Authors: Marta Hernández-Ruiz^{1,2,*}, Esther Barber-Lluch^{1,2}, Antero Prieto^{1,2}, Ramiro Logares³,

Eva Teira^{1,2}

Title: Response of pico-nano eukaryotes to inorganic and organic nutrient additions

Affiliations:

1 Centro de Investigación Mariña da Universidade de Vigo (CIM-UVigo), Illa de Toralla s/n,
36331 Vigo

2- Grupo de Oceanografía Biológica, Departamento de Ecoloxía e Bioloxía Animal,
Universidade de Vigo, 36310 Vigo, Spain.

3- Departamento de Biología Marina y Oceanografía, Institut de Ciències del Mar (ICM-CSIC),
08003 Barcelona, Spain.

***Corresponding author:** mhernandez@uvigo.es, (+34) 986 81 87 90

Código de campo cambiado

ORCID

Marta Hernández-Ruiz: 0000-0003-1880-9257

Esther Barber-Lluch: 0000-0001-7970-7663

Eva Teira: 0000-0002-4333-0101

Keyword: Small Eukaryotes, nutrient enrichment, DNA sequencing, organic nutrients,
phytoplankton.

23

24 *Abstract*

25 Organic nutrient loading in coastal waters has considerably increased due to anthropogenic
26 activities over the last decades. In particular, the increase in the proportion of organic vs.
27 inorganic nitrogen may have an impact on microbial plankton communities, particularly within
28 the small size classes, where mixotrophy is likely to be widespread. Here we explore the change
29 in taxonomic composition of small eukaryotes ($< 20 \mu\text{m}$) populating a productive coastal
30 system in response to inorganic and/or organic nutrient amendments using 18S rRNA gene
31 sequencing. Major changes in the community were observed in the mixed treatment, containing
32 both inorganic and organic nutrients, which mostly affected phytoplankton taxa. Adding mixed
33 nutrients had a positive effect on pigmented groups, when compared to additions of inorganic
34 nutrients. The responding pigmented groups included dinoflagellates, cryptophyceans,
35 chrysophyceans, bolidophyceans or dictyochophyceans, which comprise mixotrophic species,
36 but also groups that are considered to be strictly autotrophs, such as diatoms or
37 haptophyceans. Our results highlight the importance of organic nutrients for growth of small
38 phytoplankton. The combination of both, autotrophic and heterotrophic capabilities, is possibly
39 an advantage for small phytoplankton to thrive when inorganic nutrients or light are limiting or
40 suboptimal.

41

42

43 *Acknowledgements*

44 This research was supported by the Xunta de Galicia through DIMENSION project (grant
45 EM2013/023) and by Spanish Ministry of Economy, Industry and Competitiveness (MINECO)
46 through ENVISION (grant CTM2014-59031-P) and INTERES (CTM2017-83362-R) projects in
47 collaboration with the “Instituto Español de Oceanografía” (IEO) RADIALES project. Esther
48 Barber-Lluch was funded by an F.P.I. MINECO fellowship. We thank all the people involved in
49 the projects DIMENSION, in particular to E. Barber-LLuch and A. Prieto for their help with
50 sampling and analytical work, especially to G. Casas, A. Miranda, M. P. Lorenzo and A.
51 Fuentes-Lema. From IIM-CSIC, V. Vieitez performed the inorganic nutrient analyses and M.J.
52 Pazó the carbon, nitrogen and fluorescence of dissolved organic matter measurements. We also
53 thank the crew of the R/V J. M. Navaz for their help during the work at sea. R. Logares was
54 supported by a Ramón y Cajal fellowship (RYC-2013-12554, MINECO, Spain). Bioinformatics
55 analyses were performed at the MARBITS platform of the Institut de Ciències del Mar (ICM;
56 <http://marbits.icm.csic.es>). We thank Caterina R. Giner and Ramon Massana at the ICM-CSIC
57 for their assistance in sequence analyses and data interpretation.

Introduction

Coastal systems are experiencing growing anthropogenic pressures associated with the rapid expansion of urban, industrial, and agricultural activities (Crutzen, 2002; Glibert and Burford, 2017; Hounshell et al., 2017; Rockström et al., 2009). A noticeable consequence of the human footprint is the qualitative and quantitative change in nutrient loading, specifically nitrogen (N), to coastal areas (Glibert, 2017; Howarth, 2008; Vitousek et al., 1997). Recent studies point consistently to increasing loads of N relative to phosphorous (P) (Peñuelas et al., 2012) and to a higher proportion of dissolved organic N (DON) to inorganic nitrogen associated with these N inputs to aquatic systems (Glibert, 2017; Pellerin et al., 2006). Such global trends are of relevance given that nitrogen is the main limiting element for primary production in marine ecosystems (Bristow et al., 2017; Moore, 2013), and, thus, alterations in the amount and form of N entering coastal waters may have important impacts on the phytoplankton community and on the functioning of the microbial food web.

A significant fraction of DON is bioavailable and contributes significantly to phytoplankton nutrition (Bronk et al., 2007; Moneta et al., 2014; Moschonas et al., 2017). DON is a complex, heterogeneous and poorly characterized pool of dissolved matter (Benner, 2002). The characterized fraction includes urea, amino acids, amino sugars, amides, nucleic acids, and humic and fulvic substances. The different forms of DON become available to phytoplankton via a number of mechanisms. Phytoplankton may obtain the N of organic compounds using cell surface oxidases (Palenik, 1989; Palenik and Morel, 1990, 1991). The smallest forms, such as urea or some amino acids or amides, can be incorporated by active transport (Antia et al., 1991; Bronk et al., 2007; Mulholland and Lomas, 2008; Palenik and Henson, 1997). High molecular weight forms, such as humic substances, can be directly taken up by pinocytosis or phagocytosis, as reported for dinoflagellates and euglenoids (Kivic and Vesik, 1974; Klut et al., 1987; Legrand and Carlsson, 1998; Lewitus, 2006), or be broken into assimilable compounds by the use of extracellular enzymes, or through photodegradation (Bronk et al., 2007; Mulholland and Lomas, 2008).

Recent studies evaluating the effect of inorganic and organic nutrients amendments on microbial plankton community composition (Altman and Paerl, 2012; Davidson et al., 2007; Donald et al., 2013; Shangguan et al., 2017; Teira et al., 2011) indicate that organic nitrogen tends to benefit dinoflagellates, chlorophytes, cryptophytes, small flagellates and cyanobacteria, while nitrate tends to favor diatoms. Moreover, increases on phytoplankton growth and changes in community composition frequently occur after mixed (inorganic plus organic) nutrient additions (Davidson et al., 2007; Teira et al., 2011).

The recognition of the relevance of organic compounds to phytoplankton metabolism recently led to the broadening of the famous Margalef's phytoplankton mandala (Margalef, 1978) to also include the relative proportion between inorganic and organic nutrient forms and mixotrophy, that is, the ability to combine autotrophic and heterotrophic metabolic traits (Glibert, 2016). According to this revised framework, small phytoplankton (cell size <20 μm), appears to be the most benefited by a relative increase in organic nitrogen. In addition, mixotrophy, mostly associated with prey ingestion, is more common in small plankton, and, particularly relevant in harmful algal bloom (HAB) species (Burkholder et al., 2008; Glibert and Burkholder, 2011). Unfortunately, the taxonomic composition of small phytoplankton has been largely overlooked in the above cited nutrient amendment experiments, where diversity analyses were based on light microscopy, cytometry or pigment markers.

The coastal area off the Ría de Vigo (NW Spain), is characterized by intermittent upwelling of subsurface cold and inorganic nutrient-rich waters between March-April and September-October and the downwelling of open ocean surface water from October to March (Aristegui et al., 2009; Figueiras et al., 2002). This area is moderately polluted due to high population density, industrial activities and fish and shellfish exploitation activities (Fernández et al., 2016) and, therefore, affected by allochthonous inputs of nutrients through atmospheric and riverine discharge (Gago et al., 2005; Rodríguez and Macías, 2006; Teira et al., 2013). Previous studies in shelf waters off this area estimated that small eukaryotes (< 20 μm) account for ca. 80% of the total microbial plankton biomass (Espinoza-González et al., 2012), and

follow a well-defined seasonal pattern of community composition (Hernández-Ruiz *et al.*, 2018a). Yet, reactions of different microbial taxa to the input of different nitrogen forms remains unexplored.

The aim of the present study is to evaluate short-term changes in small eukaryote (< 20 µm) community composition after inorganic, organic (amino acids and glucose), and mixed (inorganic and organic) nutrient additions to surface water collected from the coastal waters off the Ría de Vigo (NW Spain) in spring, summer and autumn. The organic additions were intended to be representative of the bioavailable DON fraction in atmospheric deposition, wastewater effluents, and/or continental discharge (Meybeck 1993, Peierls and Paerl 1997, Seitzinger and Sanders 1999 Pehlivanoglu-Mantas *et al* 2008). Atmospheric deposition and river discharge contain not only organic N but also labile dissolved organic C (DOC) (Meybeck 1993, Jacobson *et al.* 2000, Cauwet 2002, Jurado *et al.* 2008). Therefore, we included both glucose and amino acids in the organic treatment, as they are among the most abundant organic labile substances identified in seawater (Benner 2002). Community composition and diversity were explored using high-throughput amplicon sequencing of the 18S rRNA gene. Based on the recently reviewed phytoplankton mandala (Glibert, 2016), we hypothesize that major changes in the taxonomic composition of small autotrophic eukaryotes would occur in response to mixed nutrient amendments.

Material and Methods

Sampling was carried out on board R/V José Navaz at a shelf station off the Ría de Vigo (position 42.14° N, 8.96° W; water depth 97 m) in April, June and October 2014. Full-depth vertical profiles of temperature, salinity and chlorophyll-fluorescence were obtained with a SBE-25 CTD equipped with a Seapoint *in situ* fluorometer at each sampling date.

Surface water (ca. 2 m depth) was sampled with 5 L Niskin bottles to measure size-fractionated chlorophyll-*a*, inorganic nutrients, dissolved organic carbon (DOC), nitrogen

(DON) concentration, bacterial biomass and to analyze small eukaryote community composition.

Experimental design

Water for microcosm experiments was prefiltered through a 200 μm pore size mesh to remove large predators. Hermetic and transparent to the entire light spectrum Whirl-pack® bags were used as experimental units. Each experiment included 2-L triplicates for a series of 4 treatments: (1) control treatment: no additions made; (2) inorganic addition treatment: 5 $\mu\text{mol l}^{-1}$ nitrate (NO_3^-), 5 $\mu\text{mol l}^{-1}$ ammonium (NH_4^+), 1 $\mu\text{mol l}^{-1}$ phosphate (HPO_4^{2-}); (3) organic addition treatment: 5 $\mu\text{mol l}^{-1}$ glucose and 5 $\mu\text{mol l}^{-1}$ of an equimolar mixture of 18 amino acids (all protein amino acids, except cysteine and tyrosine); (4) mixed addition treatment: combination of inorganic and organic additions: 5 $\mu\text{mol l}^{-1}$ nitrate (NO_3^-), 5 $\mu\text{mol l}^{-1}$ ammonium (NH_4^+), 1 $\mu\text{mol l}^{-1}$ phosphate (HPO_4^{2-}), 5 $\mu\text{mol l}^{-1}$ glucose and 5 $\mu\text{mol l}^{-1}$ of an equimolar mixture of 18 amino acids (all protein amino acids, except cysteine and tyrosine). The experimental units were maintained during 72 h under natural irradiance and temperature conditions by submerging the bags into open tanks located ashore, where surface water from the Ría de Vigo was continuously circulating.

At the end of the experiment, each experimental unit was sampled to measure size-fractionated chlorophyll-*a* and bacterial biomass. Thereafter, water from treatment triplicates was pooled and 2 L of each treatment were sequentially filtered through 20 and 3 μm pore size polycarbonate filters and 0.2 μm pore size sterivex filter and immediately frozen in liquid nitrogen and conserved at -80 °C until DNA extraction.

Inorganic nutrients and chlorophyll-*a*.

Size-fractionated chlorophyll-*a* concentration was determined from acetone extracts (extraction with 90% acetone at 4°C overnight in darkness) of plankton retained by 20, 3 and 0.2 μm polycarbonate filters and measured by fluorometry (Parsons et al., 1984). Total chlorophyll-*a* was calculated as the sum of the chlorophyll-*a* measured in each of the three size fractions.

Aliquots for inorganic nutrients determination (ammonium, nitrite, nitrate, phosphate and silicate) were collected in polyethylene bottles and frozen at -20°C until analysis by standard colorimetric methods with a Bran-Luebbe segmented flow analyzer (Hansen and Grasshoff, 1983).

Dissolved organic matter.

Water for the analysis of dissolved organic carbon (DOC) and total dissolved nitrogen (TDN) was filtered through $0.2\ \mu\text{m}$ filters (Pall, Supor membrane Disc Filter) using an all-glass filtration system under positive pressure of N_2 , collected into pre-combusted (450°C , 12 h) 10 mL glass ampoules, and acidified with H_3PO_4 to $\text{pH} < 2$ before heat sealing. Samples were measured in a Shimadzu TOC-V analyzer (Pt-catalyst) following Álvarez-Salgado and Miller (Álvarez-Salgado and Miller, 1998) method. Dissolved organic nitrogen (DON) was obtained by subtracting ammonium + nitrite + nitrate from TDN.

Heterotrophic Bacterial Biomass (BB)

The BB of seawater samples (1.8 mL) preserved with 1% paraformaldehyde + 0.05% glutaraldehyde and kept at -80°C after liquid nitrogen immersion for 15mins was determined. Analysis of the samples was carried out using a Becton Dickinson FACSCalibur flow cytometer equipped with a laser emitting at 488nm (Gasol and Del Giorgio, 2000) and staining bacteria with SybrGreen DNA fluorochrome. Bacterial abundance was calculated considering green fluorescence (SybrGreen staining) and side scatter signals. Bio volume (BV) was estimated from the empirical calibration between light side scatter a cell diameter described by Calvo-Díaz and Morán (2009), and converted into biomass using the allometric relationship described in Norland (1993).

Pico- and nanoeukaryote community composition.

Microbial community DNA retained in the $3\ \mu\text{m}$ filters (referred to as nanoeukaryotes) and $0.2\ \mu\text{m}$ sterivex (referred to as picoeukaryotes) was extracted, respectively, with the PowerSoil DNA isolation kit (MoBio Laboratories Inc., CA, USA) and the PowerWater DNA isolation kit (MoBio Laboratories Inc., CA, USA), following the manufacturer's protocol. The

DNA concentration was quantified using a Qubit® 2.0 fluorometer and the Qubit dsDNA HS Assay Kit (Thermo Fischer Scientific Inc., Massachusetts, USA). DNA was amplified using the primers TAREukFWD1 (5'-CCAGCASCYGC GGTAATTCC-3') and TAREukREV3 (5'-ACTTTCGTTCTTGATYRA-3') (Logares et al., 2014) and sequenced with the Illumina MiSeq platform (iTags) paired-end reads (2×250bp) by the Fasteris Laboratory (Geneva, Switzerland). Amplicon sequences of this study are publicly available at the European Nucleotide Archive (<http://www.ebi.ac.uk/ena>); the initial samples are available under the accession number PRJEB23729 and the samples of the end of the experiment under the accession number PRJEB32238.

The results of Illumina MiSeq were processed following an in-house pipeline (Logares, 2017). Briefly, raw reads were corrected using the BayesHammer (Nikolenko et al., 2013) procedure according to Schirmer et al. (2015). After being corrected, paired-end reads were subsequently merged with PEAR (Zhang et al., 2014) and sequences > 200bp were quality-checked (maximum expected errors 0.5) and dereplicated using USEARCH (Edgar, 2010). UPARSE V8.1.1756 (Edgar, 2013) was used to delineate Operational Taxonomic Units (OTUs) at 99% similarity. To obtain OTU abundances, reads were mapped back to OTUs at 99% similarity using an exhaustive search (-maxaccepts 20 -maxrejects 50,000). Chimera check and removal was performed both *de novo* and using the SILVA reference database (Quast et al., 2013). Taxonomy was assigned to 18S OTUs via BLAST (Altschul et al., 1990) comparisons of representative sequences against the PR² database (Guillou et al., 2013) as well as two in-house marine protist databases (available at <https://github.com/ramalok>). The previous two databases are either based in a collection of Sanger sequences from molecular surveys (Pernice et al., 2013) or based on 454 reads from the BioMarKs project (Massana et al., 2015). Metazoan, Charophyta and nucleomorph OTUs were removed. Blast hits were filtered prior to taxonomy assignment considering a percentage of identity >90%, a coverage >70%, a minimum alignment length of 200bp and an e-value > 0.00001. Computing was performed at the MARBITS bioinformatics platform of the Institut de Ciències del Mar (ICM; <http://marbits.icm.csic.es>).

Statistical procedures

The raw OTU table was subsampled to the number of reads present in the sample with the lowest number of reads, which was 2,380. As many OTUs were present in both size fractions, we combined datasets deriving from the 0.2 and the 3 μm filters during community analyses. We normalized the number of reads from each filter by the filter DNA yield, as recommended in Dupont *et al.* (Dupont et al., 2015).

Prior to statistical analyses, OTU abundances were transformed using the centered log ratio (Fernandes et al., 2014; Gloor et al., 2017). Zeros were replaced by the minimum value that is larger than 0 divided by 2. Variance partitioning by redundancy analysis (RDA) filtering for the top 200 most abundant OTUs was used to analyse community composition variation against sampling month and experimental treatment. Permutation tests of the factors included in Redundancy Analysis (RDA) (month and treatment) were conducted to test for significance. Log-transformation and RDA were done using the web-based program Calypso 8.62. Differential read abundance of major taxonomic groups between treatments was tested using the non-parametric Wilcoxon rank sum test, as transformed data did not comply with normality. The t-test (two-tailed) was applied to compare the mean abundance of the top 200 most abundant OTUs in the inorganic, organic and mixed treatments to that in the control. The p-value was standardized as proposed by Good (Good, 1982) in order to overcome the low number of samples. Univariate tests were run with SPSS 22.

Results

Initial environmental conditions

The initial conditions were different in each experiment. Dissolved inorganic nitrogen (DIN) concentration was high in April and October ($>4 \mu\text{M}$) and very low in June ($0.47 \mu\text{M}$), while the lowest DIN to phosphate ratio (DIN:P) occurred in April (Table1), associated with very high phosphate (P) concentration ($0.84 \mu\text{M}$). Dissolved organic carbon (DOC) and nitrogen (DON) concentrations were higher in April and October than in June. Phytoplankton

biomass, as estimated from total chlorophyll-*a* concentration, and bacterial biomass were highest in October. Overall, small cells (<20 µm) dominated the phytoplankton community, contributing to >80% of total chlorophyll-*a* concentration (Table 1).

Phytoplankton and bacterial biomass response to nutrient additions

In June and October, phytoplankton biomass increased significantly after inorganic nutrient addition (t-test, $p < 0.005$) (Fig. 1a). In April and October, the phytoplankton community responded negatively to the organic addition (Fig. 1a). The response to the mixed addition was higher than that of the inorganic addition in June, which was the only month when phytoplankton responded positively to organic addition (Fig. 1a). Remarkably, small (< 20 µm) phytoplankton responded more to the mixed addition than to the inorganic or organic additions alone in June and October (t-test, $p < 0.01$) (Fig. 1b), while large (>20 µm) phytoplankton similarly responded to inorganic and mixed treatments (Fig. 1c). Bacterial biomass increased significantly after organic and mixed addition in the three experiments (t-test, $p < 0.01$). However, the response was significantly higher after mixed than organic additions in June and October (t-test, $p < 0.001$). In October, the bacterial biomass increased significantly after inorganic addition too (Fig. 1d).

Changes in small eukaryote community composition

Overall, the initial small eukaryote community was dominated by phytoplankton groups (65±11% of total reads). The initial small eukaryote community in April differed largely from that in June and October (Fig. 2). Mamiellophyceae was the dominant group in April, accounting for 62% of the total reads (Fig. 2). *Ostreococcus* was the dominant Mamiellophyceae in the three sampling months (April, June and October), followed by *Micromonas* and *Bathycoccus* (data not shown). By contrast, marine alveolate (MALV) lineages, followed by Mamiellophyceae, and Cryptophyceae dominated the small eukaryote community in June and October (Fig. 2). Overall, after 72 h of incubation in the experimental bags, the contribution of heterotrophic taxa, such as marine stramenopiles (MAST), the

Cercozoan subphylum Monadofilosa, or Bicosoecida, to the small eukaryote community increased (Fig. 2). The nutrient addition treatments did not significantly affect richness (number of OTUs), Shannon-Wiener diversity, and Pielou's evenness (paired t-test, $p>0.112$) (Table 2). It is important to note that in June and October, the lowest number of OTUs occurred in the mixed treatment (Table 2)

The small eukaryote community composition after 72 h of incubation was mainly determined by the sampling month (Permutation test, $p=0.001$) (Fig. 3). Regardless of the lack of a global significant effect of nutrient treatments (Permutation test, $p=0.295$), most noticeable changes in community composition relative to the control treatment occurred in the mixed and inorganic treatments in June and October (Fig. 3). The contribution of diatoms largely increased, and that of Mamiellophyceae decreased, after organic additions compared to the other treatments in April (Fig. 2). In June, the abundance of diatoms increased, and that of Bolidophyceae or Dictyochophyceae decreased in the mixed compared to the other treatments, while MAST and Chrysophyceae were more abundant in the inorganic and organic than in the control treatments (Fig. 2). The most remarkable changes in October were the large increase of Mamiellophyceae abundance in the mixed treatment and that of Bicosoecida in the organic treatment compared to the control (Fig. 2).

Response to mixed versus inorganic nutrient addition of specific taxonomic groups

In order to evaluate if the response to the mixed addition of the different taxonomic groups was just associated with the inorganic nutrient addition, we compared the abundance of reads in the mixed with that in the inorganic treatments (Fig. 4). Most of the algal groups, including Mamiellophyceae, Bacillariophyceae, Chrysophyceae, and Bolidophyceae, responded more to the mixed than to the inorganic addition (Wilcoxon test, $p<0.002$), resulting in a greater number of OTUs showing high abundances when both inorganic and organic nutrients are added (Fig. 4). By contrast, Dinophyceae, Cryptophyceae and Dictyochophyceae responded similarly to mixed and inorganic additions. Among the heterotrophic groups, MALV-II, Monadofilosa and MAST responded more to mixed than to inorganic additions (Wilcoxon test,

p<0.001) (Fig. 4). On average, the phytoplankton and heterotrophic groups that increased significantly in abundance in the mixed compared to the inorganic enrichment collectively represented 42±15% and 21±7%, respectively, of the total reads at the beginning of the experiments.

Response of the most abundant protist populations to nutrient additions

In order to evaluate the effect of the different treatments at the level of individual OTUs we selected the 200 most abundant OTUs. These selected OTUs included representatives of all the major protist groups and represented, on average, 87% of the total reads in samples. Only 30 OTUs showed significant responses to nutrient treatments (Fig. 5), of which, 19 were phytoplankton representatives.

Within the phytoplankton, only a dinoflagellate (*Prorocentrum minimum_1*) and a haptophyte (*Chrysochromulina simplex*) showed a significant positive response to inorganic nutrient addition (t-test, p<0.034). A negative response to inorganic nutrients occurred in a dinoflagellate (*Pelagodinium beii_1*), a Mamiellophyceae (*Micromonas pusilla*) and a Chlorodentrales (t-test, p<0.037) (Fig. 5). Within the heterotrophs, two OTUs, belonging to MALV-II and MAST-3D clades, showed a positive response to inorganic nutrients, and another two, belonging to Monadofilosa and the MAST-1C clade, showed a negative response (t-test, p<0.039).

The organic additions caused mainly negative responses within phytoplankton (5 out of 19) OTUs (Fig. 5), including dinoflagellates, diatoms and chlorophytes (t-test, p<0.037). Three dinoflagellate OTUs showed a significant positive response to organic additions (t-test, p<0.044) (Fig. 5). On the contrary, the significant responses of the heterotrophs to organic additions (4 out of 11 OTUs) were only positive (t-test, p<0.044) (Fig. 5).

The mixed additions caused more positive responses than the inorganic additions within the phytoplankton (Fig. 5), including a dinoflagellate, two Mamiellophyceae (*Bathycoccus prasinos* and *Crustomastix stigmatica*), the Prasinophyceae *Pyramimonas australis*, the

Bolidophyceae *Bolidomonas pacifica* and the Dictyochophyceae *Florenciella parvula* (t-test, $p < 0.047$). These six OTUs responded exclusively to mixed additions. There were also negative responses to the mixed additions within phytoplankton, including three dinoflagellates, a Chlorophyta, a diatom, and a Chrysophyceae (t-test, $p < 0.027$) (Fig. 5). The mean read abundance of the phytoplankton OTUs responding positively to mixed additions was 7-fold higher than the mean abundance of those OTUs responding negatively to the mixed additions (data not shown). Within the heterotrophic OTUs, two showed a positive response, and two a negative response to mixed additions (t-test, $p < 0.044$) (Fig. 5).

Discussion

As previously reported, the response of the microbial community to controlled nutrient manipulation largely differs depending of the sampling period (Martínez-García et al., 2010; Riis et al., 2018; Teira et al., 2015, 2011). The 200 μm prefiltration to exclude large zooplankton probably altered the small eukaryote community composition during the incubations because of the strong grazing pressure over the smallest plankton components (Burkill et al., 1987; Caron et al., 2000; Escaravage and Prins, 2002; Landry and Hassett, 1982; Lignell et al., 2003; Martínez-García et al., 2010). In fact, predominantly picoplanktonic taxa, such as Mamiellophyceae or MALV-II clade, were reduced in the control compared to the initial community (Fig. 2).

The small eukaryote community composition response patterns are coherent with those of small autotrophic biomass (Figs. 1, 3), which suggests that phytoplankton taxa were the main responders to nutrient enrichment. This is further supported by the fact that the number and mean abundance of phytoplankton OTUs responding significantly to the additions were, respectively, 1.7 and 2.1-fold higher than those of heterotrophic OTUs (data not shown). The most prominent changes in biomass and community composition occurred in the mixed and inorganic treatments in June and October, when DIN concentrations were low to moderate (Table 1), (Fig. 3). It is important to note that the effect of nutrient enrichment on small

eukaryote community composition was not significant when pooling the three experiments, likely due to the strong effect of the sampling month on community composition (Figs 2, 3).

Phytoplankton biomass increased significantly upon mixed and inorganic additions (Fig. 1c), as previously observed in coastal areas (Davidson et al., 2007; Joint et al., 2002; Martínez-García et al., 2010) (Fig. 1). The marginal response in April suggests non-limiting conditions, which agrees with the high concentration of nutrients at the beginning of this experiment (Table 1). During summer and autumn, small phytoplankton benefited more from additions containing both inorganic and organic nutrients than from inorganic nutrient additions alone (Fig. 1a), which suggests diverse nutritional strategies within this size fraction. The initial small eukaryote communities in June and October were more diverse than in April (Table 2). Summer and autumn communities included heterotrophic groups and several phytoplankton groups that are known to include autotrophic and mixotrophic species such as Dinophyceae, Chrysophyceae, Cryptophyceae, Dictyochophyceae and Bolidophyceae (Fig. 2) (Frias-Lopez et al., 2009a; Hoef-Emden and Archibald, 2016; Massana, 2011; Raven et al., 2009).

The combined addition of inorganic and organic nutrients compared to that of inorganic nutrients alone significantly increased the abundance of most protist groups (Fig. 4). While some of the groups benefiting from the combined addition include mixotrophic representatives, such as Chrysophyceae and Bolidophyceae, or are heterotrophic, such as MALV-II, MAST or Monadofilosa; Bacillariophyta (diatoms) and Mamiellophyceae are considered to include strict autotrophic taxa (Flynn et al., 2013). In particular, it is remarkable that the increase in the relative abundance of mamiellophyceans in the mixed treatment in October (Fig. 2), coincided with a moderate concentration of DIN, a high DON concentration, and high bacterial biomass (Table 1). The contribution of cryptophyceans and dinoflagellates, that include many mixotrophic representatives, was also highest at the beginning of the autumn experiment, which suggest that the environmental conditions at this sampling time were favorable for mixotrophs. The response of phytoplankton taxa to the mixed addition could be due to bacterial remineralization. However, given the short incubation time (72 h), and the large amount of

inorganic nutrients added (10 μ M of N), it is highly improbable that the additional benefit in the mixed treatment derives from bacterial remineralization. Therefore, the response in the mixed compared to the inorganic treatment suggests the mixotrophic behavior of many phytoplankton taxa, which supports the direct utilization of organic substrates (i.e. through osmotrophy), including the added glucose and/or amino acids or secondary metabolites (e.g. vitamins) produced by other microorganisms responding to the organic nutrients. Although many phytoplankton species, including diatoms and Mamiellophyceae, show a significant uptake of glucose, amino acids or other organic nitrogenous compounds (Berman and Bronk, 2003; Flynn and Butler, 1986; Glibert and Legrand, 2006; M Hernández-Ruiz et al., 2018; Terrado et al., 2015), it is commonly assumed that osmotrophy by itself cannot support algal growth in marine ecosystems (Caron et al., 2017; Flynn et al., 2013). Alternatively, the benefit of the mixed additions could be the ingestion of prey which responded to organic substrates, such as bacteria or other small protists (i.e. through phagotrophy) (Flynn et al., 2013; Selosse et al., 2017; Worden et al., 2015).

Bacterial biomass increased significantly in the organic and mixed treatment in the three experiments (Fig. 1d), which could, in turn, promote the growth of bacterivores. The positive effect of organic or mixed additions on *Telonema subtile*, and OTUs affiliated to *Pharaphysomonas* genus, MAST-4A clade and Monadofilosa subphylum (Fig. 5) suggests an indirect response associated to bacterial biomass increase, as all these taxa are bacterivores (Jürgens and Massana, 2008; Massana, 2011; Massana et al., 2006, 2004; Shalchian-Tabrizi et al., 2006). *Picomonas* has been reported to ingest colloidal particles (Moreira and López-García, 2014), which are likely more abundant in the organic and mixed treatments (Chin et al., 1998). Within phytoplankton, phagotrophy has been also described for dinoflagellates, *Bolidomonas pacifica* and *Florenciella parvula*, all significantly and exclusively stimulated by mixed additions (Fig. 5). Even if, due to their small size, Mamiellophyceae are not expected to be phagotrophs (Massana, 2011), such capability remains controversial, as bacterivory has been documented for *Micromonas* in cultures (Gonzalez et al., 1993; McKie-Krisberg and Sanders,

2014; Sanders and Gast, 2012), but not in natural waters (Unrein et al., 2014). Within the Chlorophyta, a positive effect of mixed additions occurred for the picoplanktonic Mamiellophyceae *Bathycoccus prasinos* and *Crustomastix stigmatica*, and the Prasinophyceae *Pyramimonas australis* (Fig. 5), of which only *Pyramimonas* species has been reported to be phagotrophic (Bell and Laybourn-Parry, 2003; Raven et al., 2009). Then again, considering that phagotrophy is absent in diatoms and unlikely in the Mamiellophyceae (Flynn et al., 2013), the significant increase in abundance of populations within these taxa in the mixed compared with the inorganic amendments (Figs. 4, 5) is most likely related to osmotrophy. Hernández-Ruiz *et al* (M Hernández-Ruiz et al., 2018) demonstrated that *Bathycoccus prasinos*, and other mamiellophyceans are able to use amino acids in this sampling area during the summer stratification period. Nevertheless, semi-extracellular phagocytosis (pomacytosis) has been recently proposed as a potential predation mechanism in very small eukaryotes (Kamennaya et al., 2018), and thus phagotrophy cannot be entirely disregarded for picophytoplankton.

The positive phytoplankton response to the organic addition observed in June (Fig. 1) suggests the utilization of amino acids to alleviate N limitation either by enzymatically cleaving the amine group (Palenik and Morel, 1990, 1991) or by direct uptake (Bronk et al., 2007; Raven and Giordano, 2016). Phagotrophic phytoplankton could also benefit from the bacterial biomass increase in response to the organic additions (Fig. 1d). Phytoplankton could also profit from nutrient release by bacterial remineralization, albeit this is highly unlikely, because bacteria would mineralize N from organic substrates only under conditions of C limitation (Goldman and Dennett, 1991), which was prevented by the concomitant addition of glucose to the organic and mixed treatments. The increase of the relative abundance of Dictyochophyceae and Chrysophyceae in the organic treatment during the summer (Fig. 2) is coherent with phagotrophy or osmotrophy, as both capabilities have been observed within these groups (Frias-Lopez et al., 2009b; Raven et al., 2009; Terrado et al., 2015).

Negative responses to organic nutrient additions were also found in terms of phytoplankton biomass in April and October (Fig. 1), as previously reported in the same

sampling area (Martínez-García *et al.*, 2010; Prieto *et al.*, 2015). The organic addition would stimulate bacterial growth, including pathogenic bacteria, that, in turn, could inhibit or outcompete phytoplankton for the available inorganic nutrients (Joint *et al.*, 2002). The addition of organic substrates could also stimulate herbivore or omnivore microzooplankton. The positive response of heterotrophic populations to the organic addition, including *Telonema subtile*, *Paraphysomonas* sp. and a MAST-4A OTU support this hypothesis (Fig. 5). Orsi *et al.* (2018) demonstrated that although *Telonema* and MAST flagellates prey on bacteria, they appear to also ingest picoalgae. Unexpectedly, nutrient enrichment caused more negative than positive responses at the level of phytoplankton OTUs (Fig. 5), although the mean abundance of negatively responding OTUs was ca. 3-fold lower than that of positively responding OTUs (data not shown). Interestingly, those OTUs showing negative responses did not show any positive response, which suggest either low competitive abilities or strong grazing susceptibility. The read abundance of *Pelagodinium bei*-2 and that of a Chlorodentrales OTU were systematically reduced after nutrient enrichment (Fig. 5). *Pelagodinium bei* is a symbiotic dinoflagellate widely distributed in the marine environments over the world (Siano *et al.*, 2010). We speculate that nutrient enrichment might have increased grazing pressure on these two OTUs., although the mechanisms remain to be unraveled.

The results obtained in this work contribute to the understanding of the microbial community response to nutrient additions in a future global change scenario, when the amount of organic nutrient forms reaching coastal waters is expected to increase. Small eukaryotic phytoplankton responded to mixed nutrients more than to inorganic nutrients, showing the largest biomass increase when DIN concentration was the lowest. The beneficial effect of the mixed addition compared to the single inorganic nutrient enrichment was apparent not only among potentially mixotrophic taxa, such as dinoflagellates, cryptophytes, chrysophytes, bolidophytes and dictyochophytes, but also within groups that are mostly considered strict autotrophs, such as diatoms or mamiellophytes. Our results highlight the importance of

455 organic nutrients for small phytoplankton, which may explain, to some extent, their great
456 ecological success in marine ecosystems.

457

458 *References*

- 459 Altman, J.C., Paerl, H.W., 2012. Composition of inorganic and organic nutrient sources
460 influences phytoplankton community structure in the New River Estuary, North Carolina.
461 *Aquat. Ecol.* 46, 269–282. <https://doi.org/10.1007/s10452-012-9398-8>
- 462 Altschul, S.F., Gish, W., Miller, W., Myers, E.W., Lipman, D.J., 1990. Basic local alignment
463 search tool. *J. Mol. Biol.* 215, 403–10. [https://doi.org/10.1016/S0022-2836\(05\)80360-2](https://doi.org/10.1016/S0022-2836(05)80360-2)
- 464 Álvarez-Salgado, X.A., Miller, A.E.J., 1998. Simultaneous determination of dissolved organic
465 carbon and total dissolved nitrogen in seawater by high temperature catalytic oxidation:
466 Conditions for precise shipboard measurements. *Mar. Chem.* 62, 325–333.
467 [https://doi.org/10.1016/S0304-4203\(98\)00037-1](https://doi.org/10.1016/S0304-4203(98)00037-1)
- 468 Antia, N.J., Harrison, P.J., Oliveira, L., 1991. The role of dissolved organic nitrogen in
469 phytoplankton nutrition, cell biology and ecology. *Phycologia* 30, 1–89.
470 <https://doi.org/10.2216/i0031-8884-30-1-1.1>
- 471 Arístegui, J., Barton, E.D., Álvarez-Salgado, X.A., Santos, A.M.P., Figueiras, F.G., Kifani, S.,
472 Hernández-León, S., Mason, E., Machú, E., Demarcq, H., 2009. Sub-regional ecosystem
473 variability in the Canary Current upwelling. *Prog. Oceanogr.* 83, 33–48.
474 <https://doi.org/10.1016/j.pocean.2009.07.031>
- 475 Bell, E.M., Laybourn-Parry, J., 2003. Mixotrophy in the antarctic phytoplagellate, *Pyramimonas*
476 *gelidicola* (Chlorophyta: Prasinophyceae). *J. Phycol.* 39, 644–649.
477 <https://doi.org/10.1046/j.1529-8817.2003.02152.x>
- 478 Benner, R., 2002. Chemical Composition and Reactivity, in: Hansell, D.A., Carlson, C.A.
479 (Eds.), *Biogeochemistry of Marine Dissolved Organic Matter*. Academic Press, pp. 59–90.
480 <https://doi.org/10.1016/B978-012323841-2/50005-1>
- 481 Berman, T., Bronk, D., 2003. Dissolved organic nitrogen: a dynamic participant in aquatic

ecosystems. *Aquat. Microb. Ecol.* 31, 279–305. <https://doi.org/10.3354/ame031279>

Bristow, L.A., Mohr, W., Ahmerkamp, S., Kuypers, M.M.M., 2017. Nutrients that limit growth in the ocean. *Curr. Biol.* 27, R474–R478. <https://doi.org/10.1016/j.cub.2017.03.030>

Bronk, D.A., See, J.H., Bradley, P., Killberg, L., 2007. DON as a source of bioavailable nitrogen for phytoplankton. *Biogeosciences* 3, 283–296. <https://doi.org/10.5194/bgd-3-1247-2006>

Burkholder, J.M., Glibert, P.M., Skelton, H.M., 2008. Mixotrophy, a major mode of nutrition for harmful algal species in eutrophic waters. *Harmful Algae* 8, 77–93. <https://doi.org/10.1016/j.hal.2008.08.010>

Burkill, P.H., Mantoura, R.F.C., Llewellyn, C.A., Owens, N.J.P., 1987. Microzooplankton grazing and selectivity of phytoplankton in coastal waters. *Mar. Biol.* 93, 581–590. <https://doi.org/10.1007/BF00392796>

Calvo-Díaz, A., Morán, X.A.G., 2009. Empirical leucine-to-carbon conversion factors for estimating heterotrophic bacterial production: Seasonality and predictability in a temperate coastal ecosystem. *Appl. Environ. Microbiol.* 75, 3216–3221. <https://doi.org/10.1128/AEM.01570-08>

Caron, D.A., Alexander, H., Allen, A.E., Archibald, J.M., Armbrust, E.V., Bachy, C., Bell, C.J., Bharti, A., Dyhrman, S.T., Guida, S.M., Heidelberg, K.B., Kaye, J.Z., Metzner, J., Smith, S.R., Worden, A.Z., 2017. Probing the evolution, ecology and physiology of marine protists using transcriptomics. *Nat. Rev. Microbiol.* 15, 6–20. <https://doi.org/10.1038/nrmicro.2016.160>

Caron, D.A., Dennett, M.R., Lonsdale, D.J., Moran, D.M., Shalapyonok, L., 2000. Microzooplankton herbivory in the Ross Sea, Antarctica. *Deep Sea Res. Part II Top. Stud. Oceanogr.* 47, 3249–3272. [https://doi.org/10.1016/S0967-0645\(00\)00067-9](https://doi.org/10.1016/S0967-0645(00)00067-9)

Cauwet, G., 2002. DOM in the Coastal Zone. In Hansell DA and Carlson CA (ed),

Biogeochemistry of marine dissolved organic matter . Academic Press. San diego, CA, , p
579-609

Chin, W.-C., Orellana, M. V., Verdugo, P., 1998. Spontaneous assembly of marine dissolved
organic matter into polymer gels. *Nature* 391, 568–572. <https://doi.org/10.1038/35345>

Crutzen, P.J., 2002. Geology of mankind. *Nature* 415, 23–23. <https://doi.org/10.1038/415023a>

Davidson, K., Gilpin, L.C., Hart, M.C., Fouilland, E., Mitchell, E., Á lvarez Calleja, I., Laurent,
Cé., Miller, A.E.J., Leakey, R.J.G., 2007. The influence of the balance of inorganic and
organic nitrogen on the trophic dynamics of microbial food webs. *Limnol. Oceanogr.* 52,
2147–2163. <https://doi.org/10.4319/lo.2007.52.5.2147>

Donald, D.B., Bogard, M.J., Finlay, K., Bunting, L., Leavitt, P.R., 2013. Phytoplankton-
Specific Response to Enrichment of Phosphorus-Rich Surface Waters with Ammonium,
Nitrate, and Urea. *PLoS One* 8, e53277. <https://doi.org/10.1371/journal.pone.0053277>

Dupont, C.L., McCrow, J.P., Valas, R., Moustafa, A., Walworth, N., Goodenough, U., Roth, R.,
Hogle, S.L., Bai, J., Johnson, Z.I., Mann, E., Palenik, B., Barbeau, K. a, Venter, J.C.,
Allen, A.E., 2015. Genomes and gene expression across light and productivity gradients in
eastern subtropical Pacific microbial communities. *ISME J.* 9, 1076–92.
<https://doi.org/10.1038/ismej.2014.198>

Edgar, R.C., 2013. UPARSE: highly accurate OTU sequences from microbial amplicon reads.
Nat. Methods 10, 996–8. <https://doi.org/10.1038/nmeth.2604>

Edgar, R.C., 2010. Search and clustering orders of magnitude faster than BLAST.
Bioinformatics 26, 2460–2461. <https://doi.org/10.1093/bioinformatics/btq461>

Escaravage, V., Prins, T.C., 2002. Silicate availability, vertical mixing and grazing control of
phytoplankton blooms in mesocosms, in: *Sustainable Increase of Marine Harvesting:
Fundamental Mechanisms and New Concepts*. Springer Netherlands, Dordrecht, pp. 33–
48. https://doi.org/10.1007/978-94-017-3190-4_4

532 Espinoza-González, O., Figueiras, F., Crespo, B., Teixeira, I., Castro, C., 2012. Autotrophic and
 533 heterotrophic microbial plankton biomass in the NW Iberian upwelling: seasonal
 534 assessment of metabolic balance. *Aquat. Microb. Ecol.* 67, 77–89.
 535 <https://doi.org/10.3354/ame01584>

536 Fernandes, A.D., Reid, J.N.S., Macklaim, J.M., McMurrough, T.A., Edgell, D.R., Gloor, G.B.,
 537 2014. Unifying the analysis of high-throughput sequencing datasets: characterizing RNA-
 538 seq, 16S rRNA gene sequencing and selective growth experiments by compositional data
 539 analysis. *Microbiome* 2, 15. <https://doi.org/10.1186/2049-2618-2-15>

540 Fernández, E., Álvarez-Salgado, X.A., Beiras, R., Ovejero, A., Méndez, G., 2016. Coexistence
 541 of urban uses and shellfish production in an upwelling-driven, highly productive marine
 542 environment: The case of the Ría de Vigo (Galicia, Spain). *Reg. Stud. Mar. Sci.* 8, 362–
 543 370. <https://doi.org/10.1016/j.rsma.2016.04.002>

544 Figueiras, F.G., Labarta, U., Reiriz, M.J.F., 2002. Coastal upwelling, primary production and
 545 mussel growth in the Rías Baixas of Galicia, in: Vadstein, O., Olsen, Y. (Eds.),
 546 Sustainable Increase of Marine Harvesting: Fundamental Mechanisms and New Concepts:
 547 Proceedings of the 1st Maricult Conference Held in Trondheim, Norway, 25--28 June
 548 2000. Springer Netherlands, Dordrecht, pp. 121–131. [https://doi.org/10.1007/978-94-017-](https://doi.org/10.1007/978-94-017-3190-4_11)
 549 [3190-4_11](https://doi.org/10.1007/978-94-017-3190-4_11)

550 Flynn, K.J., Butler, I., 1986. Nitrogen sources for the growth of marine microalgae: role of
 551 dissolved free amino acids. *Mar. Ecol. Prog. Ser.* <https://doi.org/10.2307/24821281>

552 Flynn, K.J., Stoecker, D.K., Mitra, A., Raven, J.A., Glibert, P.M., Hansen, P.J., Granéli, E.,
 553 Burkholder, J.M., 2013. Misuse of the phytoplankton-zooplankton dichotomy: The need to
 554 assign organisms as mixotrophs within plankton functional types. *J. Plankton Res.* 35, 3–
 555 11. <https://doi.org/10.1093/plankt/fbs062>

556 Frias-Lopez, J., Thompson, A., Waldbauer, J., Chisholm, S.W., 2009a. Use of stable isotope-
 557 labelled cells to identify active grazers of picocyanobacteria in ocean surface waters.

558 Environ. Microbiol. 11, 512–525. <https://doi.org/10.1111/j.1462-2920.2008.01793.x>

559 Frias-Lopez, J., Thompson, A., Waldbauer, J., Chisholm, S.W., 2009b. Use of stable isotope-

560 labelled cells to identify active grazers of picocyanobacteria in ocean surface waters.

561 Environ. Microbiol. 11, 512–525. <https://doi.org/10.1111/j.1462-2920.2008.01793.x>

562 Gago, J., Álvarez-Salgado, X.A., Nieto-Cid, M., Brea, S., Piedracoba, S., 2005. Continental

563 inputs of C, N, P and Si species to the Ría de Vigo (NW Spain). Estuar. Coast. Shelf Sci.

564 65, 74–82. <https://doi.org/10.1016/j.ecss.2005.05.008>

565 Gasol, J.M., Del Giorgio, P.A., 2000. Using flow cytometry for counting natural planktonic

566 bacteria and understanding the structure of planktonic bacterial communities. Sci. Mar. 64,

567 197–224. <https://doi.org/10.3989/scimar.2000.64n2197>

568 Glibert, P., Burford, M., 2017. Globally Changing Nutrient Loads and Harmful Algal Blooms:

569 Recent Advances, New Paradigms, and Continuing Challenges. Oceanography 30, 58–69.

570 <https://doi.org/10.5670/oceanog.2017.110>

571 Glibert, P.M., 2017. Eutrophication, harmful algae and biodiversity — Challenging paradigms

572 in a world of complex nutrient changes. Mar. Pollut. Bull. 124, 591–606.

573 <https://doi.org/10.1016/j.marpolbul.2017.04.027>

574 Glibert, P.M., 2016. Margalef revisited: A new phytoplankton mandala incorporating twelve

575 dimensions, including nutritional physiology. Harmful Algae 55, 25–30.

576 <https://doi.org/10.1016/j.hal.2016.01.008>

577 Glibert, P.M., Burkholder, J.A.M., 2011. Harmful algal blooms and eutrophication: “strategies”

578 for nutrient uptake and growth outside the Redfield comfort zone. Chinese J. Oceanol.

579 Limnol. 29, 724–738. <https://doi.org/10.1007/s00343-011-0502-z>

580 Glibert, P.M., Legrand, C., 2006. The Diverse Nutrient Strategies of Harmful Algae: Focus on

581 Osmotrophy, in: Ecology of Harmful Algae. Springer Berlin Heidelberg, pp. 163–175.

582 https://doi.org/10.1007/978-3-540-32210-8_13

583 Gloor, G.B., Macklaim, J.M., Pawlowsky-Glahn, V., Egozcue, J.J., 2017. Microbiome Datasets
584 Are Compositional: And This Is Not Optional. *Front. Microbiol.* 8, 1–6.
585 <https://doi.org/10.3389/fmicb.2017.02224>

586 Goldman, J.C., Dennett, M.R., 1991. Ammonium regeneration and carbon utilization by marine
587 bacteria grown on mixed substrates. *Mar. Biol.* 109, 369–378.
588 <https://doi.org/10.1007/BF01313502>

589 Gonzalez, J.M., Sherr, B.F., Sherr, E.B., 1993. Digestive enzyme activity as a quantitative
590 measure of protistan grazing: the acid lysozyme assay for bacterivory. *Mar. Ecol. Prog.*
591 *Ser.* 100, 197–206. <https://doi.org/10.3354/meps100197>

592 Good, I.J., 1982. C140. Standardized tail-area probabilities. *J. Stat. Comput. Simul.* 16, 65–66.
593 <https://doi.org/10.1080/00949658208810607>

594 Guillou, L., Bachar, D., Audic, S., Bass, D., Berney, C., Bittner, L., Boute, C., Burgaud, G., De
595 Vargas, C., Decelle, J., Del Campo, J., Dolan, J.R., Dunthorn, M., Edvardsen, B.,
596 Holzmann, M., Kooistra, W.H.C.F., Lara, E., Le Bescot, N., Logares, R., Mahé, F.,
597 Massana, R., Montresor, M., Morard, R., Not, F., Pawlowski, J., Probert, I., Sauvadet,
598 A.L., Siano, R., Stoeck, T., Vaulot, D., Zimmermann, P., Christen, R., 2013. The Protist
599 Ribosomal Reference database (PR2): A catalog of unicellular eukaryote Small Sub-Unit
600 rRNA sequences with curated taxonomy. *Nucleic Acids Res.* 41, 597–604.
601 <https://doi.org/10.1093/nar/gks1160>

602 Hansen, H.P., Grasshoff, K., 1983. Automated chemical analysis, in: Grasshoff, K., Kremling,
603 K., Ehrhardt, M. (Eds.), *Methods of Seawater Analysis*. Verlag Chemie, Weinheim, pp.
604 347–395. <https://doi.org/10.1002/9783527613984>

605 Hernández-Ruiz, M., Barber-Lluch, E., Prieto, A., Álvarez-Salgado, X.A., Logares, R., Teira,
606 E., 2018. Seasonal succession of small planktonic eukaryotes inhabiting surface waters of
607 a coastal upwelling system. *Environ. Microbiol.* 20, 2955–2973.
608 <https://doi.org/10.1111/1462-2920.14313>

609 Hernández-Ruiz, M., Prieto, A., Barber-Lluch, E., Teira, E., 2018. Amino acid utilization by
 610 eukaryotic picophytoplankton in a coastal upwelling system. *Mar. Ecol. Prog. Ser.* 588,
 611 43–57. <https://doi.org/10.3354/meps12435>

612 Hoef-Emden, K., Archibald, J.M., 2016. Cryptophyta (Cryptomonads), in: *Handbook of the*
 613 *Protists*. Springer International Publishing, Cham, pp. 1–41. [https://doi.org/10.1007/978-3-](https://doi.org/10.1007/978-3-319-32669-6_35-1)
 614 [319-32669-6_35-1](https://doi.org/10.1007/978-3-319-32669-6_35-1)

615 Hounshell, A.G., Peierls, B.L., Osburn, C.L., Paerl, H.W., 2017. Stimulation of Phytoplankton
 616 Production by Anthropogenic Dissolved Organic Nitrogen in a Coastal Plain Estuary.
 617 *Environ. Sci. Technol.* 51, 13104–13112. <https://doi.org/10.1021/acs.est.7b03538>

618 Howarth, R.W., 2008. Coastal nitrogen pollution: A review of sources and trends globally and
 619 regionally. *Harmful Algae* 8, 14–20. <https://doi.org/10.1016/j.hal.2008.08.015>

620 Joint, I., Hendrikson, P., Fonnes, G.A., Bourne, D., Thingstad, T.F., Rieman, B., 2002.
 621 Competition for inorganic nutrients between phytoplankton and bacterioplankton in
 622 nutrient manipulated mesocosms. *Aquat. Microb. Ecol.* 29, 145–159.
 623 <https://doi.org/10.3354/ame029145>

624 Jurado, E., Dachs, J., Duarte, C.M., Simó, R., 2008 Atmospheric deposition of organic and
 625 black carbon to the global oceans. *Atm. Environ.* 4, 7931–7939

626 Jürgens, K., Massana, R., 2008. Protistan Grazing on Marine Bacterioplankton, in: Kirchman,
 627 D.L. (Ed.), *Microbial Ecology of the Oceans*. John Wiley & Sons, Inc., Hoboken, NJ,
 628 USA, pp. 383–441. <https://doi.org/10.1002/9780470281840.ch11>

629 Kamennaya, N.A., Kennaway, G., Fuchs, B.M., Zubkov, M. V, 2018. “Pomacytosis”—Semi-
 630 extracellular phagocytosis of cyanobacteria by the smallest marine algae. *PLOS Biol.* 16,
 631 e2003502. <https://doi.org/10.1371/journal.pbio.2003502>

632 Kivic, P.A., Vesik, M., 1974. Pinocytotic uptake of protein from the reservoir in *Euglena*. *Arch.*
 633 *Microbiol.* 96, 155–159. <https://doi.org/10.1007/BF00590172>

634 Klut, M.E., Bisalputra, T., Antia, N.J., 1987. Some observations on the structure and function of
635 the dinoflagellate pusule. *Can. J. Bot.* 65, 736–744. <https://doi.org/10.1139/b87-098>

636 Landry, M.R., Hassett, R.P., 1982. Estimating the grazing impact of marine micro-zooplankton.
637 *Mar. Biol.* 67, 283–288. <https://doi.org/10.1007/BF00397668>

638 Legrand, C., Carlsson, P., 1998. Uptake of high molecular weight dextran by the dinoflagellate
639 *Alexandrium catenella*. *Aquat. Microb. Ecol.* 16, 81–86.
640 <https://doi.org/10.3354/ame016081>

641 Lewitus, A.J., 2006. Osmotrophy in marine microalgae, in: Subba Rao, D. V. (Ed.), *Algal*
642 *Cultures, Analogues of Blooms and Applications*. Science Publishers, Enfield, New
643 Hampshire.

644 Lignell, R., Seppälä, J., Kuuppo, P., Tamminen, T., Andersen, T., Gismervik, I., 2003. Beyond
645 bulk properties: Responses of coastal summer plankton communities to nutrient
646 enrichment in the northern Baltic Sea. *Limnol. Oceanogr.* 48, 189–209.
647 <https://doi.org/10.4319/lo.2003.48.1.0189>

648 Logares, R., 2017. Workflow for Analysing MiSeq Amplicons based on Uparse v1.5.
649 <https://doi.org/https://doi.org/10.5281/zenodo.259579>

650 Logares, R., Sunagawa, S., Salazar, G., Comejo-Castillo, F.M., Ferrera, I., Sarmento, H.,
651 Hingamp, P., Ogata, H., de Vargas, C., Lima-Mendez, G., Raes, J., Poulain, J., Jaillon, O.,
652 Wincker, P., Kandels-Lewis, S., Karsenti, E., Bork, P., Acinas, S.G., 2014. Metagenomic
653 16S rDNA Illumina tags are a powerful alternative to amplicon sequencing to explore
654 diversity and structure of microbial communities. *Environ. Microbiol.* 16, 2659–2671.
655 <https://doi.org/10.1111/1462-2920.12250>

656 Margalef, R., 1978. Life-forms of phytoplankton as survival alternatives in an unstable
657 environment. *Oceanol. Acta* 1 1, 493–509.

658 Martínez-García, S., Fernández, E., Álvarez-Salgado, X., González, J., Lønborg, C., Marañón,

659 E., Morán, X., Teira, E., 2010. Differential responses of phytoplankton and heterotrophic
 660 bacteria to organic and inorganic nutrient additions in coastal waters off the NW Iberian
 661 Peninsula. *Mar. Ecol. Prog. Ser.* 416, 17–33. <https://doi.org/10.3354/meps08776>
 662 Martínez-García, S., Fernández, E., Calvo-Díaz, A., Marañón, E., Morán, X.A.G., Teira, E.,
 663 2010. Response of heterotrophic and autotrophic microbial plankton to inorganic and
 664 organic inputs along a latitudinal transect in the Atlantic Ocean. *Biogeosciences* 7, 1701–
 665 1713. <https://doi.org/10.5194/bg-7-1701-2010>
 666 Massana, R., 2011. Eukaryotic picoplankton in surface oceans. *Annu. Rev. Microbiol.* 65, 91–
 667 110. <https://doi.org/10.1146/annurev-micro-090110-102903>
 668 Massana, R., Castresana, J., Balague, V., Guillou, L., Romari, K., Groisillier, A., Valentin, K.,
 669 Pedros-Alio, C., 2004. Phylogenetic and Ecological Analysis of Novel Marine
 670 Stramenopiles. *Appl. Environ. Microbiol.* 70, 3528–3534.
 671 <https://doi.org/10.1128/AEM.70.6.3528-3534.2004>
 672 Massana, R., Gobet, A., Audic, S., Bass, D., Bittner, L., Boutte, C., Chambouvet, A., Christen,
 673 R., Claverie, J.M., Decelle, J., Dolan, J.R., Dunthorn, M., Edvardsen, B., Forn, I., Forster,
 674 D., Guillou, L., Jaillon, O., Kooistra, W.H.C.F., Logares, R., Mahé, F., Not, F., Ogata, H.,
 675 Pawlowski, J., Pernice, M.C., Probert, I., Romac, S., Richards, T., Santini, S., Shalchian-
 676 Tabrizi, K., Siano, R., Simon, N., Stoeck, T., Vaultot, D., Zingone, A., de Vargas, C., 2015.
 677 Marine protist diversity in European coastal waters and sediments as revealed by high-
 678 throughput sequencing. *Environ. Microbiol.* 17, 4035–4049. [https://doi.org/10.1111/1462-](https://doi.org/10.1111/1462-2920.12955)
 679 2920.12955
 680 Massana, R., Terrado, R., Forn, I., Lovejoy, C., Pedrós-Alió, C., 2006. Distribution and
 681 abundance of uncultured heterotrophic flagellates in the world oceans. *Environ. Microbiol.*
 682 8, 1515–1522. <https://doi.org/10.1111/j.1462-2920.2006.01042.x>
 683 McKie-Krisberg, Z.M., Sanders, R.W., 2014. Phagotrophy by the picoeukaryotic green alga
 684 *Micromonas*: implications for Arctic Oceans. *ISME J.* 8, 1953–61.

685 <https://doi.org/10.1038/ismej.2014.16>

686 Meybeck, M., 1993. C, N, P and S in rivers: from sources to global inputs In Wollast R,
687 Mackenzie FT, Chou L (ed) Interactions of C, N, P and S. Biogeochemical Cycles and
688 Global Change. Springer-Verlag, Berlin, New York, p 163-194

689 Moneta, A., Veuger, B., van Rijswijk, P., Meysman, F., Soetaert, K., Middelburg, J.J., 2014.
690 Dissolved inorganic and organic nitrogen uptake in the coastal North Sea: A seasonal
691 study. *Estuar. Coast. Shelf Sci.* 147, 78–86. <https://doi.org/10.1016/j.ecss.2014.05.022>

692 Moore, L.R., 2013. More mixotrophy in the marine microbial mix. *Proc. Natl. Acad. Sci.* 110,
693 8323–8324. <https://doi.org/10.1073/pnas.1305998110>

694 Moreira, D., López-García, P., 2014. The rise and fall of Picobiliphytes: How assumed
695 autotrophs turned out to be heterotrophs. *BioEssays* 36, 468–474.
696 <https://doi.org/10.1002/bies.201300176>

697 Moschonas, G., Gowen, R.J., Paterson, R.F., Mitchell, E., Stewart, B.M., McNeill, S., Glibert,
698 P.M., Davidson, K., 2017. Nitrogen dynamics and phytoplankton community structure: the
699 role of organic nutrients. *Biogeochemistry* 134, 125–145. [https://doi.org/10.1007/s10533-](https://doi.org/10.1007/s10533-017-0351-8)
700 [017-0351-8](https://doi.org/10.1007/s10533-017-0351-8)

701 Mulholland, M.R., Lomas, M.W., 2008. Nitrogen Uptake and Assimilation, in: Capone, D.G.,
702 Bronk, D.A., Mulholland, M.R., Carpenter, E.J. (Eds.), *Nitrogen in the Marine*
703 *Environment*. Elsevier, San Diego, pp. 303–384. [https://doi.org/10.1016/B978-0-12-](https://doi.org/10.1016/B978-0-12-372522-6.00007-4)
704 [372522-6.00007-4](https://doi.org/10.1016/B978-0-12-372522-6.00007-4)

705 Nikolenko, S.I., Korobeynikov, A.I., Alekseyev, M.A., 2013. BayesHammer : Bayesian
706 clustering for error correction in single-cell sequencing. *BMC Genomics* 14, 1–11.
707 [https://doi.org/https://doi.org/10.1186/1471-2164-14-S1-S7](https://doi.org/10.1186/1471-2164-14-S1-S7)

708 Norland, S., 1993. The relationship between biomass and volume of bacteria., in: Kemp, P.F.,
709 Sherr, B.F., Sherr, E.B., Cole, J.J. (Eds.), *Handbook of Methods in Aquatic Microbial*

710 Ecology. Lewis Publication, pp. 303–308.

711 Orsi, W.D., Wilken, S., del Campo, J., Heger, T., James, E., Richards, T.A., Keeling, P.J.,
712 Worden, A.Z., Santoro, A.E., 2018. Identifying protist consumers of photosynthetic
713 picoeukaryotes in the surface ocean using stable isotope probing. *Environ. Microbiol.* 00.
714 <https://doi.org/10.1111/1462-2920.14018>

715 Palenik, B., 1989. Organic nitrogen utilization by phytoplankton: the role of cell-surface
716 deaminases. Massachusetts Inst Of Tech Cambridge

717 Palenik, B., Henson, S.E., 1997. The use of amides and other organic nitrogen sources by the
718 phytoplankton *Emiliana huxleyi*. *Limnol. Oceanogr.* 42, 1544–1551.
719 <https://doi.org/10.4319/lo.1997.42.7.1544>

720 Palenik, B., Morel, F.M., 1990. Amino acid utilization by marine phytoplankton: A novel
721 mechanism. *Limnol. Oceanogr.* 35, 260–269. <https://doi.org/10.4319/lo.1990.35.2.0260>

722 Palenik, B., Morel, F.M., 1991. Amine oxidases of marine phytoplankton. *Appl. Environ.*
723 *Microbiol.* 57, 2440–3.

724 Parsons, T.R., Maita, Y., Lalli, C.M., 1984. A Manual of Chemical & Biological Methods for
725 Seawater Analysis. Elsevier. <https://doi.org/10.1016/B978-0-08-030287-4.50002-5>

726 Pehlivanoglu-Mantas, E., Sedlak, D. L., 2008. Measurement of dissolved organic nitrogen
727 forms in wastewater effluents: concentrations, size distribution and NDMA formation
728 potential. *Water Res.*, 42(14), 3890–3898

729 Peierls, B.L., Paerl, H.W., 1997. Bioavailability of atmospheric organic nitrogen deposition to
730 coastal phytoplankton. *Limnol. Oceanogr.* 42, 1819–1823

731 Pellerin, B.A., Kaushal, S.S., McDowell, W.H., 2006. Does Anthropogenic Nitrogen
732 Enrichment Increase Organic Nitrogen Concentrations in Runoff from Forested and
733 Human-dominated Watersheds? *Ecosystems* 9, 852–864. [https://doi.org/10.1007/s10021-](https://doi.org/10.1007/s10021-006-0076-3)
734 [006-0076-3](https://doi.org/10.1007/s10021-006-0076-3)

735 Peñuelas, J., Sardans, J., Rivas-ubach, A., Janssens, I.A., 2012. The human-induced imbalance
736 between C, N and P in Earth's life system. *Glob. Chang. Biol.* 18, 3–6.
737 <https://doi.org/10.1111/j.1365-2486.2011.02568.x>

738 Pernice, M.C., Logares, R., Guillou, L., Massana, R., 2013. General Patterns of Diversity in
739 Major Marine Microeukaryote Lineages. *PLoS One* 8.
740 <https://doi.org/10.1371/journal.pone.0057170>

741 Prieto, A., Barber-Lluch, E., Hernández-Ruiz, M., Martínez-García, S., Fernández, E., Teira, E.,
742 2015. Assessing the role of phytoplankton–bacterioplankton coupling in the response of
743 microbial plankton to nutrient additions. *J. Plankton Res.* 38, 55–63.
744 <https://doi.org/10.1093/plankt/fbv101>

745 Quast, C., Priesse, E., Yilmaz, P., Gerken, J., Schweer, T., Yarza, P., Peplies, J., Glöckner,
746 F.O., 2013. The SILVA ribosomal RNA gene database project: Improved data processing
747 and web-based tools. *Nucleic Acids Res.* 41, 1–7. <https://doi.org/10.1093/nar/gks1219>

748 Raven, J.A., Beardall, J., Flynn, K.J., Maberly, S.C., 2009. Phagotrophy in the origins of
749 photosynthesis in eukaryotes and as a complementary mode of nutrition in phototrophs:
750 relation to Darwin's insectivorous plants. *J. Exp. Bot.* 60, 3975–3987.
751 <https://doi.org/10.1093/jxb/erp282>

752 Raven, J.A., Giordano, M., 2016. Combined Nitrogen, in: *The Physiology of Microalgae*.
753 Springer International Publishing, Cham, pp. 143–154. [https://doi.org/10.1007/978-3-319-](https://doi.org/10.1007/978-3-319-24945-2_7)
754 [24945-2_7](https://doi.org/10.1007/978-3-319-24945-2_7)

755 Rii, Y.M., Bidigare, R.R., Church, M.J., 2018. Differential Responses of Eukaryotic
756 Phytoplankton to Nitrogenous Nutrients in the North Pacific Subtropical Gyre. *Front. Mar.*
757 *Sci.* 5, 1–17. <https://doi.org/10.3389/fmars.2018.00092>

758 Rockström, J., Steffen, W., Noone, K., Persson, Å., Chapin, F.S., Lambin, E.F., Lenton, T.M.,
759 Scheffer, M., Folke, C., Schellnhuber, H.J., Nykvist, B., de Wit, C.A., Hughes, T., van der

760 Leeuw, S., Rodhe, H., Sörlin, S., Snyder, P.K., Costanza, R., Svedin, U., Falkenmark, M.,
 761 Karlberg, L., Corell, R.W., Fabry, V.J., Hansen, J., Walker, B., Liverman, D., Richardson,
 762 K., Crutzen, P., Foley, J.A., 2009. A safe operating space for humanity. *Nature* 461, 472–
 763 475. <https://doi.org/10.1038/461472a>

764 Rodríguez, L., Macías, F., 2006. Eutrophication trends in forest soils in Galicia (NW Spain)
 765 caused by the atmospheric deposition of nitrogen compounds. *Chemosphere* 63, 1598–
 766 1609. <https://doi.org/10.1016/j.chemosphere.2005.08.072>

767 Sanders, R.W., Gast, R.J., 2012. Bacterivory by phototrophic picoplankton and nanoplankton in
 768 Arctic waters. *FEMS Microbiol. Ecol.* 82, 242–253. [https://doi.org/10.1111/j.1574-](https://doi.org/10.1111/j.1574-6941.2011.01253.x)
 769 6941.2011.01253.x

770 Schirmer, M., Ijaz, U.Z., D’Amore, R., Hall, N., Sloan, W.T., Quince, C., 2015. Insight into
 771 biases and sequencing errors for amplicon sequencing with the Illumina MiSeq platform.
 772 *Nucleic Acids Res* 43. <https://doi.org/10.1093/nar/gku1341>

773 Seitzinger, S.P., Sanders, R.W., 1999. Atmospheric inputs of dissolved organic nitrogen
 774 stimulate estuarine bacteria and phytoplankton. *Limnol. Oceanogr.* 44, 721–730

775 Selosse, M.-A., Charpin, M., Not, F., 2017. Mixotrophy everywhere on land and in water: the
 776 grand écart hypothesis. *Ecol. Lett.* 20, 246–263. <https://doi.org/10.1111/ele.12714>

777 Shalchian-Tabrizi, K., Eikrem, W., Klaveness, D., Vaultot, D., Minge, M., Le Gall, F., Romari,
 778 K., Throndsen, J., Botnen, A., Massana, R., Thomsen, H., Jakobsen, K., 2006. *Telonemia*,
 779 a new protist phylum with affinity to chromist lineages. *Proc. R. Soc. B Biol. Sci.* 273,
 780 1833–1842. <https://doi.org/10.1098/rspb.2006.3515>

781 Shangguan, Y., Glibert, P.M., Alexander, J., Madden, C.J., Murasko, S., 2017. Phytoplankton
 782 assemblage response to changing nutrients in Florida Bay: Results of mesocosm studies. *J.*
 783 *Exp. Mar. Bio. Ecol.* 494, 38–53. <https://doi.org/10.1016/J.JEMBE.2017.05.006>

784 Siano, R., Montresor, M., Probert, I., Not, F., de Vargas, C., 2010. *Pelagodinium* gen. nov. and

785 *P. béii* comb. nov., a Dinoflagellate Symbiont of Planktonic Foraminifera. *Protist* 161,
786 385–399. <https://doi.org/10.1016/J.PROTIS.2010.01.002>

787 Teira, E., Hernando-Morales, V., Cornejo-Castillo, F.M., Alonso-Sáez, L., Sarmiento, H.,
788 Valencia-Vila, J., Serrano Catalá, T., Hernández-Ruiz, M., Varela, M.M., Ferrera, I.,
789 Gutiérrez Morán, X.A., Gasol, J.M., 2015. Sample Dilution and Bacterial Community
790 Composition Influence Empirical Leucine-to-Carbon Conversion Factors in Surface
791 Waters of the World's Oceans. *Appl. Environ. Microbiol.* 81, 8224–8232.
792 <https://doi.org/10.1128/AEM.02454-15>

793 Teira, E., Hernando-Morales, V., Martínez-García, S., Figueiras, F.G., Arbones, B., Álvarez-
794 Salgado, X.A., 2013. Response of bacterial community structure and function to
795 experimental rainwater additions in a coastal eutrophic embayment. *Estuar. Coast. Shelf*
796 *Sci.* 119, 44–53. <https://doi.org/10.1016/j.ecss.2012.12.018>

797 Teira, E., Martínez-García, S., Carreira, C., Morán, X., 2011. Changes in bacterioplankton and
798 phytoplankton community composition in response to nutrient additions in coastal waters
799 off the NW Iberian Peninsula. *Mar. Ecol. Prog. Ser.* 426, 87–104.
800 <https://doi.org/10.3354/meps09008>

801 Terrado, R., Monier, A., Edgar, R., Lovejoy, C., 2015. Diversity of nitrogen assimilation
802 pathways among microbial photosynthetic eukaryotes. *J. Phycol.* 51, 490–506.
803 <https://doi.org/10.1111/jpy.12292>

804 Unrein, F., Gasol, J.M., Not, F., Forn, I., Massana, R., 2014. Mixotrophic haptophytes are key
805 bacterial grazers in oligotrophic coastal waters. *ISME J.* 8, 164–76.
806 <https://doi.org/10.1038/ismej.2013.132>

807 Vitousek, P.M., Aber, J.D., Howarth, R.W., Likens, G.E., Matson, P.A., Schindler, D.W.,
808 Schlesinger, W.H., Tilman, D.G., 1997. Technical Report: Human Alteration of the Global
809 Nitrogen Cycle: Sources and Consequences. *Ecol. Appl.* 7, 737.
810 <https://doi.org/10.2307/2269431>

811 Worden, A.Z., Follows, M.J., Giovannoni, S.J., Wilken, S., Zimmerman, A.E., Keeling, P.J.,
812 2015. Rethinking the marine carbon cycle: Factoring in the multifarious lifestyles of
813 microbes. *Science* (80-.). 347. <https://doi.org/10.1126/science.1257594>

814 Zhang, J., Kobert, K., Flouri, T., Stamatakis, A., 2014. PEAR: A fast and accurate Illumina
815 Paired-End reAd mergeR. *Bioinformatics* 30, 614–620.
816 <https://doi.org/10.1093/bioinformatics/btt593>

817

818

Table 1

Table 1: Abiotic variables in surface water. Temperature (°C) (T), Salinity (S), Nitrate ($\mu\text{mol L}^{-1}$) (NO_3), Nitrite ($\mu\text{mol L}^{-1}$) (NO_2), Ammonium ($\mu\text{mol L}^{-1}$) (NH_4), Phosphate ($\mu\text{mol L}^{-1}$) (PO_4), Silicate ($\mu\text{mol L}^{-1}$) (SiO_2), Dissolved Organic Carbon ($\mu\text{mol L}^{-1}$) (DOC), Dissolved Organic Nitrogen ($\mu\text{mol L}^{-1}$) (DON), Dissolved Inorganic Nitrogen ($\mu\text{mol L}^{-1}$) (DIN), Nitrogen:Phosphate ratio (DIN/P), Humic-like Dissolved Organic Matter Fluorescence (ppb QS) (peak-M), Protein-like Dissolved Organic Matter Fluorescence (ppb TRP) (Peak-T), Chlorophyll-a total (mg L^{-1})(Chl), % Chlorophyll-a > 20 μm and Bacterial Biomass ($\mu\text{gC L}^{-1}$)(BB).

	April	June	October
Temperature (°C)	13.55	17.53	19.45
Salinity	34.92	35.32	34
Nitrate ($\mu\text{mol L}^{-1}$)	3.63	0.39	3.41
Nitrite ($\mu\text{mol L}^{-1}$)	0.28	0.01	0.24
Ammonium ($\mu\text{mol L}^{-1}$)	2.11	0.07	0.69
Phosphate ($\mu\text{mol L}^{-1}$)	0.84	0.03	0.25
Silicate ($\mu\text{mol L}^{-1}$)	5.92	1.25	6.23
Dissolved Organic Carbon ($\mu\text{mol L}^{-1}$)	91.19	79.57	91.65
Dissolved Organic Nitrogen ($\mu\text{mol L}^{-1}$)	8.67	6.77	9.45
Dissolved Inorganic Nitrogen ($\mu\text{mol L}^{-1}$)	6.01	0.47	4.34
Nitrogen:Phosphate ratio	7.12	17.1	17.64
Humic-like Dissolved Organic Matter Fluorescence (ppb QS)	1.99	1.12	1.83
Protein-like Dissolved Organic Matter Fluorescence (ppb TRP)	1.65	1.88	1.97
Chlorophyll-a total (mg L^{-1})	0.57	0.41	2.01
% Chlorophyll-a > 20 μm	16.76	12.23	4.93
Bacterial Biomass ($\mu\text{gC L}^{-1}$)	15.09	10.01	21.36

Table 2

Table 2: Richness (number of OTUs), Shannon-Wiener diversity, and Pielou's evenness in the different treatments and experiments obtained from the subsampled OTU table. (t0) initial sample, (CON) control, (INORG) inorganic treatment, (ORG) organic treatment, (MIX) mixed treatment.

	Small Eukaryotes								
	April			June			October		
	Richnes s	Shanno n	Pielo u	Richnes s	Shanno n	Pielo u	Richnes s	Shanno n	Pielou
t0	247	2.389	0.301	371	4.613	0.540	476	4.541	0.511
CON	273	3.804	0.470	376	4.657	0.544	480	4.933	0.554
INOR	270	3.936	0.487	360	4.609	0.543	466	4.657	0.525
G	317	4.245	0.511	344	4.374	0.519	466	4.757	0.537
ORG	303	4.036	0.490	275	4.466	0.551	391	4.075	0.473
MIX									

Figure Captions:

Figure 1: Response ratios calculated by dividing the mean chlorophyll-a concentration or bacterial biomass in each treatment by the corresponding value in the non-amended control. The treatments were (INORG) inorganic, (ORG) organic and (MIX) mixed. (a) total chlorophyll-a, (b) <20 μm chlorophyll-a, action), (c) 20-200 μm chlorophyll-a. (d) Bacterial biomass response ratio. Asterisks indicate statistically significant differences between each treatment and the control and error bars represent standard errors.

Figure 2: Relative contribution of reads to the major taxonomic groups of small eukaryotes in each experiment: (a) April, (b) June and (c) October. Each bar represents a treatment: initial time (t0), control (CON), inorganic nutrients (INORG), organic nutrients (ORG) and mixed (MIX).

Figure 3: Redundancy analysis (RDA) plot constrained to visualize the variance in OTU abundances explained by treatment: control (CON), inorganic (INORG), organic (ORG), and Mixed (MIX). Axes show the percent of the total variation described by each principal component.

Figure 4: Histograms of the CLR-transformed abundance of the major taxonomic groups at the end of the experiments in the mixed treatment (grey bars) and in the inorganic treatment (blue bars). The counts in the Y-axis represent the number of OTUs within each abundance class. Asterisks indicate statistically significant differences between mixed treatment and inorganic treatment using the Wilcoxon test. Light-grey and white shadowed plots indicate phytoplankton and heterotrophic groups, respectively.

Figure 5: CLR-transformed abundance at the end of the experiments of OTUs showing a significant response to at least one treatment. The crosses represent the CLR-transformed abundance of each experiment and the solid circle the average of the three experiments. The error bars represent the standard errors. The treatments were control (CON), inorganic (INORG), organic (ORG), and Mixed (M). Light-grey and white shadowed plots indicate

866 phytoplankton and heterotrophic groups, respectively. Asterisks in the treatment abbreviations
867 represent significant differences between that treatment and the corresponding control.