9, p<0.05, pseudo-F = 3.7, p<0.05 and pseudo-F = 5.8, p< 0.05, respectively) but did not contribute significantly to the linear distance model. Salinity was not a significant driver of variation in the chlorophyta community. However, all 7 parameters mentioned above were included in the best distant linear model solution (selection criteria: $R^{2,}$ selection procedure: forward, R^2= 0.31). According to model results, 31.4% of total variation between samples in the chlorophyta community can be explained for by the cumulative effect of the seven model parameters (Fig. 17).

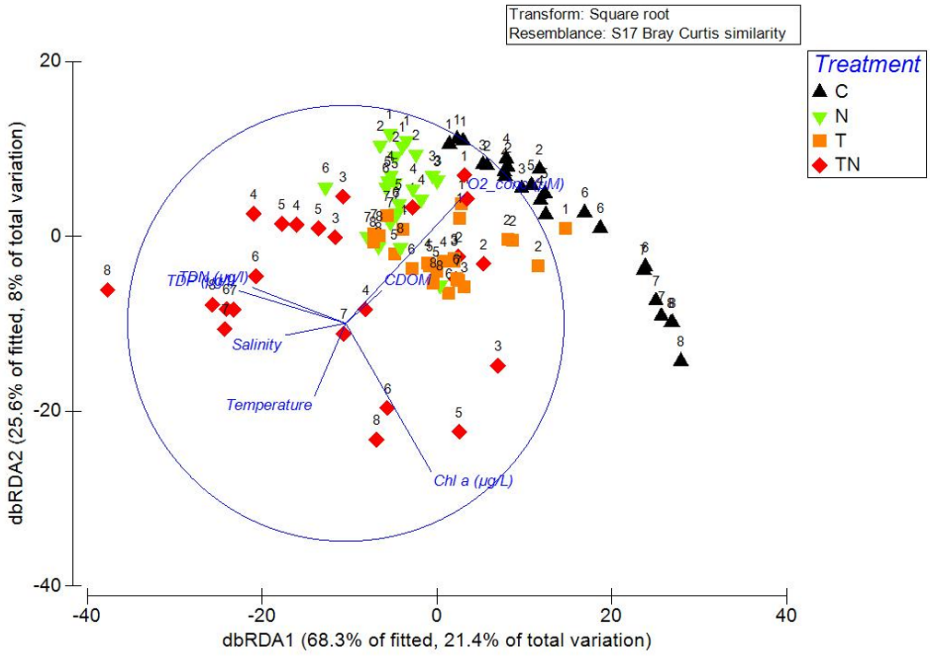

Figure 17. Distance based linear mode for chlorophyta communities. Results presented as a distance-based redundancy analysis (dbRDA) plot. All parameters contributing to the linear model are shown, specifically Temperature, Salinity, TDP; Total Dissolved Phosphorus (µgL^{-1}), O_2 concentration (µM), TDN; Total Dissolved Nitrogen (µgL-1) ; CDOM; Coloured Dissolved Organic Matter (µgL^{-1}),Chl a; Chlorophyll a (µgL^{-1}). Positive/negative association of parameters and relative influence on community composition denoted through vector length and direction respectively. Community similarity based on Bray-Curtis index of square root transformed abundance data. Control samples in black, N samples in green, T in orange and TN in red. Numbers above symbols denote experimental day.

3.7. Other taxa

Other taxa encountered in the samples include those of Table 1. Significant differences in abundance between treatments were only observed in Cyanobacteria, *Aphanizomenon sp.* in particular (RM ANOVA, F(3) = 4.6, p<0.05). *Woronichinia sp*. abundance in all treatments and days was not considered as viewing resolution did not permit counting of cells in colonies.

Table 1. Other taxa (phyla) observed among all samples (all treatments and experimental days.

Other taxa	
Cyanobacteria	
Charophyta	Cercozoa
Cryptophyta	Choanozoa
Haptophyta	Euglenozoa

4. Discussion and conclusions

In the present mesocosm experiment two plankton communities were simulated in the tanks, corresponding to two seasons: winter (Control) and summer (TN treatment), while the N treatments and T treatments provide a fully factorial experiment with the possibility to also analyze for interactive effects. Temperature showed the main effect on most organism groups studied. Significant effects of the interaction of experimental factors ("time", "treatment") on community dynamics of Diatoms, Dinoflagellates and Ciliates occurred during the experiment, and showed effects of the experimental manipulations on the dynamics of major microbial plankton groups. Abundance and composition alterations of *Ochrophyta*, *Chlorophyta* and other taxa was also observed, even though they do not strictly conform to the definition of microplankton.

Overall mean abundance of diatoms in all treatments was orders of magnitude higher than that of dinoflagellates and ciliates, which constitute the formation of a herbivorous food web sensu Legendre and Rassoulzadegan, (1995). In this scenario diatoms are directly consumed by larger zooplankton, which were observed in the single end-sample analysis (data not reported in this study). Summer temperature applied in treatments T and TN hampered diatom growth (despite nutrient addition in TN) as demonstrated by lower mean abundances in comparison to treatments C and N. Notably, mean diatom abundance in treatments T and TN crashed simultaneously after D6. This may also be influenced by increased competition with bacterioplankton, as growth and abundance of those were observed to increase in the T and TN treatments (Verma et al. 2022, submitted). This is in line with studies of phytoplankton in the Gulf of Bothnia according to which diatoms prevail during spring, while smaller forms of

phytoplankton are dominant in the summer (Andersson and Hagstrom, 1994;Andersson et al., 1996). In winter condition treatment (C), diatom abundance in the Control mesocosms continued to peak until the end of the experiment, possibly due to lack of a shading effect and competition for resources with heterotrophic bacteria brought about by DOM addition in the N mesocosms (Båmstedt and Wikner, 2016) .

Mean dinoflagellate abundance formed two distinct patterns between treatments (Controls, N treatment and treatments T, TN). Generally, mean dinoflagellate abundance was lower in tanks where summer temperature was applied, similarly to diatoms, thus concurring with field reports from the study area that show lower abundances in summer (Andersson and Hagstrom, 1994; Andersson et al., 1996). Comparatively higher abundances in N and Control mesocosms could reflect dinoflagellates benefiting from growth of diatom prey (Sherr and Sherr, 1994) in these mesocosms. However, the crash of mean dinoflagellate abundance in C and N after D6 indicates that dinoflagellates were experiencing strict control within the food web. The same seemed to be true in T and TN treatments, where dinoflagellates started experiencing an increasing trend at the beginning (like C, N) which however did not continue. This levelling off could also be associated with appearance of mesozooplankton in the end of the experiment (especially TN and T treatments). Mixotrophy among dinoflagellates (Stoecker, 1999) may have further complicated abundance patterns in this taxon.

Mean ciliate abundances in treatments T and TN appeared to be clearly inhibited after D4 and D5 respectively. In Controls mean ciliate abundance was kept low but constant, whilst in N ciliates grew unhindered. Such differences among treatments with higher temperature setting (T, TN) and those with lower (C, N) resembles the seasonality of ciliate predators; in the northern Baltic Sea copepods are found at low abundances during winter(Viitasalo et al., 1994) therefore ciliates are released of top-down pressure. DOM amendment in N treatment, by boosting heterotrophy, may have invertedly fueled the microbial loop, thereby allowing enhanced ciliate growth. Strong top-down pressure on ciliates in treatments T, TN was possibly exerted late in the experiment due to copepod life cycle characteristics(Allan, 1976), especially considering that temperature increase favors copepod egg production. One could reasonably argue that if nutrient amendment in TN enhanced ciliate growth compared to T at the first half of the experiment, then metazoans capitalized earlier on this growth. Hence, ciliate abundance crashed earlier in TN, as seen by the time lag of an entire experimental Day between ciliate abundances in treatments TN and T. The ability of ciliates to proliferate in summer conditions without nutrient amendment (T), at low diatom abundances, could be attributed to mechanisms such as mixotrophy, especially since low algal density provides a competitive advantage for mixotrophic ciliates compared to heterotrophs (Stoecker et al., 2017).

As stressed earlier, the interaction of “time” and the different “treatments” had a significant effect on diatom, dinoflagellate and ciliate community composition. The effect of temperature setting at 10°C, regardless of nutrient amendment, induced more than a third (dinoflagellates) and up to half (ciliates) of total variation between samples in these groups. Therefore, seasonal variability between summer and winter are clearly reflected in ciliate and

dinoflagellate communities and to a lesser extent in diatoms. Regardless of season, in all three groups few dominant species were observed in terms of abundance which is typical of the Gulf of Bothnia (Andersson et al., 1996). Diatom, ciliate and dinoflagellate species observed were typical of the Baltic Sea, such as the diatoms *Chaetoceros* sp., *Skeletonema* sp., oligotrich ciliates and the dinoflagellate *Peridiniella* sp. (Andersson et al., 1996; Setälä and Kivi, 2003; Camarena-Gómez et al., 2021).

In all three taxa a codominance of two or more genera was observed, indicating that conditions were not favourable for a particular genus. For instance, although the *Skeletonema* sp. was the most abundant diatom, *Chaetoceros* sp. comparative abundance remained high in samples. Regarding dinoflagellates, *Peridiniella* sp. (identified as *Peridiniella catenanta* based on the web key in Nordic Microalgae (SMHI, 2019)) was observed in all samples and codominated with athecate dinoflagellates of the order *Gymnodiniales.* Concerning ciliates, it is noteworthy that a higher abundance of *Mesodinium* sp. (most likely *Mesodinium rubrum*), compared to other ciliate genera, in treatments T, TN was not observed in the samples, which would have been expected based on Andersson et al. (1996). Additionally, tintinnids were favoured by winter conditions. The fixation method used in this study (Lugol's solution) possibly led to taxonomic uncertainty (as noted in Setälä and Kivi, 2003), which was addressed by marking shrinked or deformed ciliates as unidentified.

Only in the case of two phyla, *Chlorophyta* and *Ochrophyta* did we observe almost complete dominance of a single genus. The predominant chlorophyte was *Monoraphidium* sp. (most likely *M. contortum* (SMHI, 2019)). *Chlorophyta* abundance was generally higher in treatments T and TN and as such mirrored natural variability of *Monoraphidium* sp. which is more abundant during the summer, after the spring bloom in the northern Bothnian Sea. *Ochrophyta* were primarily represented by *Dinobryon* sp. found as single individuals in samples. This genus is typical of freshwater, but notably three species have been recorded in marine waters, including the Baltic Sea (Havskum and Riemann, 1996; Unrein et al., 2010). In the northern Bothnian Sea, *Dinobryon sp.* is a winter species (Alasaarela, 1979; Lkavalko and Thomsen, 1997), which could explain higher abundances in Controls and N treatment (winter temperatures). Bacterivory has been observed in *Dinobryon* (Havskum and Riemann, 1996; Unrein et al., 2010), therefore nutrient amendment in N treatment could have increased its prey availability, fueling growth of this taxon.

Other taxa found in samples, described in in the Results section, were found in the samples, in abundances several orders of magnitude lower than the taxa described above, mostly due to underestimation of their abundance using the Utermöhl, (1931) method. It is however noteworthy that Cyanobacteria (e.g *Aphanizomenon* sp. filaments) were found in higher abundances in Control and N treatments, whereas based on literature, higher abundances in T, TN treatments would have been expected. Perhaps trophic interactions within taxa of lower abundances could have led to this paradox, or abiotic conditions in these mesocosms prevented Cyanobacteria from proliferating.

The analysis of abiotic drivers in the different microplankton taxa, further reinforce the influence of temperature on dinoflagellate and ciliate communities. In each of these taxa, samples form two distinct clusters (T, TN and C, N). In diatoms, chlorophyta and ochrophyta, such clustering based on temperature is not as pronounced, although it could be expected given abundance and community composition data. Of the parameters fed into the models for the identification of abiotic drivers, no single one was excluded in all taxa where these were investigated. It is notable that in diatoms and chlorophyta, ochrophyta (considered autotrophic for simplicity) while DOM (here CDOM) is a significant driver as an individual component it does not contribute significantly to the model as an environmental driver. The influence of CDOM, (predominantly negative) on primary producers has been previously recorded in the study area (Wikner and Andersson, 2012; Andersson et al., 2018).

In summary, this study targeted the influence of substrate and temperature manipulation on microplankton community. Temperature and substrate manipulations imposed winter and summer conditions in C and TN mesocosms respectively. Treatments T and N were not assignable to a particular season but contributed towards a fully factorial experiment. In this experiment warming was not induced as in Hoppe et al. (2008). In the present study the influence of temperature and nutrients on the microplankton community could be disentangled. In terms of temperature, variation in the microplankton assemblages followed natural seasonal patterns for the study area (Andersson et al., 1996). In terms of nutrient amendment, we traced a significant influence of substrate manipulations both in terms of microplankton abundances and community composition. The suppression of diatom abundance by the addition of nutrients (N) with the parallel increase in ciliate, dinoflagellate abundance in this treatment is indicative of the shift in heterotrophy brought about by increased DOM concentration observed by Wikner and Andersson (2012) and Andersson et al. (2018) in the study area. Such a shift could invariably spread to higher trophic levels eventually impacting fish, therefore fisheries (Andersson et al., 2018) . Further research could enhance our understanding as to how the two parameters (temperature and nutrients) control summer and winter protist assemblages in sub-arctic environments. Knowledge of such mechanisms is critical if we are to understand the impact of climate change on microplankton assemblages, especially in areas highly influenced by climate variability such as the Baltic Sea(HELCOM, 2021).

The pelagic food web is a network (Andersson et al., 2017), and shifts at the basic (bacteria,nano-,micro-plankton) level could lead to major changes higher up (Sarmento et al., 2010). This is clearly demonstrated in the present study where it is noted that shifts in the bacterial community affect microplankton (and possibly higher trophic levels). This could have a profound influence on the ecosystem and humans. As such, the present study is valuable not only from an ecological perspective by providing insight into food web dynamics. It is also valuable from a coastal management perspective because food web function affects major activities at sea (i.e fisheries, climate change impact reduction) and how these are regulated.

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