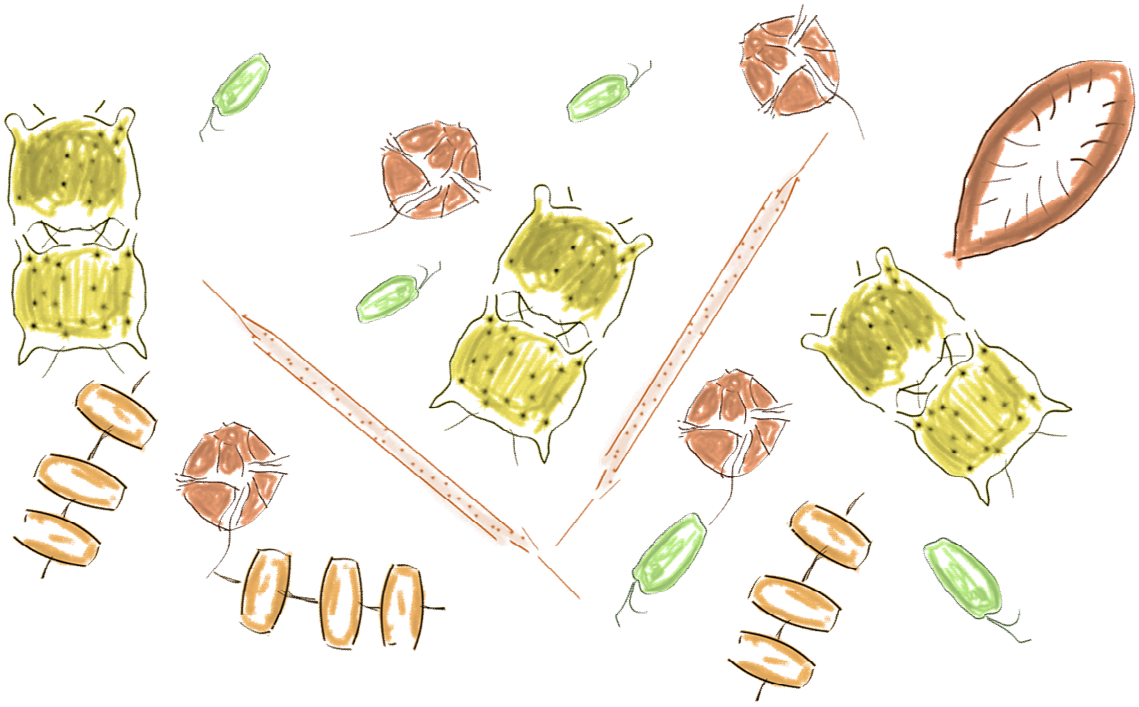
Interreg Project *Wasserqualität – Waterkwaliteit*



Analysis of the Dutch and German phytoplankton datasets of the Wadden Sea

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List of abbreviations

ABBREVIATIONS	FULL DESCRIPTION
BMU	Federal Ministry for the Environment
BOCHTVWTM	Monitoring station: "Bocht van Watum"
BOOMKDP	Monitoring station: "Boomkensdiep"
BORK_W_1	Monitoring station: "Westerems, Emshörn Rinne"
C	Carbon
CHL	Chlorophyll a
CN	Carbon to nitrogen ratio
DANTZGT	Monitoring station: "Dantziggat"
DE	Germany
DOOVBWT	Monitoring station: "Doove Balg west"
EC	European Community
ENS	Effective Number of Species
EU	European Union
GF/F	Glass fiber filter
GROOTGND	Monitoring station: "Groote Gat noord"
HIFMB	Helmholtz Institute for Functional Marine Biodiversity
HPLC	High-performance liquid chromatography
HUIBGOT	Monitoring station: "Huibertgat oost"
ICBM	Institut für Chemie und Biologie des Meeres
ICC	Intra Class Correlation
ID	Identification
JABU_W_1	Monitoring station: "Wilhelmshaven Mole"
LMM	Linear mixed effect model
LN	Natural logarithm
LOESS	Locally Weighted Least Squares Regression
MARSDND	Monitoring station: "Marsdiep noord"
MSFD	Marine Strategy Framework Directive
NA	missing values represented by the symbol NA (not available)
NB	An abbreviation for the Latin phrase nota bene, meaning “note well”
NL	Netherlands
NLWKN	Niedersächsische Landesbetrieb für Wasserwirtschaft, Küsten- und Naturschutz
NNEY_W_2	Monitoring station: "Norderney (HW)"
NP	Nitrogen to Phophorus ratio
OSPAR	Oslo and Paris Conventions ("OS" for Oslo and "PAR" for Paris)
PH3	Pelagic Habitat Indicator - OSPAR Comission
PIE	Probability of Interspecific Encounters
PSU	Practical Salinity Unit
ROTTMPT3	Monitoring station: "Rottumerplaat 3 km uit de kust"
ROTTMPT50	Monitoring station: "Rottumerplaat 50 km uit de kust"
ROTTMPT70	Monitoring station: "Rottumerplaat 70 km uit de kust"

S	Species richness
SEM	Structural equation model
SER	Species Exchange Ratio
SI	Silicon
SPM	Suspended particulate matter
TERSLG10	Monitoring station: "Terschelling 10 km uit de kust"
TERSLG100	Monitoring station: "Terschelling 100 km uit de kust"
TERSLG135	Monitoring station: "Terschelling 135 km uit de kust"
TERSLG175	Monitoring station: "Terschelling 175 km uit de kust"
TERSLG235	Monitoring station: "Terschelling 235 km uit de kust"
TERSLG4	Monitoring station: "Terschelling 4 km uit de kust"
TERSLG50	Monitoring station: "Terschelling 50 km uit de kust"
TN	Total Nitrogen
TP	Total Phosphorus
VIF	Variance Inflation Factor
WEMU_W_1	Monitoring station: "Wesermündung "
WFD	Water Framework Directive
ZUIDOLWOT	Monitoring station: "Zuid Oost Lauwers oost"

Introduction

Eutrophication has been and still is a major anthropogenic pressure on marine coastal systems, including the Wadden Sea. As part of the European Union Water Framework Directive (WFD 2000), phytoplankton is a quality criterion to assess the ecosystem status of the Wadden Sea. Indeed, in combination with nutrient data, the biomass and composition of phytoplankton can provide important information on the biodiversity and functioning of the Wadden Sea. To fulfill the reporting duties within WFD, monitoring programs have been established by Rijkswaterstaat for the Dutch part of the Wadden Sea and the Lower Saxony Water Management, Coastal Defence and Nature Conservation Agency (NLWKN), for the German part of the southern Wadden Sea. In 2019, funded by the EU Interreg V A program, both agencies initiated the Water Quality project (project #201265) to harmonize the assessment of phytoplankton and eutrophication in the Wadden Sea. This report summarizes the findings from this project with respect to data analysis of the phytoplankton long time monitoring data sets and statistical modelling. We start with a general overview of the data before we respond to guiding question from an assessment perspective. Based on a summary of these results, we provide recommendations for future improvements of the monitoring setup as well as data harmonization and accessibility.

General overview of the data

Monitoring stations & sampling

For this project, we analyzed long time series data from German and Dutch monitoring stations in the Wadden Sea (Fig. 1). We focus on the coastal stations and included data available for Dutch offshore stations only when comparing coastal and offshore dynamics. The stations TERSLG4 and ZUIDOLWOT were left out since the time series did not cover recent years (Table 1).

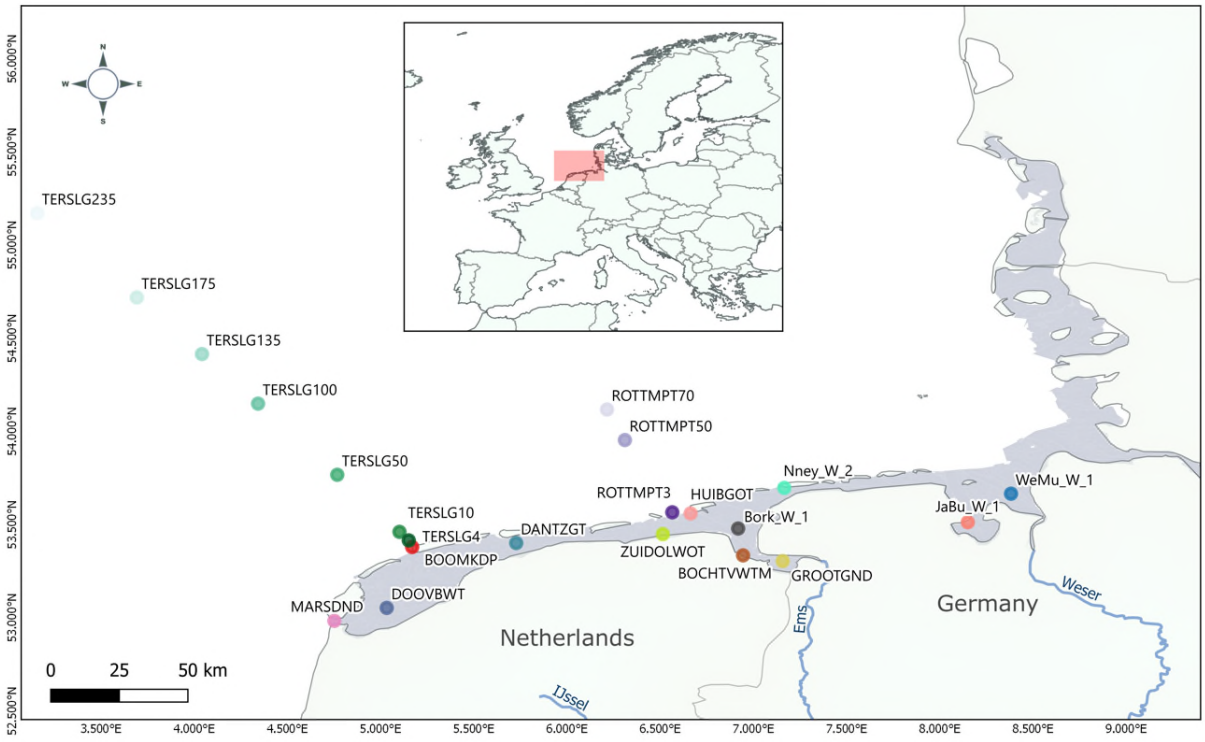


Fig. 1. Map of the study area, including the coastal stations (<50km distance from the coast-located in the grey area) and the offshore stations (>=50km distance from the coast). Data is available for four German stations (Bork_W_1, Nney_W_2, JaBu_W_1 and WeMu_W_1) and 18 Dutch stations.

We refrain from reiterating the strategy behind these monitoring stations and the details of sampling, which have been laid out elsewhere (Hanslik et al. 1998, Prins et al. 2012). We used the original data sets, but undertook a series of harmonization steps. First, we removed all species identified as purely heterotrophic. Second, we harmonized the species nomenclature between the two datasets (NL and DE). Third, species-specific biomass was estimated from biovolume using the C-conversion equations described by Menden-Deuer and Lessard (2000), which for diatoms with biovolumes >3000 µm^3 is 0.288 * Volume$^{0.811}$ = pgC cell^{-1}, for smaller diatoms and other groups: 0.216 * Volume$^{0.939}$ = pgC cell^{-1}.

Table 1. Monitoring stations in the Dutch and German monitoring scheme with information on whether they were included in all analyses or only when comparing to open North Sea conditions.

Country	Station ID	Location	Included	Observation
Netherlands	MARSDND	coastal	✓	
Netherlands	DOOVBWT	coastal	✓	
Netherlands	BOOMKDP	coastal	✓	
Netherlands	TERSLG4	coastal	✗	No data available after 2007
Netherlands	TERSLG10	coastal	✓	
Netherlands	TERSLG50	offshore	comparison	
Netherlands	TERSLG100	offshore	comparison	
Netherlands	TERSLG135	offshore	comparison	
Netherlands	TERSLG175	offshore	comparison	
Netherlands	TERSLG235	offshore	comparison	
Netherlands	DANTZGT	coastal	✓	
Netherlands	ZUIDOLWOT	coastal	✗	No data available after 2009
Netherlands	ROTTMPT3	coastal	✓	
Netherlands	ROTTMPT50	offshore	comparison	
Netherlands	ROTTMPT70	offshore	comparison	
Netherlands	HUIBGOT	coastal	✓	
Netherlands	BOCHTVWTM	coastal	✓	
Netherlands	GROOTGND	coastal	✓	
Germany	Bork_W_1	coastal	✓	
Germany	Nney_W_2	coastal	✓	
Germany	JaBu_W_1	coastal	✓	
Germany	WeMu_W_1	coastal	✓	No winter samples

Sampling frequency and annual medians

The sampling effort for each **coastal station** varied considerably across stations and years, in both environmental (Fig. 2) and phytoplankton (Fig. 3) monitoring data sets. In Bork_W_1 for example, the environmental parameters were only sampled in winter and autumn from 1994-2010 and sometimes in spring. From 2011 onwards, the station was sampled also in summer but then in a lower frequency in the winter months (Fig. 2). In this same station, phytoplankton samples were taken over the four seasons from 2007-2010, then only in spring, summer and autumn (Fig. 3). WeMu_W_1 was never sampled in winter. In some stations the sampling frequency increased over time (ex. In DOOVBWT), while in other stations it decreased over time (ex. GROOTGND and HUIBGOT). Also, the number of sampled months per season varied across stations and years. In general, the NL stations have longer time series data, especially for environmental variables, than the DE stations.

Given the discrepancies in sampling and given that the main questions of this report address long-term trends in phytoplankton biomass and biodiversity, we calculated annual medians for each parameter. The median is superior to the mean for this purpose as it is unaffected by extreme outliers and non-normal distributions of the data.

Fig. 2. Sampling frequency for the water quality parameters of the Wadden Sea stations from 1970-2020. The bars represent the number of samples per year. Each segment of the bars represents one month sampled and the colors represent the season: *winter* = Dec, Jan, Feb; *spring* = Mar, Apr, May; *summer* = Jun, Jul, Aug; *autumn* = Sep, Oct, Nov.

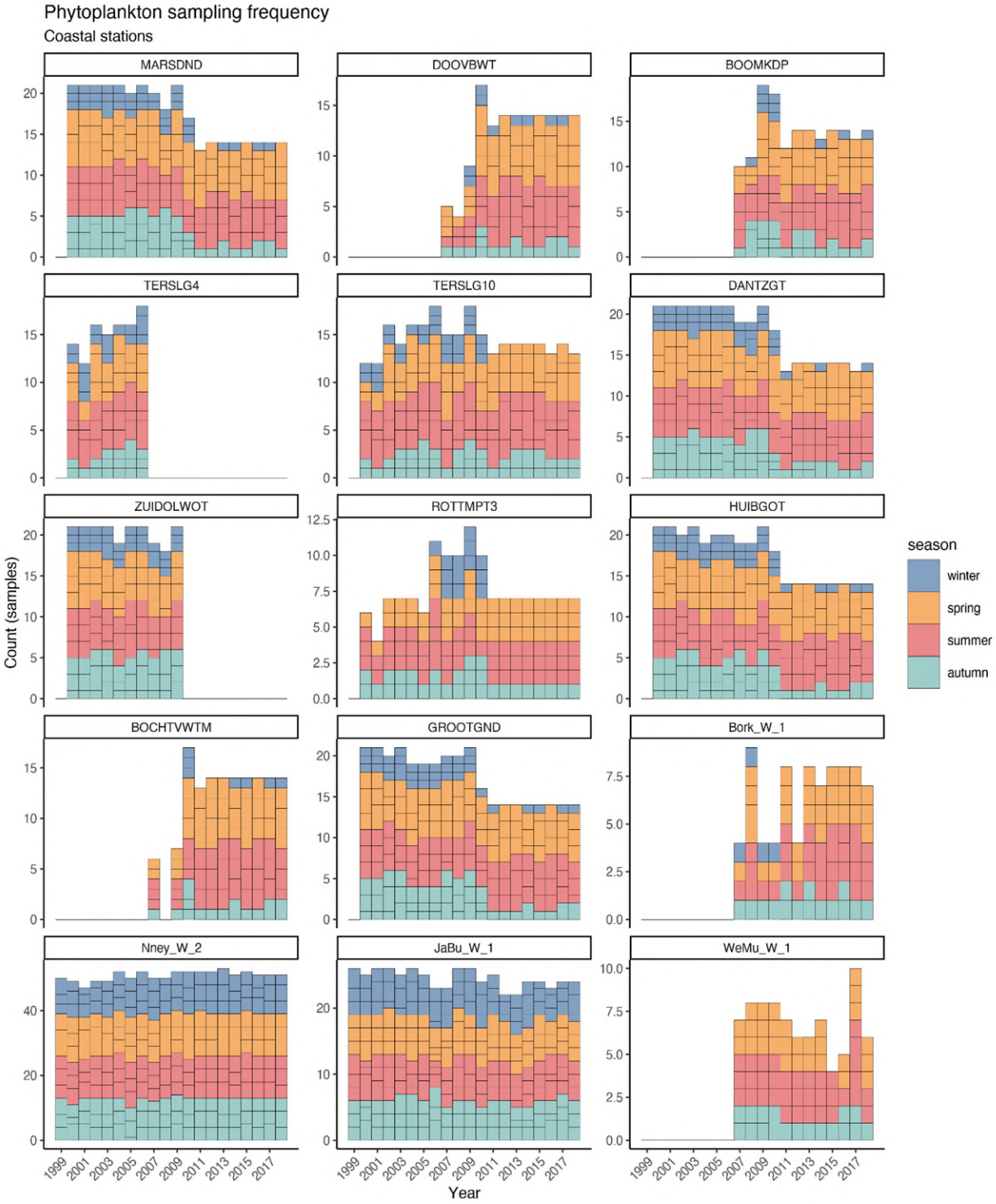


Fig. 3. Sampling frequency for phytoplankton abundance of the Wadden Sea stations from 1999-2018. The bars represent the number of samples per year. Each segment of the bars represents one month sampled and the colors represent the season: *winter* = Dec, Jan, Feb; *spring* = Mar, Apr, May; *summer* = Jun, Jul, Aug; *autumn* = Sep, Oct, Nov.

Temporal trends of environmental parameters and phytoplankton

In order to show both the temporal trends for each station, for the DE and NL monitoring programs, and for the entire data sets, we combined two different analytical approaches. First, we present the annual median per station and visualize the trend over time using a LOESS regression (Locally Weighted Least Squares Regression). The same is also done for DE and NL

data to test whether the temporal dynamics are different between countries. Second, the formal test for temporal changes relies on a linear mixed effect model (LMM, more information on page 21), where the response variable is a function of year as fixed effect and (1|StationID) as random effect. The random effect allows for different intercepts for the stations but tests for a joint (common) slope with time. It therefore explicitly tests whether the response variable shows a joint and significant linear trend across all stations. The LMMs were performed in R using the lme4 package (Bates et al. 2015). To normalize data distribution, we calculated the natural log of the annual median of each environmental and biomass parameter except for temperature, pH and salinity.

Environmental variables over time

Total nitrogen (TN) and total phosphorus (TP) varied over several orders of magnitude between the most nutrient rich stations (GROOTGND and BOCHTVWTM) and the nutrient poorest (TERSLG10), but at each station both TN and TP significantly decreased over time (Fig. 4a, c). Consequently, we observed strong significant declines in TN and TP (Table 2), which did not differentiate much between NL and DE stations (Fig. 4b, d). This decrease, which encompassed almost an order of magnitude across the last 50 years, was proportionally larger for TP than for TN. Consequently, the molar N:P ratio significantly increased within and across stations (Fig. 4e, f, Table 2), exceeding previously reported high N:P ratios from the 70s. The molar N:P ratio was constantly higher than the 16:1 Redfield ratio or the molar N:P ratio of 22:1 (Guildford and Hecky 2000), indicating a tendency towards increasing P-limitation close to the coast (Burson et al. 2016). For Si, we see a similar broad range of concentrations as for TN and TP between stations (Fig. 4g, h), but the temporal trend is much more subtle albeit overall negative (Table 2). Again, the two low salinity stations GROOTGND and BOCHTVWTM had the highest Si-concentrations, TERSLG10 the lowest (see Fig. S1 for untransformed data per station).

Suspended particulate matter (SPM) varied among the stations, again with GROOTGND and BOCHTVWTM having highest and TERSLG10 lowest concentrations (Fig. 5a). The overall temporal decline in SPM (Table 2) was steeper in DE than NL stations (Fig. 5b). Salinity increased with time (Table 2), which however was mainly visible in the Dutch stations and here the station with lowest salinity (GROOTGND) (Fig. 5c, d). The overall temperature increase of 0.4°C per decade indicates a strong warming effect (Fig. 5e,f, Table 2), which is significantly faster than for the open North Sea, which warmed by 1.3 °C from 1969 to 2017 (UBA 2019). The warming trend was generally consistent for the stations with the exception of a more variable trend at ROTTMPLT3 and WeMu_W_1 (Fig. S2). A significant but small increase in pH became visible across the NL stations, whereas the DE stations rather declined in pH over time (Fig. 5 g,h, Table 2).

When including the random effects, the LMM explained 35-92 % of the variation in environmental variables (conditional R^2 in Table 2). The temporal trends were strongest for TN, TP and their ratio as well as temperature (marginal R^2 in Table 2), whereas for SPM, Si and salinity the explained variance by the common linear term is <1%. Thus, in addition to direct human impacts on the Wadden Sea (fisheries, shipping, tourism), the ecosystem is characterized by massive multifactorial changes in the abiotic conditions, where current nutrient levels are lower than during the last 50 years, but temperatures and N:P ratios are higher than previously recorded.

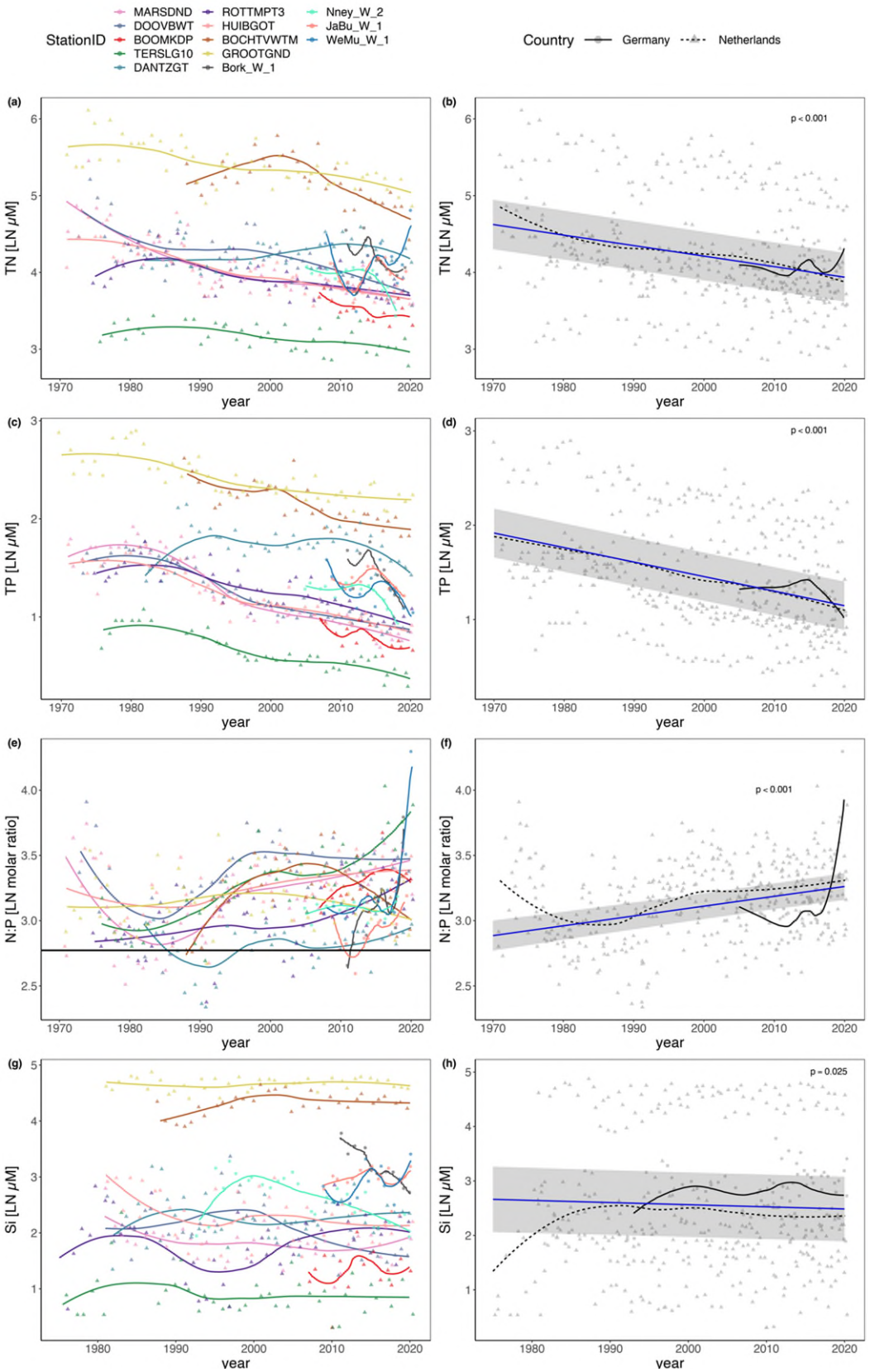


Fig. 4. Temporal trend of nutrient concentrations at the Wadden Sea coastal stations for N, P, their ratio and Si. Left column: Annual means and LOESS trend lines colored by station. Right column: Overall predicted time effects from the LMM (blue line) with their confidence interval (grey shaded area) as well as separate LOESS trends for German and Dutch stations (DE: continuous line; NL: dashed line). *Data are LN transformed, see figure S1 in the Appendix for untransformed data.*

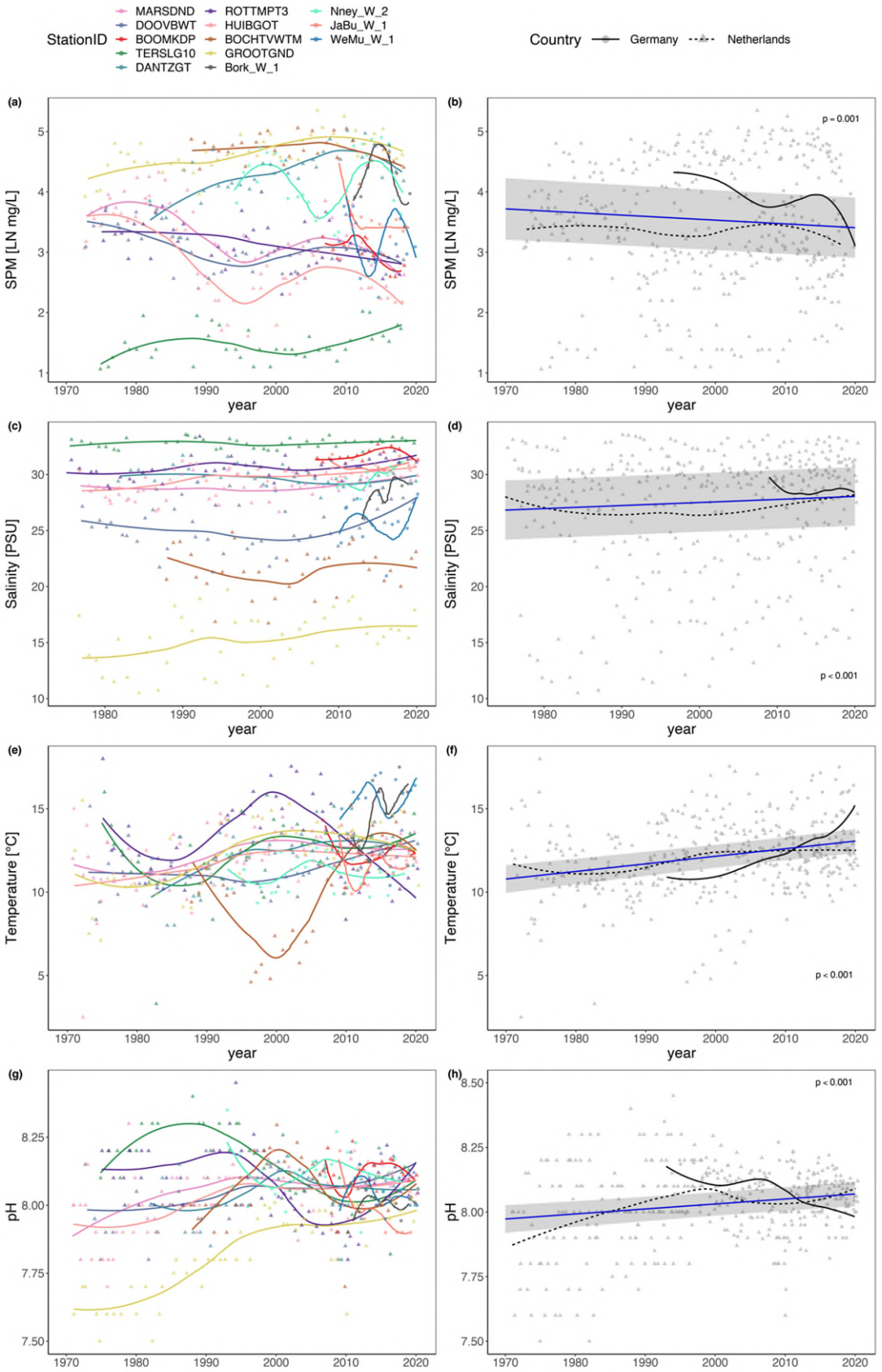


Fig. 5. Temporal trend of environmental factors at the Wadden Sea coastal stations. Left column: Annual means and LOESS trend lines colored by station. Right column: Overall predicted time effects from the LMM (blue line) with their confidence interval (grey shaded area) as well as separate LOESS trends for German and Dutch stations (DE: continuous line; NL: dashed line). *Data of SPM is LN transformed, see figure S1 in the Appendix for untransformed data*

Table 2. Results of the linear mixed effect model (LMM) to analyze temporal trends of environmental parameters over the years, considering "StationID" as a random effect. For each response variable, we give estimates for intercept (year = 0) and slope (increase or decrease per year) as well as their significance as fixed effects. For random effects, we give the residual variance (σ^2), the variance associated to the random terms (τ_{00}), the intra class correlation (ICC, how much of the overall variance is connected to the random term), and the number of stations (N). The full number of observations and the marginal and conditional R^2 values are given.

	LN.TN		**LN.TP**		**LN.NP**		**LN.Si**	
Predictors	*Estimates*	*p*	*Estimates*	*p*	*Estimates*	*p*	*Estimates*	*p*
(Intercept)	31.667	**<0.001**	32.264	**<0.001**	-11.938	**<0.001**	10.426	**0.003**
year	-0.014	**<0.001**	-0.015	**<0.001**	0.008	**<0.001**	-0.004	**0.025**
Random Effects								
σ^2	0.05		0.02		0.05		0.14	
τ_{00}	0.33 $_{StationID}$		0.22 $_{StationID}$		0.03 $_{StationID}$		1.17 $_{StationID}$	
ICC	0.86		0.91		0.36		0.89	
N	13 $_{StationID}$		13 $_{StationID}$		13 $_{StationID}$		13 $_{StationID}$	
Observations	407		411		407		392	
Marginal R^2 / Conditional R^2	0.086 / 0.877		0.160 / 0.921		0.115 / 0.429		0.002 / 0.890	

	LN.SPM		**Salinity**		**Temperature**		**pH**	
Predictors	*Estimates*	*p*	*Estimates*	*p*	*Estimates*	*p*	*Estimates*	*p*
(Intercept)	15.965	**<0.001**	-26.849	**0.035**	-78.822	**<0.001**	4.157	**<0.001**
year	-0.006	**0.001**	0.027	**<0.001**	0.045	**<0.001**	0.002	**<0.001**
Random Effects								
σ^2	0.19		2.07		3.45		0.01	
τ_{00}	0.82 $_{StationID}$		22.94 $_{StationID}$		1.44 $_{StationID}$		0.01 $_{StationID}$	
ICC	0.82		0.92		0.29		0.33	
N	13 $_{StationID}$		13 $_{StationID}$		13 $_{StationID}$		13 $_{StationID}$	
Observations	401		385		427		427	
Marginal R^2 / Conditional R^2	0.007 / 0.816		0.005 / 0.918		0.074 / 0.346		0.035 / 0.351	

Phytoplankton biomass over time

Different stations showed substantial differences both in the average phytoplankton carbon biomass and their temporal trends (Fig. 6a). Aggregating at the country level, we find that carbon biomass is two orders of magnitude higher in NL than DE (Fig. 6b). Reasons for this discrepancy are detailed and discussed in Box 3 in the section on assessments. The two countries also show different temporal dynamics with an order of magnitude increase in C biomass in NL between 1999 and 2014, followed by a slight decrease. In DE we find a decline in C biomass, if any trend. As more NL than DE stations are monitored, the overall trend turns out to be significantly positive but with low explanatory power (Table 3).

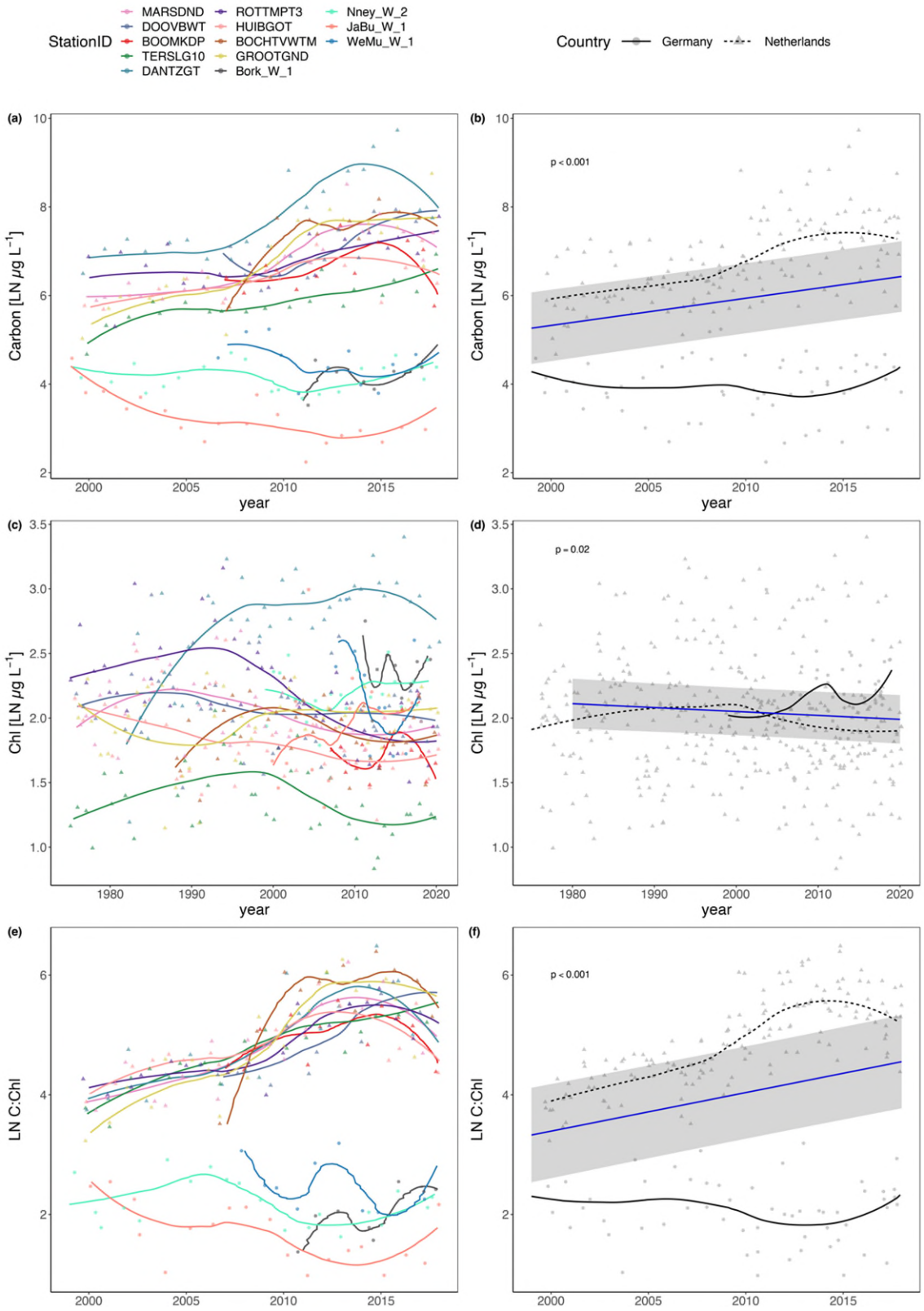


Fig. 6: Temporal trend of the phytoplankton biomass measured as carbon (**a**,**b**), chlorophyll *a* (**c**,**d**) and the C:Chl ratio (**e**,**f**) at the Wadden Sea coastal stations. Left column: Annual means and LOESS trend lines colored by station. Right column: Overall predicted time effects from the LMM (blue line) with their confidence interval (grey shaded area) as well as separate LOESS trends for German and Dutch stations (DE: continuous line; NL: dashed line). *Data are LN transformed see figure S3 in the Appendix for untransformed data*

The temporal trend for chlorophyll biomass is negative but even less prominent (Table 3), as single stations show little consistent variation, some declining since the late 1980s to early 1990s, others increasing or fluctuating (Fig. 6c,d, Table 3). In contrast to C-biomass, chlorophyll *a* didn't show strong differences between countries, but exhibited major variance among the stations (Fig. 6c,d). The C:Chl ratio significantly increased over time (Fig. 6e,f, Table 3), solely based on the NL stations and thus reflecting the increase in carbon biomass in NL.

Table 3. Results of the linear mixed effect mode, analyzing the change in phytoplankton biomass over the years, considering "StationID" as a random effect. For each response variable, we give estimates for intercept (year = 0) and slope (increase or decrease per year) as well as their significance as fixed effects. For random effects, we give the residual variance (σ^2), the variance associated to the random terms (τ_{00}), the intra class correlation (ICC, how much of the overall variance is connected to the random term), and the number of stations (N). The full number of observations and the marginal and conditional R^2 values are given.

	LN Carbon L-1		**LN Chl L-1**		**LN C:Chl**	
Predictors	*Estimates*	*p*	*Estimates*	*p*	*Estimates*	*p*
(Intercept)	-117.473	**<0.001**	8.124	**0.002**	-125.592	**<0.001**
year	0.061	**<0.001**	-0.003	**0.021**	0.064	**<0.001**
Random Effects						
σ^2	0.33		0.09		0.30	
τ_{00}	2.06 $_{StationID}$		0.11 $_{StationID}$		1.96 $_{StationID}$	
ICC	0.86		0.54		0.87	
N	13 $_{StationID}$		13 $_{StationID}$		13 $_{StationID}$	
Observations	209		408		206	
Marginal R^2 / Conditional R^2	0.044 / 0.870		0.007 / 0.540		0.050 / 0.873	

Phytoplankton biodiversity over time

We explain our approach to biodiversity analysis in Box 1, which also details how these metrics link to MSFD and OSPAR indicator discussions. Both metrics of standing diversity, richness (S) and effective number of species (ENS), decline with time across stations (Table 4), but this negative trend is only associated to the Dutch stations, which in general report more species, but lower ENS. However, this difference by country may be an artefact from DE stations being sampled over a shorter period of time: in many stations across countries, richness and ENS peaks around 2007 to 2009, then declines until 2012, after which diversity increases again, also in the NL stations (Fig. 7a,b). However, a few stations (HUIBGOT, GROOTGND) show monotonic richness declines. Annual ENS varies across stations similarly to richness, but also shows an increase in most of the stations after 2012 (Fig. 7c,d).

Turnover between neighbouring years is variable over time but does neither speed up nor slow down (Fig. 8a-d, Table 4). When accumulating over time, a clear pattern of increasing turnover becomes visible (Fig. 8e-h). Turnover is larger in the DE stations and consistently increases with increasing temporal distance for both *richness-based species exchange ratio* (SERr, see box 1 page 19-20) and *abundance-based species exchange ratio* (SERa, see box 1 page 19-20). The consistency of the pattern indicates that turnover does involve both shifts in species identity and dominance. It is also noteworthy, that the bottom right corner of the diagram is void of data, indicating that there is no "return" to a previous assemblage over long time scales, indicating a strongly directional compositional drift over time.

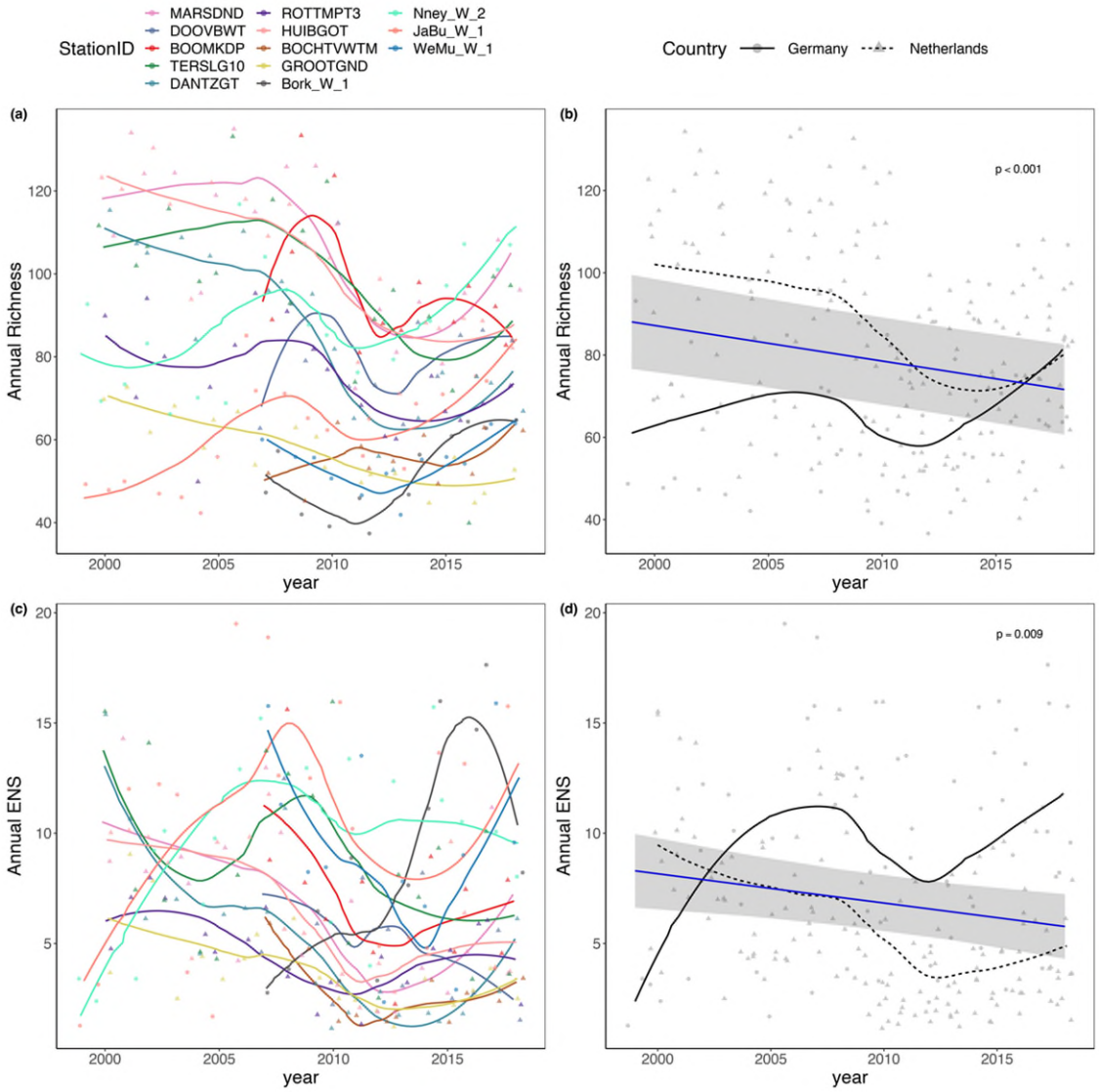


Fig. 7: Temporal trend of the phytoplankton standing diversity: richness (a, b) and effective number of species (c,d) at the Wadden Sea coastal stations. Left column: Annual means and LOESS trend lines colored by station. Right columns: Overall predicted time effects from the LMM (blue line) with their confidence interval (grey shaded area) as well as separate LOESS trends for German and Dutch stations (DE: continuous line; NL: dashed line). *Data are LN transformed.*

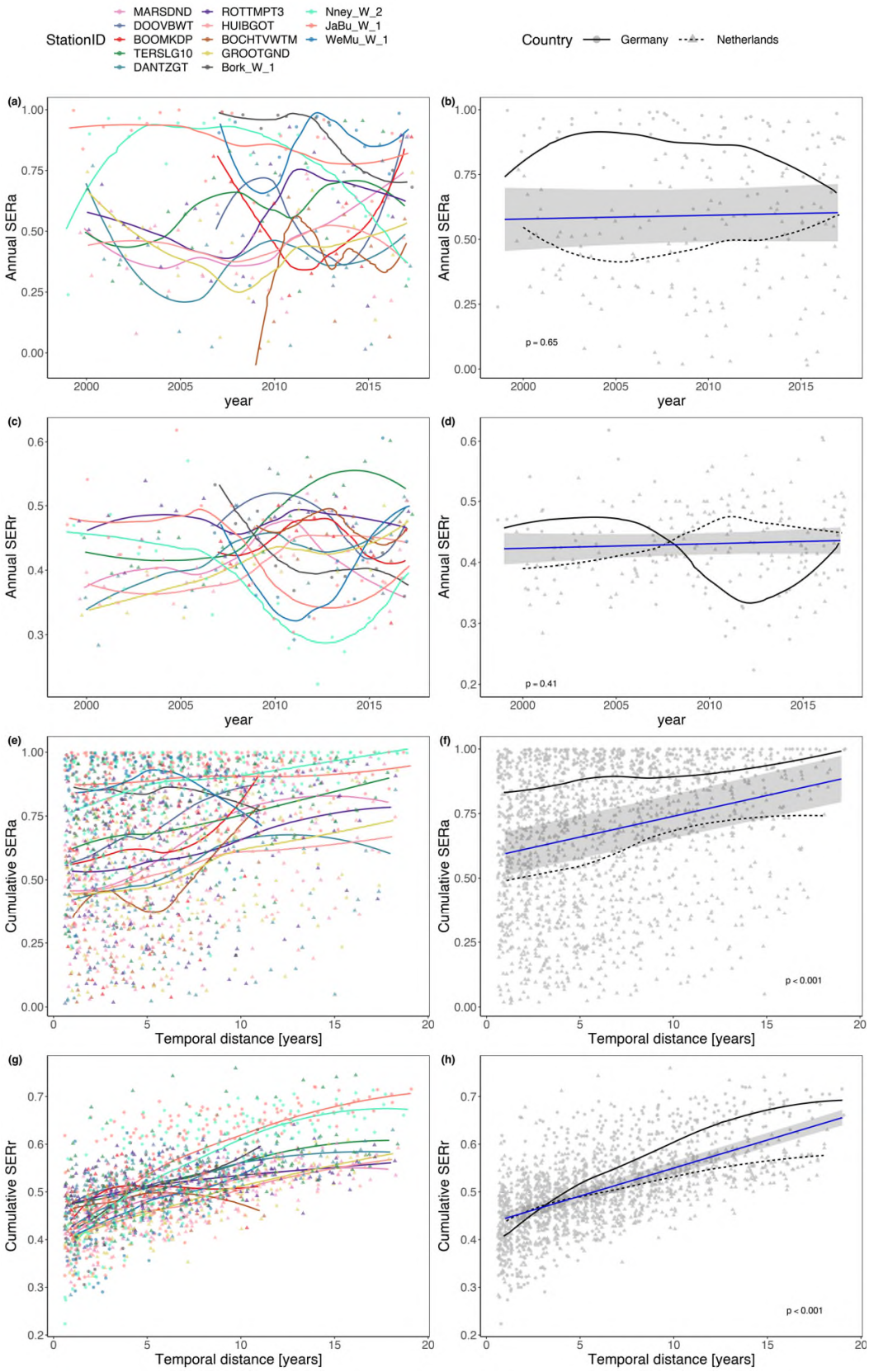


Fig. 8: Temporal trend of the phytoplankton turnover (SERa and SERr) at the Wadden Sea coastal stations. Left column: Annual means and LOESS trend lines colored by station. Right column: Overall predicted time effects from the LMM (blue line) with their confidence interval (grey shaded area) as well as separate LOESS trends for German and Dutch stations (DE: continuous line; NL: dashed line).

Table 4. Results of the linear mixed effect model, analyzing the change in phytoplankton diversity and turnover over time (year) and the cumulative turnover between years (dist). Station ID is included as random effect. All details as in Table 2. Please not that for the cumulative turnover, the predictor is not year but temporal distance in years.

	Annual Richness		**Annual ENS**		**SERa**		**SERr**		**Cumulative SERa**		**Cumulative SERr**	
Predictors	*Estimates*	*p*	*Estimates*	*p*	*Estimates*	*p*	*Estimates*	*p*	*Estimates*	*p*	*Estimates*	*p*
(Intercept)	1815.494	**<0.001**	273.709	**0.007**	-2.271	0.721	-1.073	0.556	0.579	**<0.001**	0.433	**<0.001**
year	-0.864	**<0.001**	-0.133	**0.009**	0.001	0.653	0.001	0.410				
dist									0.016	**<0.001**	0.012	**<0.001**
Random Effects												
σ^2	207.12		13.94		0.05		0.00		0.05		0.00	
τ_{00}	360.31 $_{StationID}$		4.32 $_{StationID}$		0.03 $_{StationID}$		0.00 $_{StationID}$		0.02 $_{StationID}$		0.00 $_{StationID}$	
ICC	0.63		0.24		0.40		0.16		0.34		0.15	
N	13 $_{StationID}$		13 $_{StationID}$		13 $_{StationID}$		13 $_{StationID}$		13 $_{StationID}$		13 $_{StationID}$	
Observations	213		213		199		199		1725		1725	
Marginal R^2 / Conditional R^2	0.036 / 0.648		0.027 / 0.257		0.001 / 0.405		0.003 / 0.162		0.064 / 0.380		0.382 / 0.472	

Functional group biomass over time

We calculated the annual biomass of each functional group – diatoms, dinoflagellates, flagellates, cyanobacteria and *Phaeocystis* – as the sum of carbon biomass per sample, then calculated the annual median. Diatoms had the highest biomass over all functional groups and additionally showed the clearest trend, with increasing biomass over the years at most of the NL stations (Fig. 9a,b, Table 5). Dinoflagellates showed no overall trend as their biomass varied across the stations. In some of the DE stations it presented a similar pattern to the diversity measures, with a steep drop followed by an increase in biomass in some stations (Fig. 9c,d). Flagellates increased over time in most of the stations (Fig. 9e,f), whereas cyanobacteria significantly declined over time (Fig 9.g,h). *Phaeocystis* increased, consistently in the NL stations, whereas a more non-linear pattern prevailed in the DE stations (Fig. 9i,j).

Biomass of dominant species over time

We also analyzed the species contribution to biomass over time in the Wadden Sea stations. We first calculated the annual mean biomass of each taxa and analyzed their relative biomass over years (stacked bars in Fig. 10). Taxa with less than 20% of relative biomass were colored in grey, but still separated by the black horizontal lines on each bar. In the NL stations, most of the years were dominated by the diatom genus *Thalassiosira sp*., reaching up to 90% of the total biomass in some years and stations (e.g., in DANTZGT, year 2010 and ROTTMPT3, year 2011). In the DE stations we observed a higher diversity of taxa contributing to biomass over the years, with a few years being dominated by *Phaeocystis sp.* (Fig. 10). Therefore, turnover seems to affect more the dominant species in the DE stations than in the NL stations.

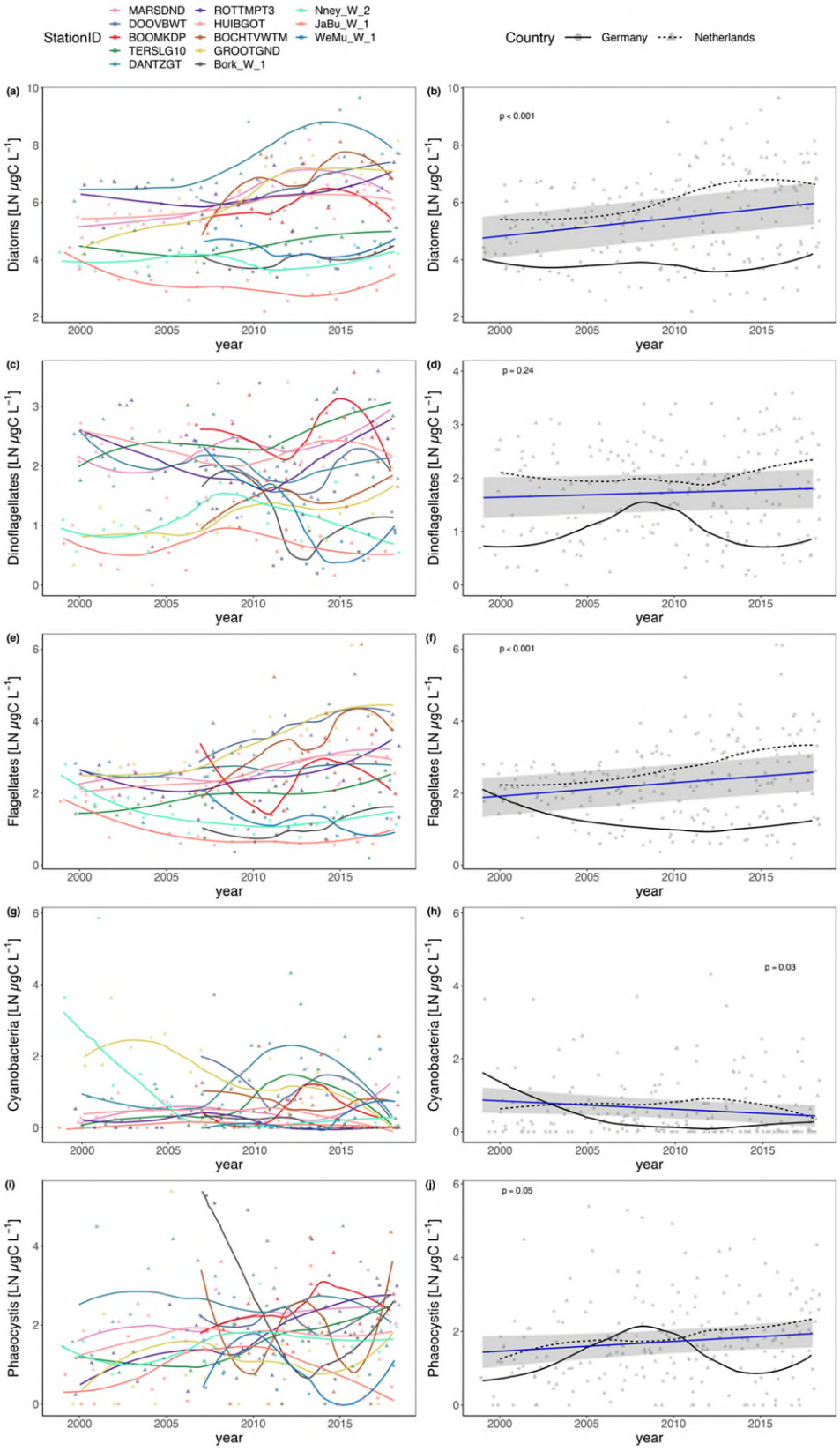


Fig. 9: Temporal trend of the phytoplankton functional groups measured as the yearly biomass median of Diatoms (**a**,**b**), Dinoflagellates (**c**,**d**), Flagellates (**e**,**f**), Cyanobacteria (**g**,**h**) and *Phaeocystis* (**i**,**j**) at the Wadden Sea coastal stations. Left column: Annual means and LOESS trend lines colored by station. Right columns: Overall predicted time effects from the LMM (blue line) with their confidence interval (grey shaded area) as well as separate LOESS trends for German and Dutch stations (DE: continuous lines; NL: dashed lines). *Data input: annual median*

Table 5. Results of the linear mixed effect model, analyzing the change in phytoplankton functional groups' biomass (LN µgC L^{-1}) over years. Station ID is included as random effect. All details as Table 2.

	Diatoms		**Dinoflagellates**		**Flagellates**		**Cyanobacteria**		**Phaeocystis**	
Predictors	*Estimates*	*p*	*Estimates*	*p*	*Estimates*	*p*	*Estimates*	*p*	*Estimates*	*p*
(Intercept)	-122.741	**<0.001**	-16.266	0.288	-71.945	**<0.001**	46.554	**0.032**	-51.467	0.062
year	0.064	**<0.001**	0.009	0.239	0.037	**<0.001**	-0.023	**0.034**	0.026	0.054
Random Effects										
σ^2	0.45		0.32		0.39		0.64		1.04	
τ_{00}	1.69 $_{StationID}$		0.38 $_{StationID}$		0.82 $_{StationID}$		0.17 $_{StationID}$		0.26 $_{StationID}$	
ICC	0.79		0.55		0.68		0.21		0.20	
N	13 $_{StationID}$		13 $_{StationID}$		13 $_{StationID}$		13 $_{StationID}$		13 $_{StationID}$	
Observations	213		213		213		213		213	
Marginal R^2 / Conditional R^2	0.052 / 0.802		0.003 / 0.546		0.031 / 0.688		0.018 / 0.221		0.015 / 0.213	

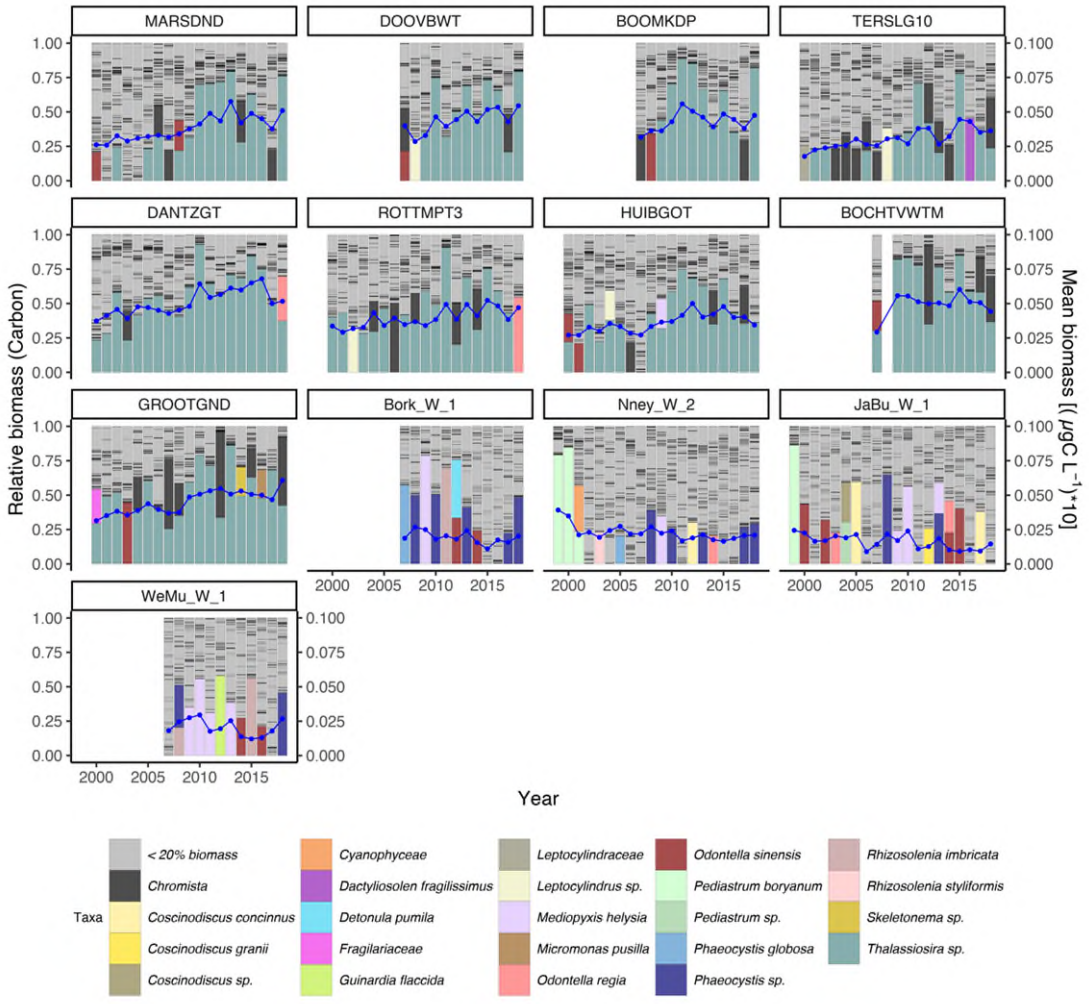


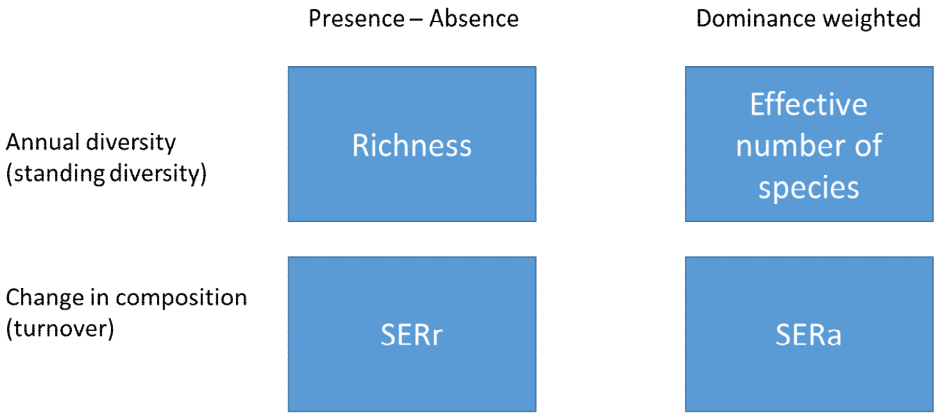
Fig. 10. Relative carbon biomass of the phytoplankton taxa over time in the Wadden Sea stations (bars). Taxa with less than 20% of biomass contribution per year were grouped and colored in grey. Annual mean biomass is shown on the right axis (blue line).

Box 1: How to analyse biodiversity in monitoring data

Biodiversity assessments need a multivariate approach as no single variable captures even the most important aspects of community composition and change (Rombouts et al. 2019). For the MSFD requirements, Rombouts et al. (2019) recommended a multivariate approach, which we in general follow with some modifications to reflect recent findings on statistical performance of these metrics (Chase and Knight 2013, Hillebrand et al. 2018).

In essence, our approach consists of a 2 x 2 combination of different assessment goals: First, we want to measure both the gross and net component of biodiversity change. Gross means that between time points, the composition changes by new species arriving (colonizations) or disappearing (extinctions) and species becoming rare or dominant. If colonizations = extinctions, the net outcome of this overall compositional change will be neutral, but if one prevails over the other, standing diversity increases or decreases. Thus, in line with recommendations for MSFD and the OSPAR indicator "Changes in Plankton Diversity" (PH3) (Rombouts et al. 2019), we combine the assessment of alpha (= standing) diversity with analyses of temporal beta (turnover) diversity.

Second, both net and gross biodiversity change can be measured on the basis of species' presence and absence or on the basis of species' relative proportion in the community. The latter mainly reflects the dominant species in a community, the former the change in the many rare species.



This leads to the following metrics:

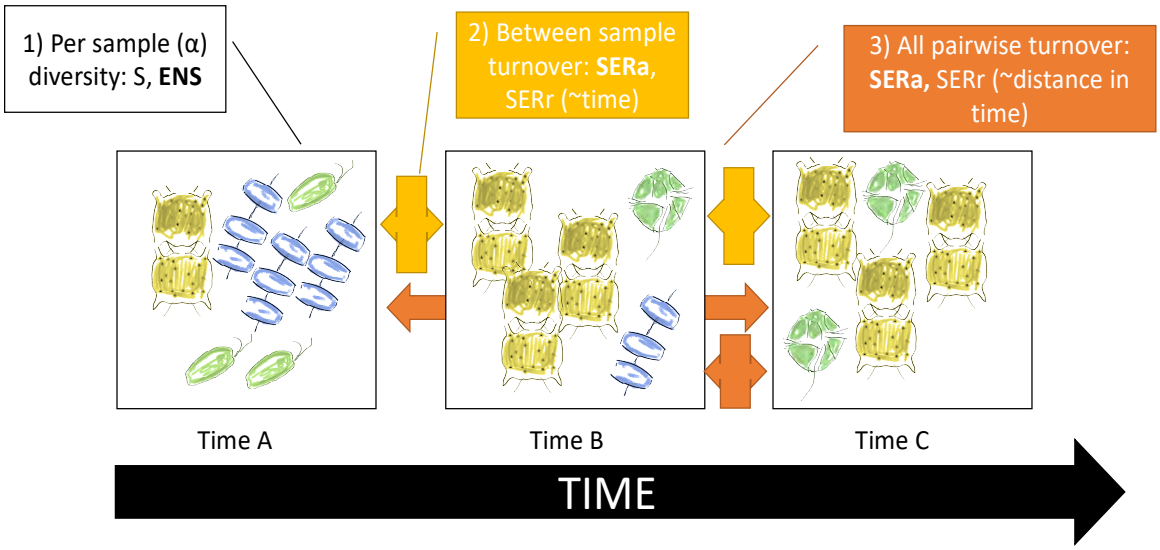
- Annual richness (S), the number of species occurring in a single year. We use S despite its use being limited by the fact that it is highly effort-dependent, i.e. more samples in a year, higher abundance of phytoplankton in a sample, more even dominance of species in a sample, more complete assessment of a sample all alter the estimate for S substantially (Chase and Knight 2013, Hillebrand et al. 2018). However, it treats rare and dominant species equally and thus especially reflects changes in the presence or absence of rare species. Moreover, S is easy communicable as the number of species present is a metric not requiring any further explanation.
- As dominant-weighted measure of standing diversity, we used **ENS**, the effective number of species. It is related to the probability of interspecific encounters (PIE, the likelihood that two random individuals belong to different species). PIE is an entropy and related to the Hurlbert metric proposed by Rombouts et al. (2019), but ENS has been shown to be the most robust metric if sampling and abundances (Chase and Knight 2013). ENS equals the number of species you would encounter in an assemblage having the same entropy (PIE) but if all species were equally abundant. It can be envisioned as the number of species effectively taking part in the community. This analysis was based on the median biomass per year, i.e, all species occurring at least once during a year contributed to annual richness and ENS.

Box 1 continued

We follow Rombouts et al (2019) in promoting that the analysis of standing diversity needs to be amended by an analysis of temporal turnover in community composition. However, we differentiate from Rombouts by pinpointing towards the fact that – in order to compare the gross to net changes in biodiversity – the employed metrics should weigh dominance in the same two different ways as S and ENS do. These measures thus correspond to richness and ENS, but in contrast to these measures of "annual diversity", SERr and SERa capture the difference in diversity between years.

- **SERr,** richness-based species exchange ratio, is a metric relying on presence and absence of species (as richness). It is identical to Jaccard dissimilarity that is often used in a spatial context and captures the proportion of the joint species from two time points that are NOT shared. Thus, if all species of time A also occur at time B, SERr = 0, if half of the species occur at one time point only, SERr = 0.5, and if time B has no species in common with time A, SERr = 1.0.
- **SERa** is the abundance-based species exchange ratio, thus the more dominant a species is, the more it influences turnover by going from rare (or absent) to dominant or from dominant to rare (or extinct). SERa also range from 0-1, with 1 = all dominant species exchanged. Like ENS, SERa weights dominance based on Simpson dominance (Hillebrand et al. 2018).

Both SERr and SERa turnover can be used for two different purposes: First, we measured **annual (immediate) turnover**, which compares consecutive years and thus reflects whether turnover from one year to the next becomes faster or slower with time. Second, we used **cumulative turnover**, which compares all samples to each other and relates this to temporal distance between the years, it thus reflects whether changes in composition continue (linear relationship between cumulative turnover and distance) or whether previous assemblages are found again (non-linear relationships returning to lower SERr or SERa at the end).



Phytoplankton, environmental parameters and nutrients

In this section, we evaluate four key questions on the relationship between aspects of phytoplankton, the environment and the assessment of the Wadden Sea Ecosystem. We combine a sequence of analyses that progress from simple bivariate correlations to more rigid multivariate analyses, as all of these analyses have (dis-)advantages and a transparent consideration of these approaches provides a more reliable basis to address the key questions.
1: **Correlations**. We use Pearson correlations between environmental variables and phytoplankton parameters across all Wadden Sea coastal stations. We provide correlation coefficients and their significance for all data as well as separately for NL and DE data, as some variables have strong differences between countries (e.g., C-biomass). This approach gives simple bivariate relationship for each combination of variable that can easily be interpreted as the correlation coefficient ranges between -1 and + 1. It has a couple of shortcomings, though: We perform multiple independent tests, which inflates the risk of finding false significant correlations. Additionally, a correlation between X and Y does not necessarily mean a causal link, as both might in fact relate to a third variable Z.
2: **Linear mixed effect models** (LMM). LMMs overcome both of these problems: The response variable is modelled as a function of multiple predictors, thus covariance is taken into account and the overall significance test is singular and thus without error inflation. Additionally, in contrast to a simple multiple regression, LMMs allow for additional variation between sample locations as these are added as random effects. Stations can thus have random intercepts, i.e., if biomass at station A and B has the same relationship with temperature, but A has 10x more biomass than B, the LMM will still find the common significant slope. LMMs need to be run separately for each of the response variables (total C-biomass, total Chl, richness S, effective number of species ENS), first for all data and then separately for DE and NL. We separated the analyses between countries because i) we already had observed differences in total C-biomass and ii) an explicit goal was to see whether the same relationships to environmental parameters exist in both data sets. Before running the LMMs, we tested for multicollinearity between the predictors, using the variance inflation factor (VIF) function in R. This function can indicate if one predictor variable can be linearly predicted from the other variables, i. e., two or more predictors (environmental variables) are strongly correlated. In our model, TN and TP were highly correlated, and keeping both variables would result in less reliable statistical inferences. For this reason, we run two models separately: with TP (shown in the main text) or with TN (in the Appendix).

Table 6. Variables included in the statistical analyses

Environmental variables	Phytoplankton variables
TN – total nitrogen in LN (µM)	**Biomass (C)** – carbon biomass in LN (µg/L)
TP – total phosphorus in LN (µM)	**Chl** – Chlorophyll *a* in LN (µg/L)
N:P – ratio	**Diatoms** – biomass in LN (µg/L)
Si – Silicon in LN (µM)	**Dinoflagellates** – biomass in LN (µg/L)
SPM – suspended particulate matter log (mg/L)	**Flagellates** – biomass in LN (µg/L)
Salinity – in PSU	**Cyanobacteria** – biomass in LN (µg/L)
Temperature – in °C	***Phaeocystis*** – biomass in LN (µg/L)
pH - dimensionless	**Annual.S** - Richness based on presence in each year
	Annual.ENS - ENS based on the median biomass of each year
	SERr and SERa – incidence and abundance based metrics of turnover

3: **Structural equation model** (SEM): SEMs allow testing all response variables in the same model, as variables can be both responses and drivers. Thereby it additionally allows testing e.g. how biomass is affected by diversity. Moreover, as the correlation structure between drivers can be explicitly modelled, it allows having highly correlated predictors such as TN and TP in the same model. However, SEM in contrast to LMM need more degrees of freedom in order to exploit this co-dependency. First, splitting the analysis by country and using StationID as categorical random effect was therefore not possible. Instead, we used longitude as a continuous random effect to control for a fraction of the variance between stations that is not captured by the environmental variables measured. Additionally, we needed to reduce the number of predictors and deleted N:P ratio and SPM, as the former depends on TN and TP concentrations, and the latter is highly correlated to biomass. As biomass is measured as C or chlorophyll we provide 2 SEMs, which mainly differ in their results for biomass. It should be noted that this partly is influenced by the C-discrepancy between NL and DE.

As **drivers** in these analyses we use the total nitrogen and phosphorus concentrations (TN and TP) and their molar ratio as well as the Si concentration (Table 6). The inclusion of either TN or TP did not systematically alter the importance of the other variables. The monitoring program does not have information on light availability (see **Recommendations**), but concentrations of suspended particulate matter (SPM) can serve as a proxy of turbidity. However, in addition to other particles, algae contribute to SPM themselves, so SPM is not only a measure of light limitation (potentially negatively correlated to biomass) but also a measure of biomass itself (and thus positively correlated). Additionally, we used salinity, temperature and pH as environmental factors. Annual median values are used for each of the analyses.

Responses were C- and Chl-based total biomass as well as C-based biomasses for each of the five functional groups used in ecosystem models of the Wadden Sea. For biodiversity, we used the approach outlined in Box 1 for the response variables. S and ENS as measures of standing diversity could be analysed with the same three approaches as detailed above. For turnover, however, the response is a dissimilarity between years, and thus can only be compared to how different the environment was between these years. We did not do whis for this report, but novel approaches such as general dissimilarity modelling (Woolley et al. 2017, Mokany et al. 2022) have successfully been used explaining compositional changes in space (Rillo et al. 2021). These could be used for temporal analyses as well.

Abiotic drivers of phytoplankton biomass

Which of the surveyed metrics (nutrients, light, temperature, or other) has the greatest impact on phytoplankton biomass (occurrence of individual species, total biomass)?

Total biomass

Environmental factors explain a substantial amount of variance in the year to year variation in total phytoplankton biomass, 16-62% for C-biomass and 24-29% for Chlorophyll in the LMM (Table 7) and 58-60% in the SEM. Adding random intercepts for stations raised the conditional explained variance up to 92% (Table 7). Correlations (Fig. 11), LMM (Table 7, Fig. 12-13) and SEM (Fig. 14-15) often were in general agreement as to which factors were driving variance in biomass, but detailed differences between the two biomass measures and between countries were abundant. The difference between biomass measures partly reflects the differences in C estimates, although C- and Chl-biomass were positively correlated in both NL and DE (Fig. 11, r=0.52 for NL, r = 0.36 for DE).

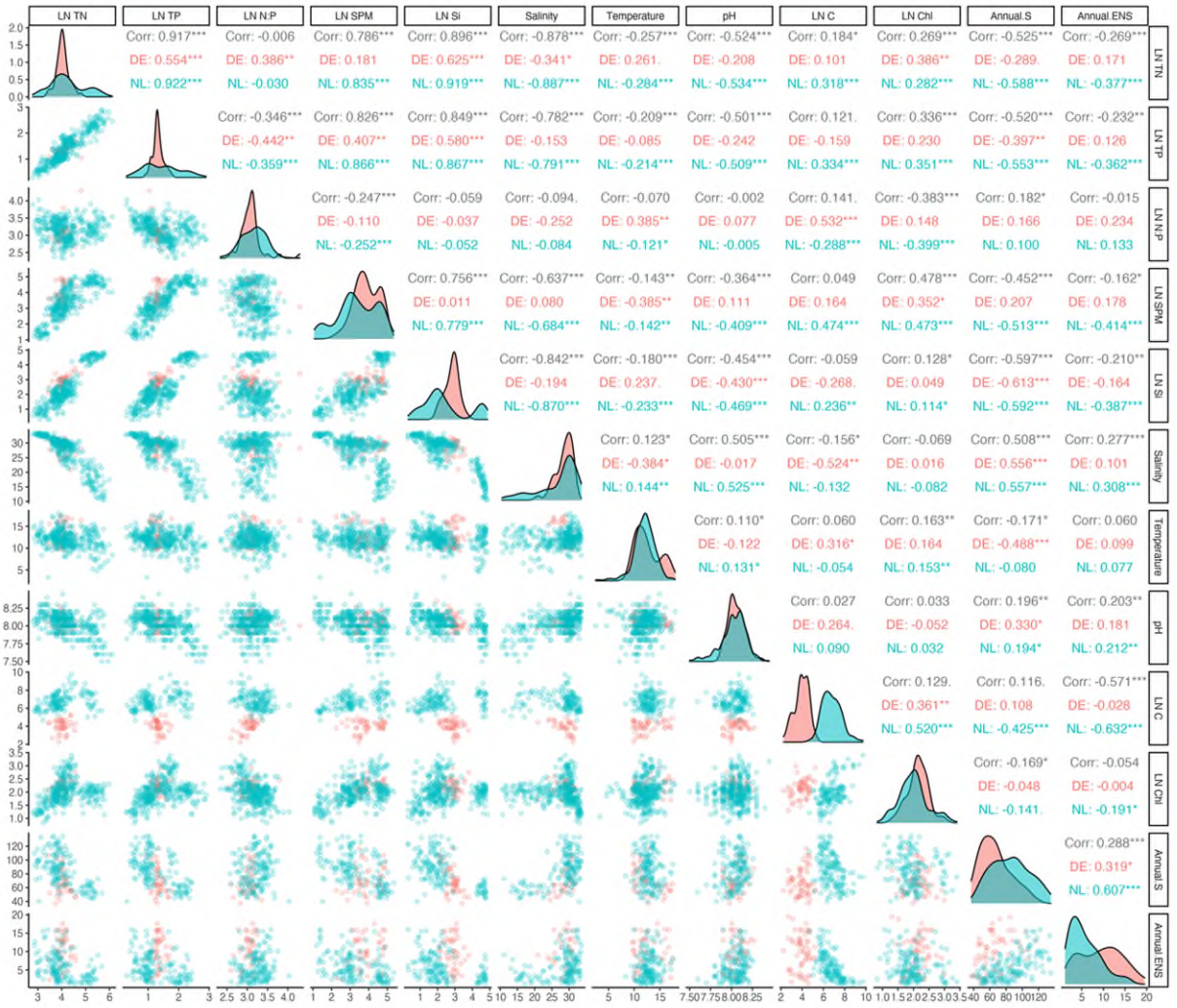


Fig. 11. Correlation matrix among all environmental parameters and phytoplankton measures. The correlation coefficients are coloured according to country, NL in blue and DE in red. Asterisks next to correlation coefficient represent the significance level: *** *p<0.001*, ***p<0.01, *p<0.05*. For more information on the variables, see table 6. *Data input: annual medians.*

We expected total biomass to increase with N and P availability. Indeed, we found positive pairwise correlations (Fig. 11) for both metrics (C and Chl) and both nutrients (N and P), which were stronger for Chl-biomass than for C-biomass and for Dutch than for German stations. LMMs detected the same positive association for TP (Table 7) and TN (Table S1) for Chl overall and in the NL data (Tables 7 and S1, Fig. 15). For C-biomass, effects were not significant as this relationship was partly covered by Si concentrations and salinity. Bivariate representation of biomass to nutrients shows a peak biomass appearing at ~90 µM TN and ~6 µM TP (Fig. 12 a-d). The decline in biomass at higher nutrient levels however is strongly associated to the low-salinity stations GROOTGND and BOCHTVWTM. C-biomass further showed the previously described difference between NL and DE, which might explain the overall lower consistency of the results for C compared to Chl. SEM found similar effects as TP was a significant driver of biomass for both C and Chl measurement, with an additional positive TN effect on Chl biomass (Fig. 14-15).

A clear separation between N or P as the main driver is difficult given the very high correlation between both nutrients. The SEM picks TP over TN for C-biomass and indeed the N:P ratios

indicate rather a P- than a N-limitation (Fig. 4 e-f, see also below). Based on correlations, biomass significantly declined with increasing N:P (indicative of more P-limitation) in NL but not DE stations, where C-biomass even increased (Fig. 11). The LMM did not find significant slopes with N:P and the SEM could not encompass this (as it incorporates both TN and TP). Based on this evidence we lean towards concluding that P-limitation is the main state of the system at present. A more detailed answer on this would need a bioassay approach (see Recommendations).

Median dissolved silicate concentrations showed a positive bivariate correlation to C-biomass and Chl-biomass in the NL data (Fig. 11), but no trend in the German data. In the multifactorial assessments this relationship turned consistently negative in the SEM (Fig. 14-15) and - for Chl-biomass – in the LMM (Table 7, Fig. 12). This conversion of effects potentially reflects the high correlation between Si and N as well as P concentrations (Fig. 11), thus the general positive nutrient – biomass trend is already captured by TN and TP. After controlling for this general trend, years and stations with higher Si concentrations obviously tended to have lower biomass.

Any conclusion on nutrient limitation based on these analyses has the caveat that a potential light limitation cannot be assessed, which is a clear recommendation (see below). The only variable related to light is SPM, which however is partly reflecting biomass in itself as phytoplankton is a major part of the suspended particles. Consequently, a positive relationship emerges between SPM and biomass (both C and Chl) in the correlations (Fig. 11) and the LMM (Table 7), which again was stronger for Chl than C and for NL than DE. SPM could not be incorporated in the SEM.

The bivariate correlation between biomass and salinity tended to be negative, which mainly reflected that the station GROOTGND, which had the lowest salinity (<20 PSU), also showed exceptionally high nutrient concentrations and thus high C-biomass (Fig. 11-13). By contrast, towards full marine salinity (>27 PSU), both C- and Chl-biomass declined with salinity again. Controlling for the nutrient-salinity interaction in the LMM and SEM found negative salinity effects on biomass.

When significant, higher temperatures were consistently associated with higher biomass, in correlations (Fig. 11) and in the LMM (Table 7, Fig. 13) for Chl-biomass in NL and C-biomass in DE. Whether this is a direct temperature effect on algal growth or an indirect effect (e.g. via higher remineralisation) cannot be obtained from the data. The SEM did not detect any significant temperature effect except for weak negative effect on C-biomass. High Chl-biomass co-occurred with high pH in both LMM (overall and NL only, Fig. 13) and SEM (Fig. 14-15). As algal photosynthesis affects the pH, the causality is not identifiable.

A major source of biomass variance in phytoplankton biomass remains elusive, as we have no direct information on the extent of zooplankton grazing and benthic filter-feeding on phytoplankton (Box 2, and recommendations).

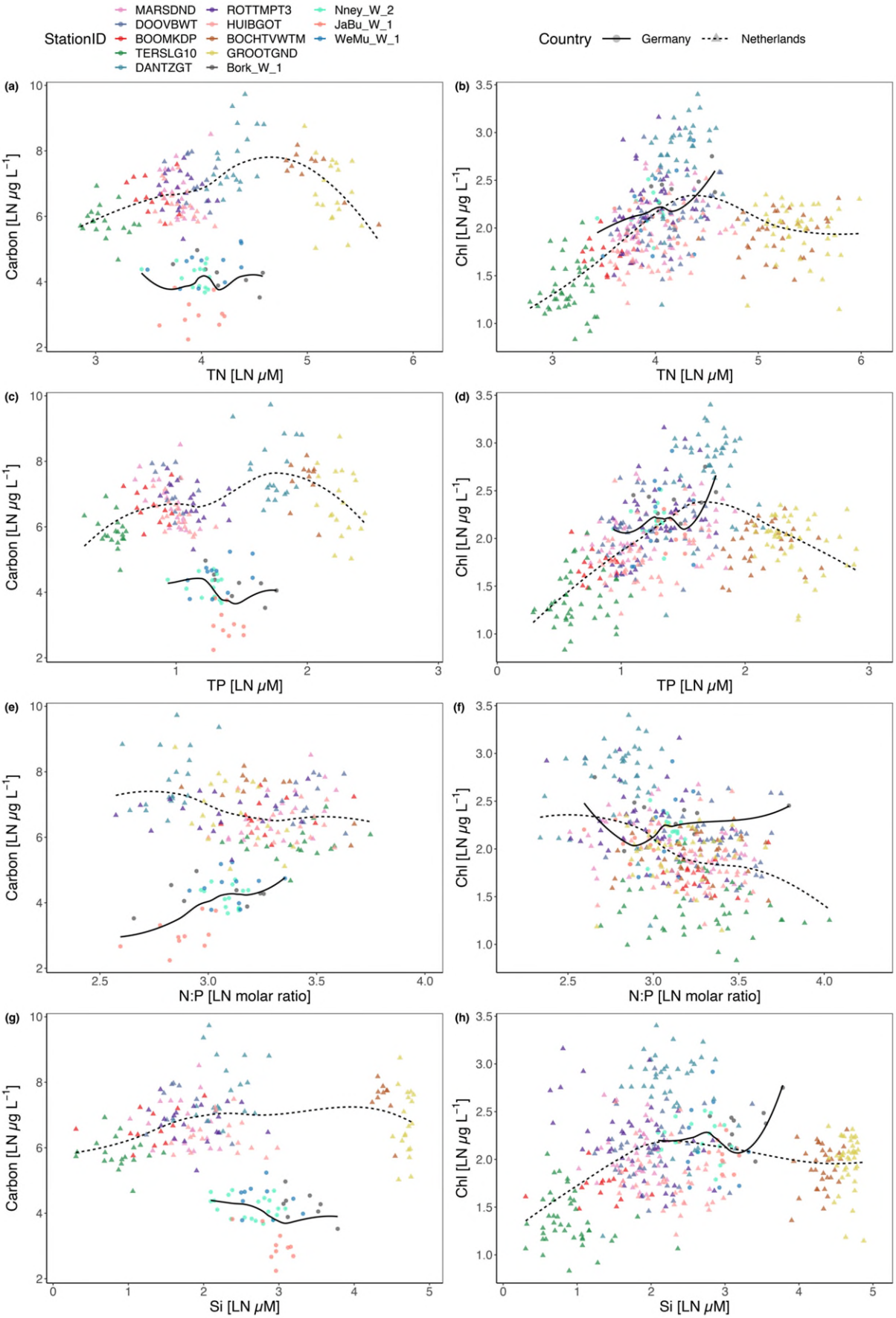


Fig. 12: Carbon (left column) and chlorophyll (right column) as phytoplankton biomass estimates plotted against nutrients N, P, their ratio, and Si. Lines represent a loess fit for NL stations (dashed) and DE stations (continuous line). Colors represent the stations as in Fig. 1. *Data input: annual scale*

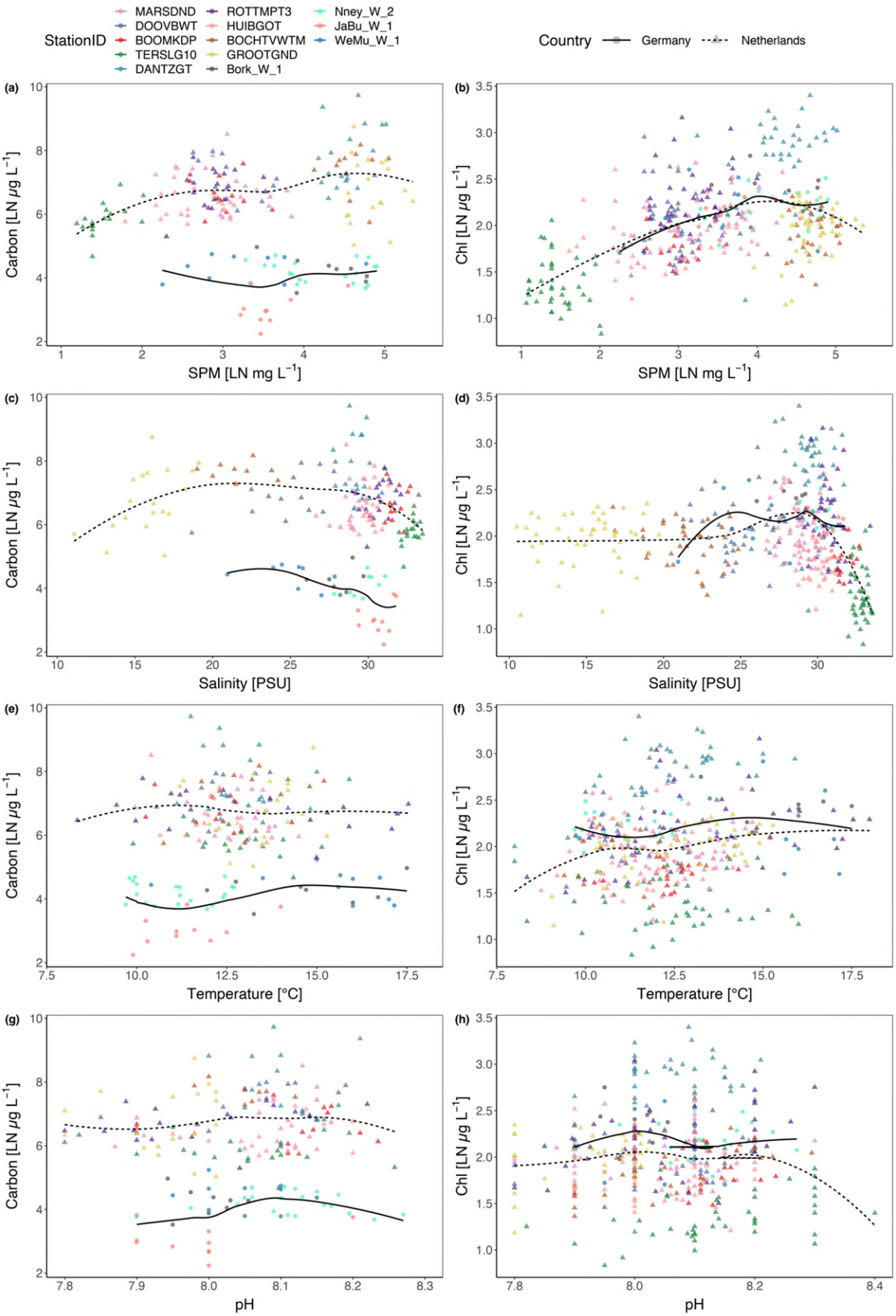


Fig. 13: Carbon (left column) and chlorophyll (right column) as phytoplankton biomass estimates plotted against SPM, salinity, temperature and pH. Dashed line represents NL stations and continuous line, DE stations. Colors represent the stations as in Fig. 1. *Data input: annual scale*

Table 7. Results of the linear mixed effect model, analyzing the effects of environmental factors on phytoplankton biomass (carbon and chlorophyll *a*), considering "StationID" as a random effect. Bold numbers indicate significant predictors. The overall model for C-biomass is highlighted as it is affected by the discrepancy in C estimates. When outputs differed between NL and DE, we highlighted the estimates in grey. Conditional R^2 for the last of the six models could not be obtained as no variance was assoiated to the random effects. Data input: *annual median*

	Carbon (LN µgL^{-1})						**Chl (LN µgL^{-1})**					
	all		**NL**		**DE**		**all**		**NL**		**DE**	
Predictors	*Estimates*	*p*	*Estimates*	*p*	*Estimates*	*p*	*Estimates*	*p*	*Estimates*	*p*	*Estimates*	*p*
(Intercept)	1.170	0.801	-3.326	0.528	10.622	0.146	-4.986	**<0.001**	-5.539	**<0.001**	1.681	0.702
LN TP	-1.760	**0.002**	-1.131	0.063	-1.027	0.229	0.419	**<0.001**	0.453	**<0.001**	-0.025	0.958
LN NP	-0.159	0.652	-0.245	0.573	0.272	0.614	0.135	0.160	0.161	0.125	0.163	0.518
LN Si	0.110	0.497	0.360	**0.037**	-0.693	**0.047**	-0.131	**0.002**	-0.136	**0.002**	-0.025	0.896
LN SPM	0.420	**0.007**	0.712	**<0.001**	0.244	0.130	0.126	**0.002**	0.128	**0.005**	0.165	**0.036**
Salinity	0.096	**0.035**	0.075	0.073	-0.151	**0.009**	-0.008	0.497	-0.009	0.450	0.013	0.477
Temperature	-0.031	0.381	-0.034	0.450	0.118	**0.011**	0.046	**<0.001**	0.047	**<0.001**	0.031	0.138
pH	0.441	0.383	0.934	0.111	-0.295	0.731	0.691	**<0.001**	0.741	**<0.001**	-0.164	0.751
Random Effects												
σ^2	0.39		0.48		0.12		0.08		0.08		0.05	
τ_{00}	3.70 $_{StationID}$		0.13 $_{StationID}$		0.10 $_{StationID}$		0.06 $_{StationID}$		0.08 $_{StationID}$		0.00 $_{StationID}$	
ICC	0.91		0.22		0.45		0.46		0.51			
N	13 $_{StationID}$		9 $_{StationID}$		4 $_{StationID}$		13 $_{StationID}$		9 $_{StationID}$		4 $_{StationID}$	
Observations	182		148		34		338		303		35	
Marginal R^2 / Condit. R^2	0.163 / 0.921		0.226 / 0.395		0.620 / 0.791		0.282 / 0.613		0.289 / 0.655		0.244 / NA	

Summary for policymakers: Phytoplankton biomass reflects changes in the Wadden Sea environment over time and between stations. Biomass generally increases with increasing nutrient concentrations, with N, P and Si contributing, but some evidence pointing towards a preponderance of P-limitation at the interannual scale. Thus, efforts to control phytoplankton biomass via nutrient reductions need to progress beyond reducing N alone. Biomass decreases towards more saline (farther away from land) and increases towards warmer conditions. So far, chlorophyll seems to achieve more consistent results between countries and approaches, reflecting that C-biomass shows a strong difference between countries. However, chlorophyll per cell is affected by light and thus part of the observed trends may derive from differences in irradiance. Light limitation and mortality via zooplankton grazing or benthic filter feeders are two potential constraints on phytoplankton biomass that are not assessed in the monitoring program.

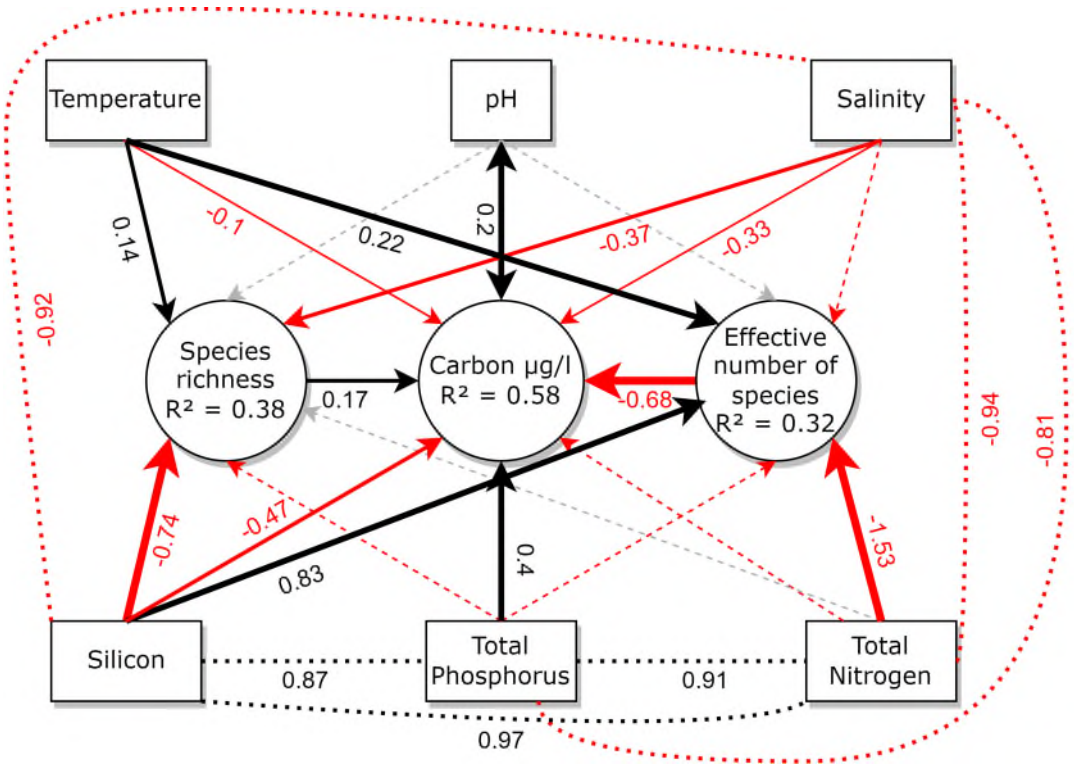


Fig. 14: Analysis of annual data using structural equation model (SEM). Yearly average phytoplankton biomass as carbon and annual diversity (raw species richness and effective species number (analogous to evenness) modelled as a response to six environmental factors, biomass additionally affected by diversity. Black arrows = positive effects, red = negative, solid lines = significant effects, dotted lines not significant. Numbers are standardized path coefficients that can be interpreted as correlation coefficients.

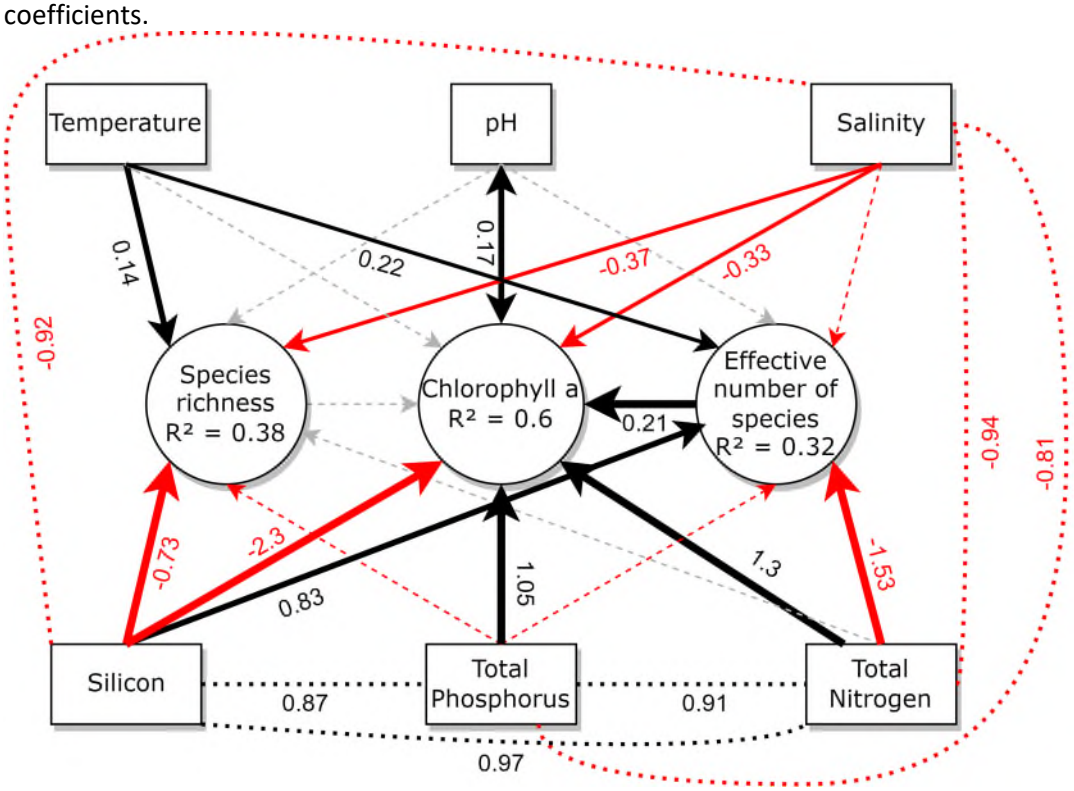
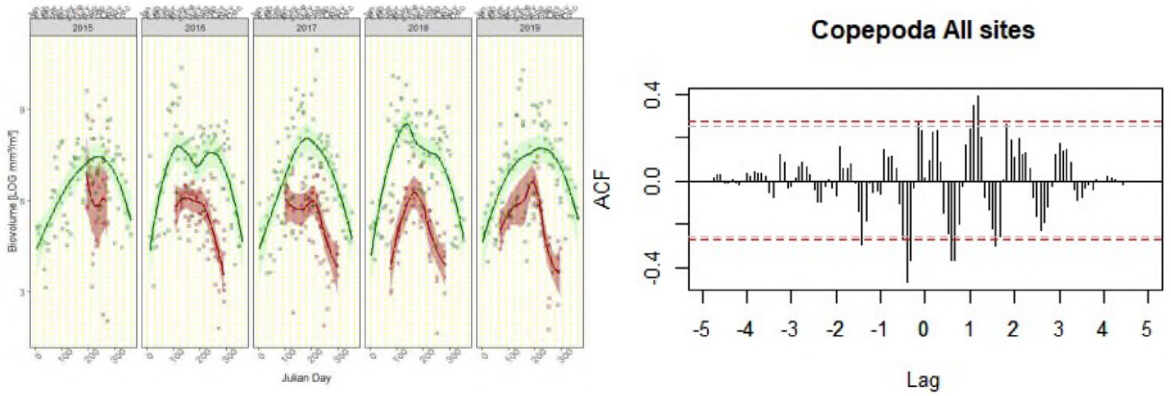


Fig. 15: Analysis of annual data using structural equation model (SEM). Yearly average phytoplankton biomass as chlorophyll a and annual diversity (raw species richness and effective species number (analogous to evenness) modelled as a response to six environmental factors, biomass additionally affected by diversity. Details as in Fig. 14.

Box 2: Zooplankton

From 2015 to 2019, NLWKN monitored zooplankton at several stations within the Wadden Sea. The time series is too short to evaluate long-term trends, but within a student project we analysed the seasonal occurrence of zooplankton and tested whether this was cross-correlated to phytoplankton biomass (student project by Jöran Paap). The locations for zooplankton sampling and the exact timing were not identical so we lumped the data and also used monthly means. Thus, the pattern described here are indicative of the results, but a further alignment of sampling will improve our capacity to incorporate trophic information.



The overall data across stations and zooplankton taxa shows clear seasonal patterns with spring/summer peaks in phytoplankton biomass (green) and total zooplankton biomass (red) later on, already indicating a trophic interaction (left panel).
As not all zooplankton feeds on phytoplankton, we tested the presence of such a trophic link using Copepoda only (the pattern using all zooplankton or all pelagic zooplankton is similar, but less significant). We used a cross-correlation analysis, which gives the correlation between both groups for any time-lag between zero and 5 years. The following graph gives the correlation on the y-axis for data that are shifted (scale is year, -1 means 1 year before), the thresholds for significant correlations is given in dashed lines. We find positive correlations with a delay of ~3 months (0.25, 1.25 year), indicating that zooplankton biomass increases a few months after the phytoplankton peak. Additionally, we see significant negative correlations with a lag of ca. -0.3 and -1.3 years and 0.7 and 1.7 years, indicating that low phytoplankton biomass follows a zooplankton peak with a few months delay.

Dominant species as indicators of nutrient conditions

To answer the second aspect of the question, which species might be indicative of the nutrient status, we first selected the dominant taxa (see Fig. S3-S4 in the appendix for total biomass and functional group raw data). For each species, we calculated its mean annual biomass, its mean proportional contribution to sample biomass and its frequency of occurrence. From 429 taxa in the data set, 106 were above median in all three categories. Of these we de-selected those that were rare (less than 1% of biomass across sampled) and infrequent (less than 75 occurrences total). From the 69 remaining species, we further reduced to 41 by focusing on species that were determined to species-level and by allowing only a few species from some

of the dominant genera. The resulting set of potential indicator species was then related to total N, total P and their ratio as well as silicate.
For each of these species at each station, we obtained an average annual species biomass (carbon) and annual mean proportion of total C-biomass. The former can be considered the absolute response to nutrients, the latter a relative response in comparison to the rest of the community. Raw data are plotted in the appendix.
Using a LMM, we calculated the slope between (log-transformed) nutrients and (log-transformed) absolute or relative biomass for each species, always using StationID as a random effect. The full results are in the Appendix (Table S2), but only half of the selected species showed significant relations between their absolute or relative biomass and any of the four nutrient axis (Fig. 16).

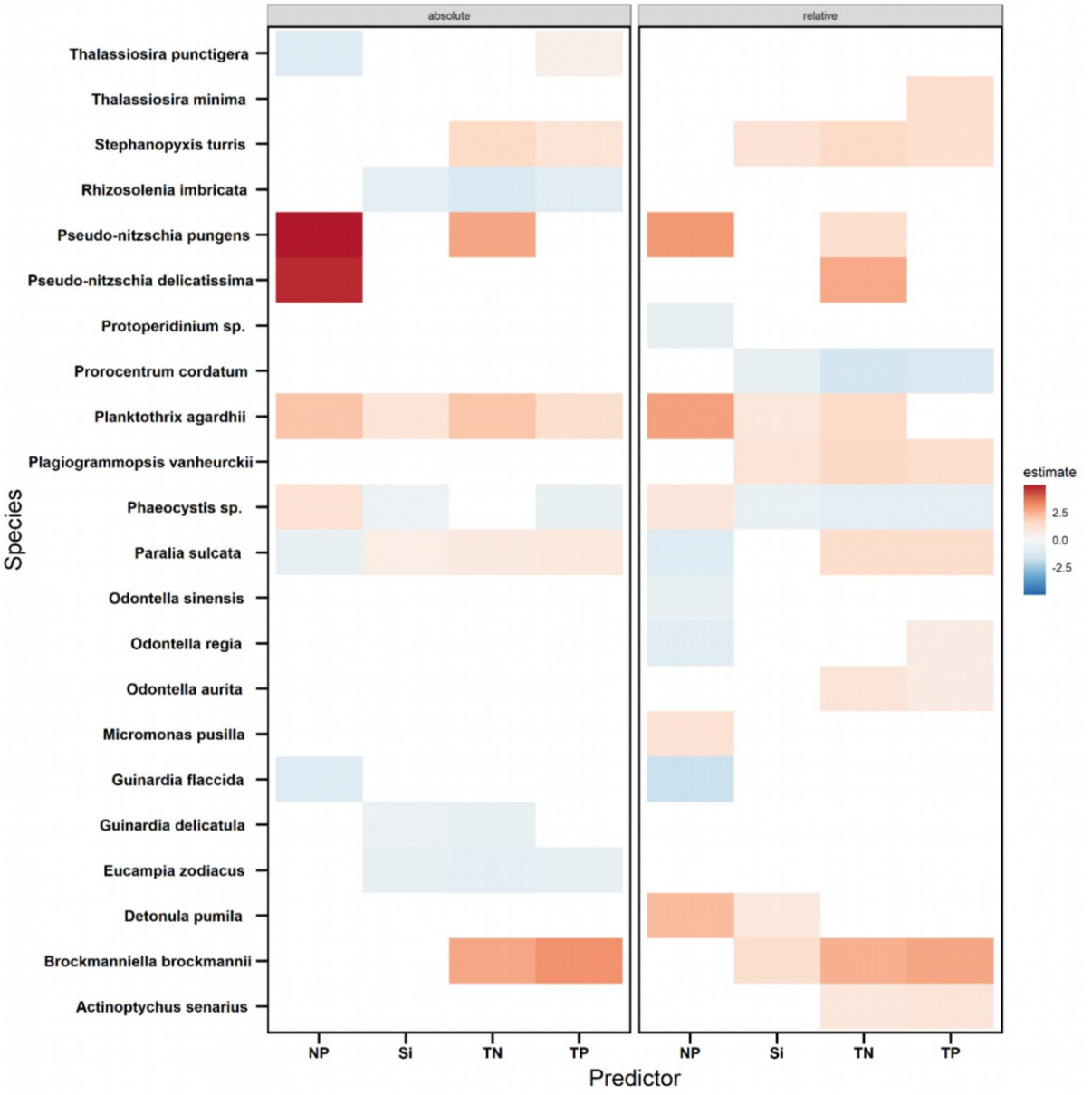


Fig. 16: Slopes of species to nutrients and their ratio derived from univariate LMM with StationID as additional random factor. Slope estimates are colour coded for each regression where $p < 0.05$, with red gradient indicating positive relationship and blue gradient negative relationship, blanks indicate non-significant regressions.

Among these, a few species stood out: The potentially toxic diatoms *Pseudo-nitzschia pungens* and *P. delicatissima* increased with increasing TN concentrations and N:P ratios, leading to a higher proportion at high N:P and TN. The diatoms *Guinardia flaccida* and *Eucampia zodiacus* declined with increasing TN. The diatom *Brockmanniella brockmanii* increased with both TN and TP in both absolute and relative terms. Most other relationships were weaker and confirmed well-known expectations such that a range of diatoms increased its relative share in biomass at high Si concentrations, whereas the proportion of *Prorocentrum cordatum* and *Phaeocystis* decreased.

Summary for policymakers: Phytoplankton species respond to nutrient gradients, but rarely so consistent that they can serve as indicator species for nutrient conditions.

Nutrient limitation

Are limitations by N and/or P detectable over the course of the year? Are these observations within the Wadden Sea different from the situation in the Southern North Sea?

In contrast to the previous section on annual mean data, we moved to sample based data to answer the first part of this question. Both TN and TP show the expected seasonal pattern with high winter concentrations followed by a reduction towards summer and then increasing concentrations in late fall (Fig. 17 a,b). This pattern was very regular for TN and more variable between stations for TP, reflecting the potential influence of rapid P-remineralization. The decline in nutrients coincides with increasing biomass in most of the algal functional groups (Fig. 18), which was especially pronounced for summer dinoflagellate blooms and spring *Phaeocystis* blooms. The overall most biomass-rich phytoplankton group, diatoms, showed a less clear seasonal pattern and was abundant throughout, but showed an early spring peak in most stations (NB: the scale in Fig. 18 is log-transformed, underestimating the differences in the most dominant group).

Decisive for the question of limitation is the N:P ratio, which again showed a very consistent seasonal pattern between years and stations (Fig. 17c): N:P ratios peak in early spring, and decline towards a minimum around August, before they increase again. Difference between stations is much less than for the concentrations. Overall, N:P is higher than 22, a proposed indicator for P-limitation (Guildford and Hecky 2000), pointing towards P-limitation. Especially during the spring bloom, P-limitation is highly likely, whereas towards summer (and dinoflagellate dominance), N limitation is at least a possibility. A test of this conclusion would be a bioassay approach to see the responses to nutrient spikes (see Recommendations). We reiterate that the conclusion of limitation is made in the absence of information on how limiting light is. We also point towards information from bioassays worldwide indicating that different species in an assemblage can be limited by different resources and co-limitation is the norm rather than an exception (Elser et al. 2007, Harpole et al. 2011).

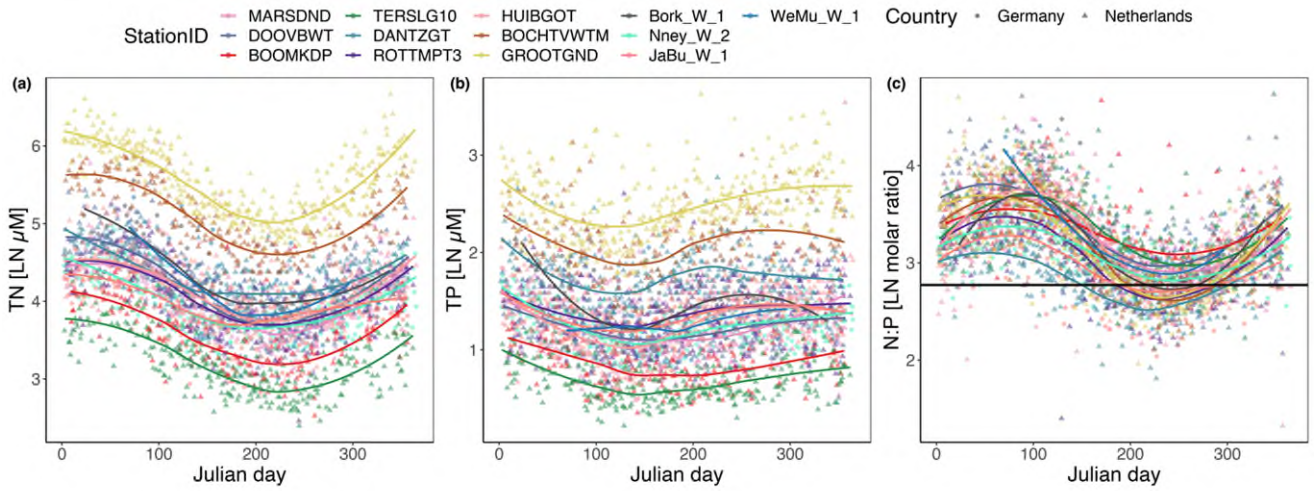


Fig. 17. Seasonal trend of total nutrients at the Wadden Sea coastal stations. Horizontal line in panel c is N:P = 16. Coloured lines are loess fits per station. *Data input: Sample data*

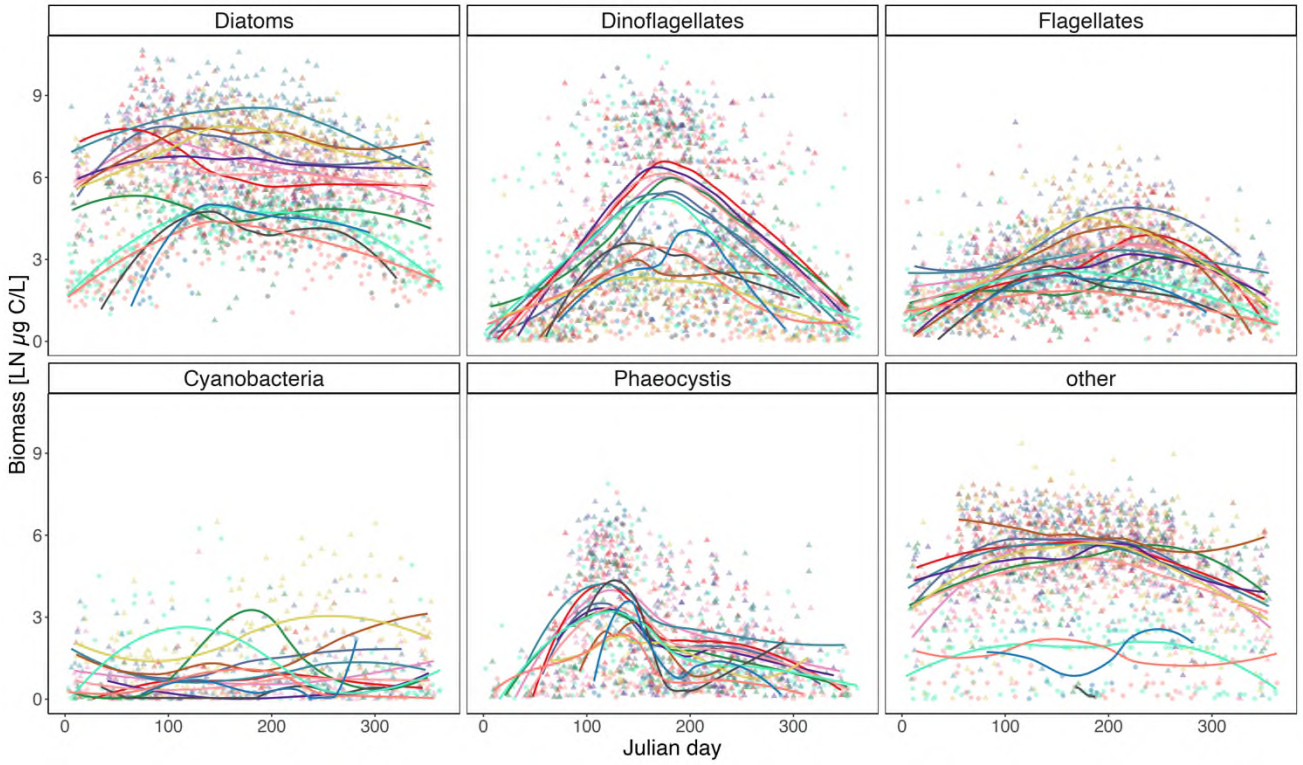


Fig. 18. Seasonal trend of the biomass of functional groups at the Wadden Sea coastal stations. *Data input: Julian day median*

In order to compare the Wadden Sea and the North Sea, we investigated the temporal trend of nutrient concentrations in the coastal and offshore stations. This analysis was done similarly to the analysis in Fig. 4, using the median of nutrient concentration per year comparing the coastal stations we addressed so far to offshore stations (Fig. 19). Carbon, chlorophyll *a* and their ratio were also plotted for the coastal and offshore stations (Fig. 20), as well as the biomass of functional groups over years (Fig. 21).

The comparison between coastal and offshore stations reveals an order of magnitude higher nutrient concentration in the Wadden Sea compared to the North Sea (Fig. 19). Similarly to the coastal stations, the nutrient concentrations also decreased over time offshore, but this decline was less intense and TN seems to increase since 2003, which might

explain the steep increase in the N:P ratio since 2006/2007. Before that, the Wadden Sea had consistently higher N:P ratios in line with previous observations (Burson et al. 2016).

While C-biomass was in comparable ranges for offshore and Wadden Sea stations, Chl-biomass is much higher in the Wadden Sea leading to higher C:Chl ratios (Fig. 20). This potentially reflects the impact of turbidity, as under dark conditions more Chl a per C is produced in the algal cells. Over time, C-biomass and Chl-biomass show weak trends until 2010, thereafter both C and C:Chl decline rapidly offshore (Fig. 20 a,c).

Separating the biomass by functional groups shows even more clearly the lower biomass at offshore compared to coastal stations (Fig. 21). Offshore, both diatoms and dinoflagellates declined in their biomass, contrasting their rather stable biomass in the Wadden Sea (Fig. 21).

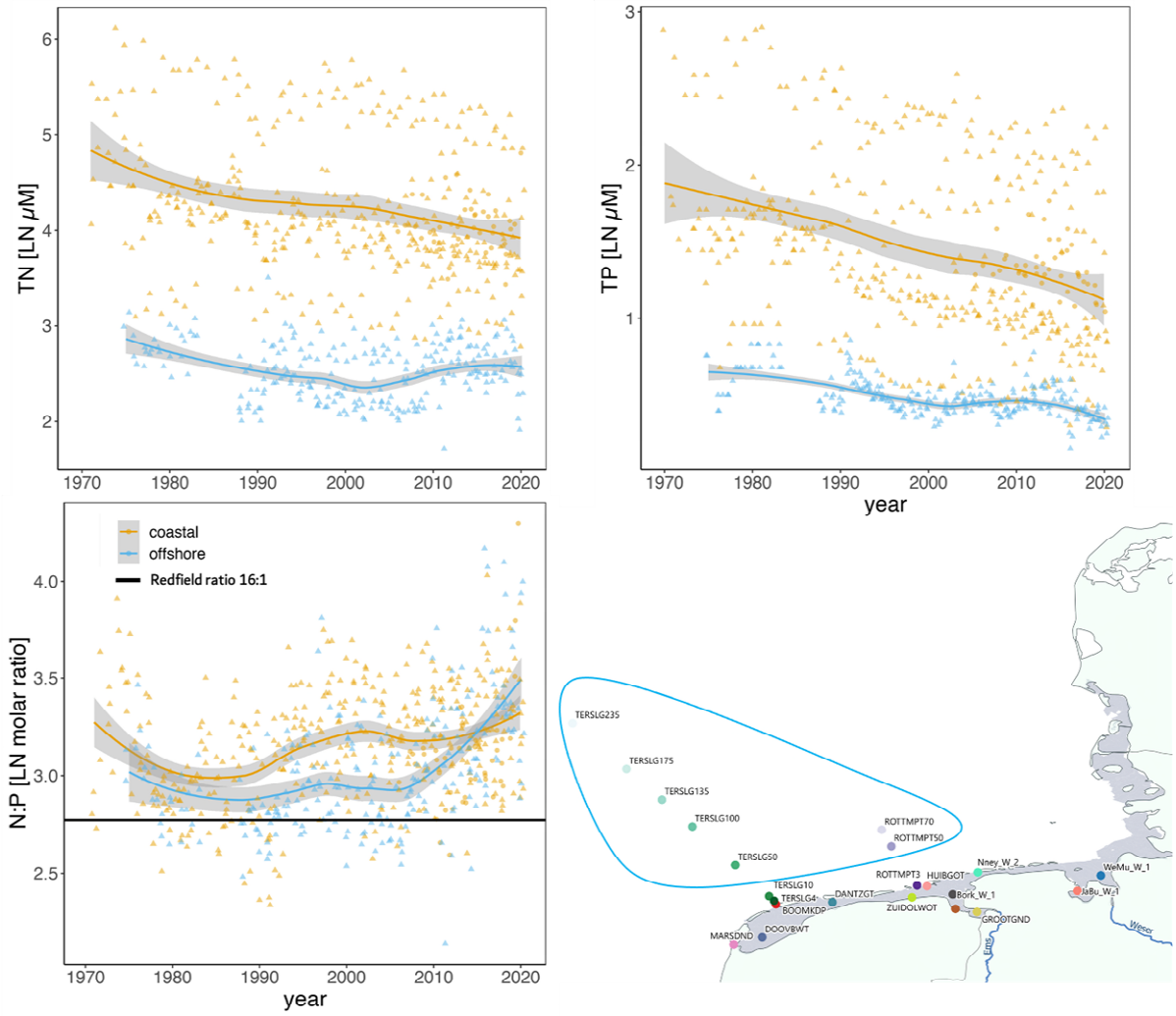


Fig. 19. Temporal trend of total nutrients at the Wadden Sea (yellow) and North Sea (blue) stations. Each dot represents the annual median in one station. Lines are loess fits.
**Untransformed data in Fig. S5 in the Appendix.*

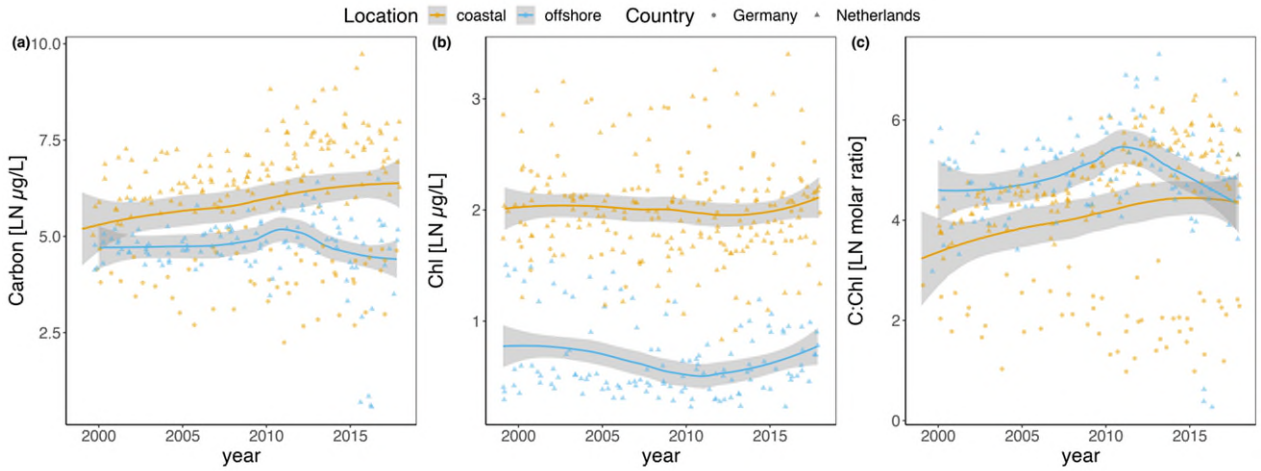


Fig. 20. Temporal trend of phytoplankton biomass as carbon (a), chl (b) and their ratio (c) at the Wadden Sea (yellow) and North Sea (blue) stations. Each dot represents the annual median in one station.

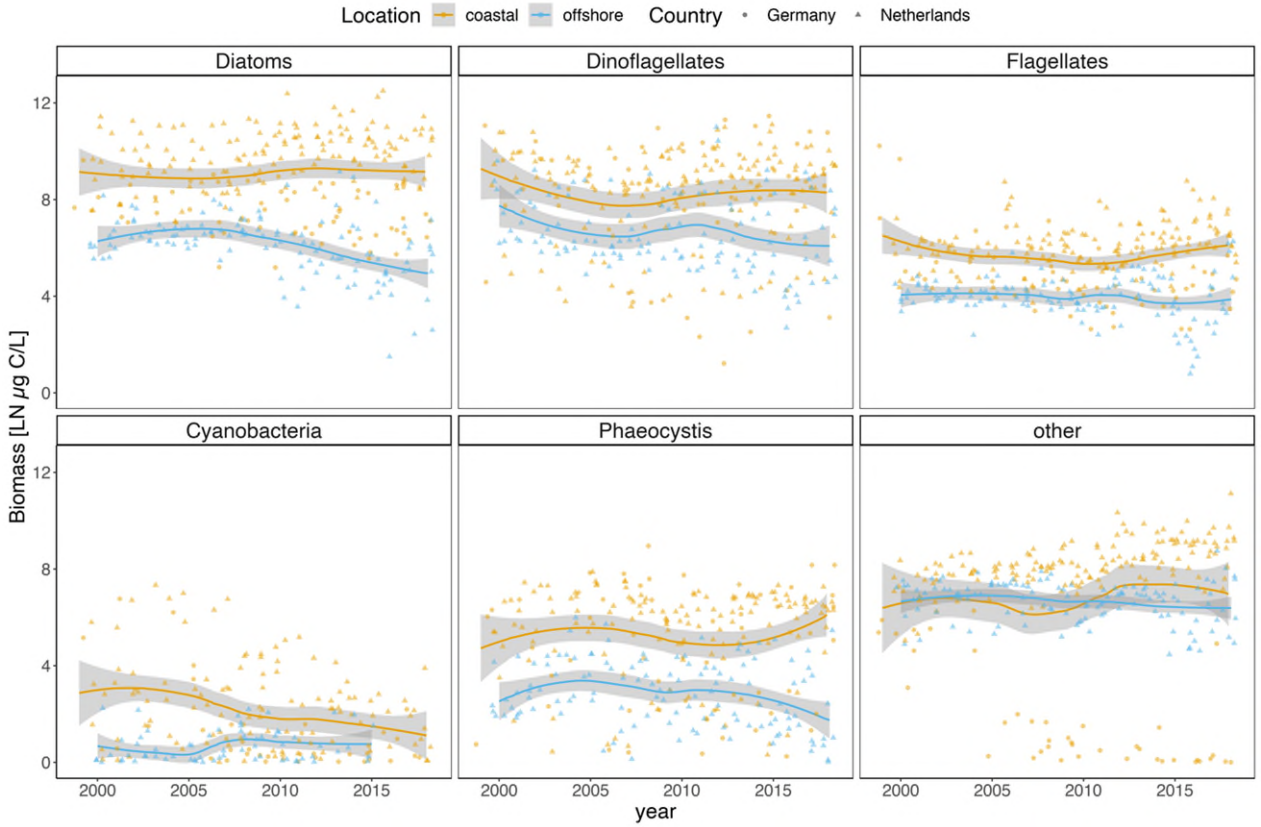


Fig. 21. Temporal trend of functional groups biomass at the Wadden Sea (yellow) and North Sea (blue) stations. Each dot represents the annual median of one station. *Data input: annual median*

Summary for policymakers: N:P ratio strongly point towards a potential P-limitation, especially for the spring and early summer phases. Lower TN concentrations in late summer at least indicate a potential for N-limitation late in the vegetation period. Thereby, the Wadden Sea differs strongly from the offshore North Sea which has lower overall nutrient concentrations, but also substantially lower N:P ratios. However, recent years saw an increase in offshore N:P. Consequently, phytoplankton biomass is lower offshore than in the Wadden Sea, especially if focusing on chlorophyll. Two of the main phytoplankton groups, diatoms and dinoflagellates, show steeper biomass declines offshore than in the coastal stations.

Phytoplankton biomass measures

In the current Lower Saxony monitoring, there are two metrics used to describe the biomass of the total phytoplankton population: biovolume (also expressed as carbon content) and chlorophyll. Based on project experience to date, can one of the two metrics be used to meaningfully describe phytoplankton biomass? Or do both have such elementary drawbacks that at best a combination is meaningful? Or is a good description of phytoplankton abundance not possible even with the combination? A brief justification of the summary would be helpful.

Biomass estimates based on carbon or chlorophyll are significantly but weakly positively correlated in each of the two datasets (r=0.361 in DE, r = 0.520 in NL, Fig. 10). Thus, on a very superficial level, estimates of low and high biomass coincide independent of the biomass measure. However, this correlation masks a series of discrepancies such as different temporal trends, different association to abiotic drivers, and different comparison between offshore and coastal biomass. These discrepancies point to the respective weaknesses of each measure, but at the same time also reflect biological characteristics of the phytoplankton.

- Chlorophyll is easily measured and therefore broadly used. However, the cellular chlorophyll content is highly dependent on the light conditions and can adapt in the range of hours, as phytoplankton generally respond to low light by increasing their investment into pigments. Thus with more turbidity, cells contain more chlorophyll per unit C, which can multiply the observed biomass without any higher abundance in the sample. The magnitude of this effect can be seen when comparing the C- and Chl-biomass difference between offshore and coastal stations, which differs by Factor 2.5 for C, but up to 5 for chlorophyll.
- The cellular carbon content is less affected by environmental conditions than chlorophyll (Hillebrand et al. 2022). However, the C-content is estimated indirectly from cell volume estimated from microscopic measurements, which is both a large effort and a potential source of variance. We observed massive discrepancies between NL and DE estimates for C-biomass, which we analysed in detail (Box 3). Additionally, empirical research has shown that different functional groups differ in their C content per cell, which again means that differences in C-biomass can be obtained without changes in biovolume but by changes in functional group dominance.

Summarizing these findings, the first take home message is that none of the two measures is “right” or “wrong” as both of these incorporate important biological processes that represent different responses to the environment beyond the biomass response (pigment or carbon per cell [volume]). Second, in the current status the chlorophyll data are the ones which allow a comparison between countries, as they align well and show rather similar responses to the main environmental drivers. Third, if the problems in the C-estimation between the countries could be aligned, C could add the chance to discuss the biomass change in functional groups. For the time being, we strongly recommend to continue both measures and to start a testing phase with alternative approaches (see recommendations) to allow a substantial comparison.

Summary for policymakers: C- and Chl- based biomass show some congruence, but also some differences in their temporal trends and relation to environmental factors. These discrepancies are partly based on differences in sampling and analysis, but partly they reflect the biology of phytoplankton. Therefore the two measures are not redundant but should be continued and compared.

Box 3: C:Chl ratios

Investigating the differences in carbon to chlorophyll a ratios (C:Chl) between the Dutch and German stations, we hypothesized that this discrepancy is partially explained by the systematic differences in the C-estimates, where NL relies on literature values and DE on per sample cell volume estimates, and partially by the difference in species composition in the data sets. The analyses were done in four major steps:

First, we tried to overcome the differences between DE and NL data by recalculating the Dutch carbon biomass using the median of cell volumes from the German data set. However, the biovolume replacement did not change the previous results, as more than 50% of the taxa in the Dutch data does not appear in the German data. We then considered only taxa identified down to species-level and present in both data sets (n=123) to recalculate carbon and C:Chl, which significantly reduced the differences between the two countries.

In order to compare the species composition between both data sets, we analyzed nearby stations located in the Ems: the German station Bork_W_1 and the Dutch station BOCHTVWTM. Interestingly, only 40,8% of the identified species are shared between the stations. This difference in species composition can be related to the taxonomic expertise and effort of analysts involved in counting and identifying the cells. In long time series data, the microscopic taxonomy can be largely influenced by the change of taxonomists involved in the identification of phytoplankton species, but also by the development and improvement of analytical tools and sampling methods (Löder et al., 2012; Nohe et al., 2018). Temporal trends in carbon content also revealed a notable difference between the two stations, with constantly much higher values in the Dutch station.

We also investigated the relationship between measured cell size (in the German data) and literature cell size (Dutch data) by taking into consideration species present in both data sets (n=123). This comparison indicated a positive association between the two biovolume estimations, however we could observe a significant variation from the 1:1 line, or 'line of equality'. We also checked the distribution of cell sizes in both data sets, which revealed a higher frequency of larger cells in the Dutch data set.

Ultimately, we compared the sampling methods between the two countries, based on the document “Specimen of a Standard Operation Procedure for laboratories of the German Marine Monitoring Programme” and Baretta-Bekker et al. (2009). We found differences related to sampling depths and sampling frequency.

To conclude, the carbon differences between the German and Dutch stations can be explained by a combination of methodological factors, ranging from biovolume estimations to taxonomic identification. Although having a globally harmonized protocol for phytoplankton monitoring programs is extremely challenging (Zingone et al., 2015), we reinforce the need of more detailed and consistent phytoplankton data sets so that better comparisons can be made.

Phytoplankton diversity

Are there significant changes in phytoplankton composition over time (referring to the 2000-2019 dataset)? If so, what is the most concise way to describe them? Can observed changes be correlated with specific environmental parameters?

Analysis of biodiversity is scale and effort dependent, which prevents the use of absolute or threshold values. Still, the diversity trends with time were conclusive overall and their relation to environmental drivers was strong, as these explained 13-32% of the variance in biodiversity alone, which increased to up to 80% by including the random terms. In the SEM, we found 44% of variance in S and 57% in ENS explained by the 6 abiotic variables. Thus, these multidimensional aspects offer a wide range of conclusions on the ongoing changes in biodiversity. As described above (Fig. 7), standing diversity (richness S and effective number of species ENS) declined in the Wadden Sea over the monitoring period analyzed (2000 – 2019). This decline was mainly driven by the NL stations. Moreover, S and ENS were positively correlated (Fig. 11, r = 0.319 for DE and r = 0.607 for NL).

Nutrients were strongly negatively correlated to diversity. S and ENS consistently declined with Si within and across countries, independent whether analysed as correlations (Fig. 11), LMM (Table 8, Fig. 22) or in the SEM (here only affecting S negatively, but ENS positively, Fig. 14-15). Likewise, increasing TN was associated to lower S (correlation) and ENS (correlation, SEM), similar relationships appeared with TP but only in the correlations. The fact that TN and TP turned non-significant as predictors in the LMM is again due to the high correlation to Si and each other. According to LMM, S further declines with increasing N:P ratios, thus in P-limited situations (overall and in NL).

Both S and ENS decrease with SPM (overall and in NL, both correlations and LMM) and increase with salinity and pH in LMM and correlations (Fig. 11 and 22, Table 8), but not in the SEM which only found a negative effect of salinity on S. By contrast, temperature effects were inconsistent as both ENS and S increased with temperature in the SEM, but decreased in the correlations.

The SEM also finds biomass to be affected by diversity, with higher ENS associated to higher Chl a biomass, but lower C-biomass, whereas higher species richness is connected to higher C-biomass (Fig. 14-15).

Turnover accumulated over time, indicating a strong compositional drift over time (see Fig. 8). These changes reflect that species composition continues to change even if some conditions are restored (such as reduction in eutrophication) as other ongoing changes (such as warming) and species immigration prevent compositional recovery.

Summary for policymakers: The diversity responses over time and with environmental factors were more consistent than the biomass response. Standing diversity declines with increasing nutrient availability, which emanated in different forms in the different statistical analysis. As diversity does not have an absolute scale, these conclusions could only be derived because of the extended time series. Thus, the information value of biodiversity lies in the trends, not in the absolute value. The temporal turnover indicates continued change in the phytoplankton composition and the absence of recovery of historic “pristine” communities.

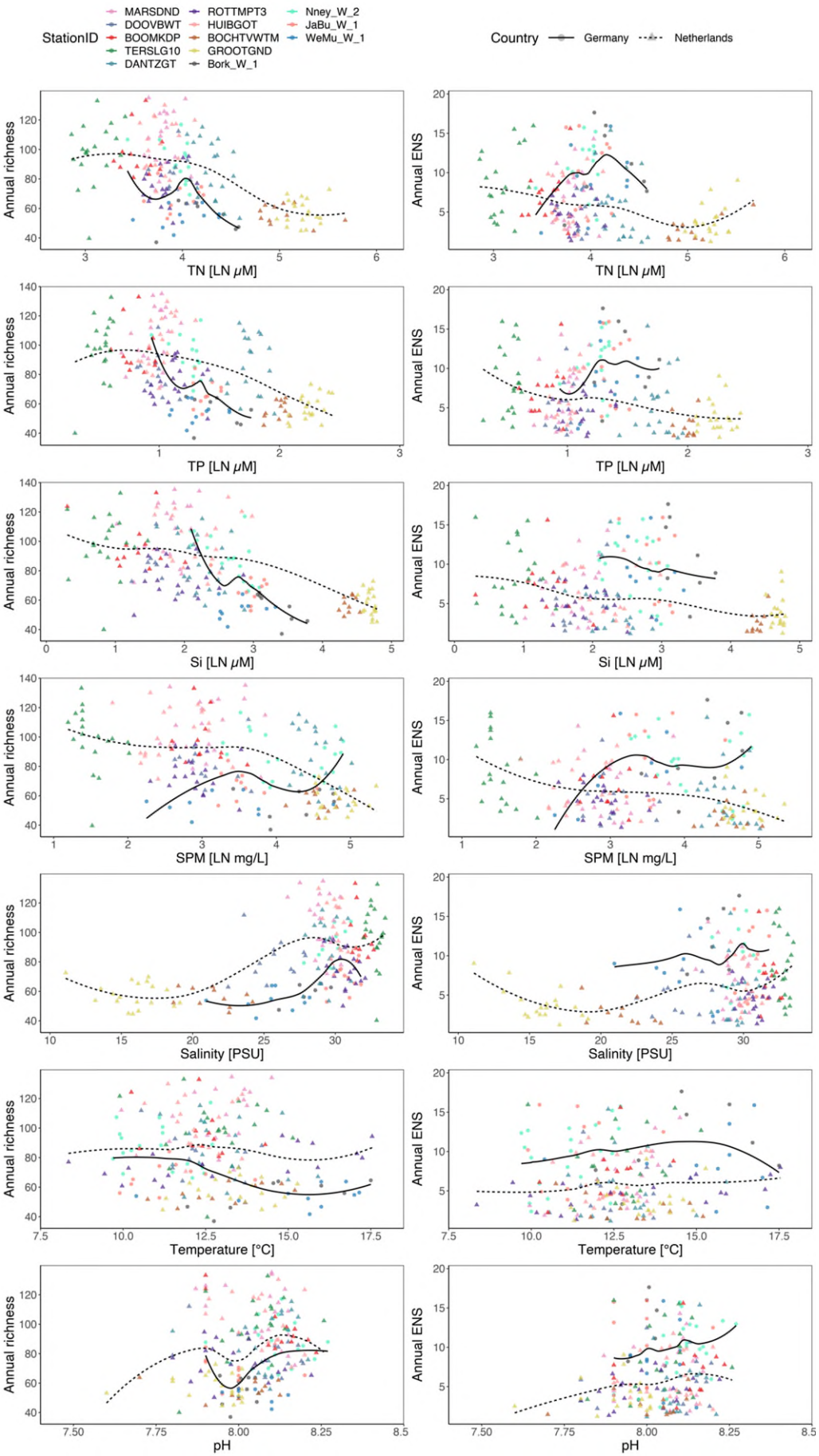


Fig. 22. Standing diversity in relation to environmental parameters. *Data input: annual median*

Table 8. Results of the linear mixed effect model, analyzing the effects of environmental factors on phytoplankton standing diversity (annual richness and ENS), considering "station" as a random effect. When outputs differed between NL and DE, we highlighted the estimates in grey. Data input: *annual median.*

	Annual Richness (S)						**Annual ENS**					
	all		**NL**		**DE**		**all**		**NL**		**DE**	
Predictors	*Estimates*	*p*	*Estimates*	*p*	*Estimates*	*p*	*Estimates*	*p*	*Estimates*	*p*	*Estimates*	*p*
(Intercept)	0.180	0.454	0.474	**0.043**	-0.668	0.076	0.046	0.847	-0.173	0.363	0.794	0.236
TP	0.489	0.161	0.554	0.153	0.281	0.698	0.187	0.669	0.450	0.287	1.578	0.405
NP	-0.195	**0.020**	-0.223	**0.018**	0.115	0.558	-0.002	0.986	0.007	0.950	0.521	0.326
Si	-0.855	**0.005**	-0.972	**0.002**	-0.876	0.205	-0.885	**0.011**	-0.658	**0.033**	-2.270	0.219
SPM	-0.263	**0.015**	-0.381	**0.011**	0.020	0.860	0.076	0.577	-0.320	0.055	0.373	0.190
Salinity	-0.271	0.227	-0.451	0.087	0.745	0.051	-0.558	**0.043**	-0.494	0.069	0.410	0.521
Temperature	0.001	0.993	-0.034	0.689	-0.047	0.657	0.154	0.085	0.131	0.175	0.195	0.366
pH	-0.174	**0.013**	-0.178	**0.026**	-0.291	**0.026**	0.122	0.175	0.075	0.397	-0.030	0.928
Random Effects												
σ^2	0.36		0.40		0.12		0.60		0.52		0.94	
τ_{00}	0.58 $_{StationID}$		0.35 $_{StationID}$		0.31 $_{StationID}$		0.45 $_{StationID}$		0.17 $_{StationID}$		0.27 $_{StationID}$	
ICC	0.62		0.47		0.72		0.43		0.25		0.23	
N	13 $_{StationID}$		9 $_{StationID}$		4 $_{StationID}$		13 $_{StationID}$		9 $_{StationID}$		4 $_{StationID}$	
Observations	182		148		34		182		148		34	
Marginal R^2 / Conditional R^2	0.223 / 0.703		0.315 / 0.637		0.283 / 0.802		0.133 / 0.505		0.143 / 0.356		0.210 / 0.389	

Assessment of phytoplankton

Within the framework of the European marine environmental directives (WFD, MSFD), it is required to assess the quality status of the water body on the basis of phytoplankton. Is it conceivable to derive assessment criteria from the available data sets (e.g. phytoplankton biomass, different forms of diversity (e.g. stability or drift of species composition, effective number of species), occurrence of blooms)?

The two lead agencies providing the data, NLWKN and Rijkswaterstaat, are to be commended for the data-rich effort to monitor phytoplankton. Combining environmental, biomass and compositional data is key to a holistic view of the role of phytoplankton in the Wadden Sea and its response to potential risks for the good environmental status. Doing this across multiple stations and having offshore stations to compare to makes a very strong case for the assessment of phytoplankton.

While it would be helpful to have binary criteria for the status of the ecosystems, this is hardly possible. First, the monitoring datasets comprise a time period with massive environmental

change, but mostly after the peak eutrophication in the 1980s. Thus, there is no information on a “pristine” status and the Wadden Sea has still higher concentrations of N and P than the North Sea despite reduced nutrient concentrations over time. Second, most changes over time and most relationships between environmental variables and phytoplankton descriptors are gradual. Therefore, fixed “threshold” values for biodiversity or biomass do not emerge from the analysis. Instead, gradual changes in drivers lead to gradual responses. Third, even if nutrients would be further reduced, the concomitant changes in other environmental factors such as temperature will not lead to a recovery of a previous species assemblage. The increase of cumulative turnover with time did not show any sign of return to a previous assemblage, which reflects that while nutrients decline, other factors change as well and lead to further changes in the composition.

In the light of these caveats, we recommend the following approaches based on a comparison of the reliability of relationships. We created a reliability metric ranging from -1 (clear consistent negative association) over 0 (no association) to +1 (fully consistent positive relationship). We derived this metric by a weighted vote count using the correlation coefficient (-1 to +1) or the path coefficients of the SEM as weights. For the LMM, we used -1 and +1 if the estimated was significant at $p<0.05$, ± 0.5 for $p< 0.1$, 0.25 for $p<0.2$ and 0.1 for $p<0.5$. Neutral relationships with $p>0.5$ in the LMM or no support in SEM were coded as 0.

Based on this assessment, the relationship between nutrients and chlorophyll as well as SPM and biomass (both C and Chl) were the most predictable positive associations (Fig. 23). Negative effects of Si, Salinity and S on chlorophyll biomass were weak or variable. Diversity (especially S) was negatively related to SPM and Si. pH and temperature had positive effects on ENS.

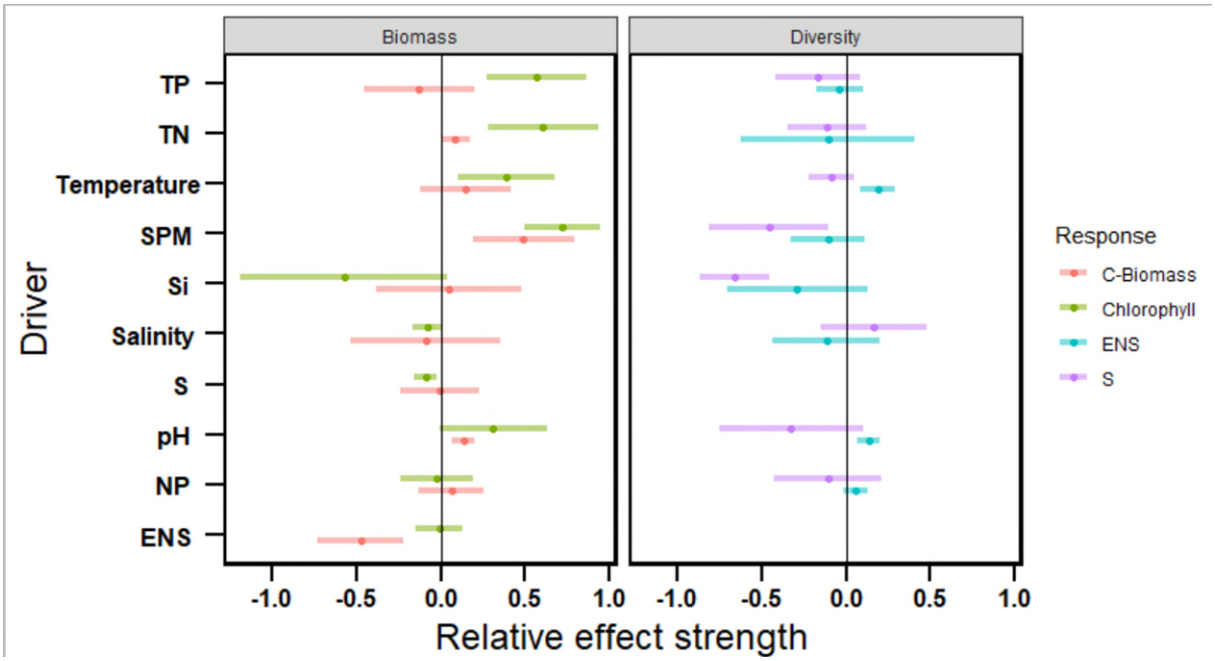


Fig. 23: Relative strength of association (mean ± confidence interval) for drivers and responses (with different colors representing different response variable).

Instead of relying on thresholds for assessment, a focus on temporal trends seems more appropriate for this highly dynamics system. Amending the current analyses with new incoming data will allow a clearer picture of the ongoing developments. Less eutrophied situations are clearly linked to lower biomass and higher standing diversity of phytoplankton, the latter even more consistently related to nutrients (Fig. 23). We especially consider the

structural equation model (SEM) a very strong approach as it takes advantage of mechanistically well proven relationships including the diversity-biomass link. But also a continued time series analysis can be used to inform the assessment. One can also use the information gathered so far to establish "internal baselines": Over the stations, a clear low biomass – high biodiversity optimum appears at TN = 25-30 µM and TP = 1.5 µM. Whether a further reduction in nutrients (towards 2.8 mg N/L from riverine sources) allows further declines in phytoplankton biomass and increases in diversity can only be observed if further actions are taken.

Summary for policymakers: The analysis of phytoplankton biomass, diversity, and species composition are important tools for understanding the aquatic system and the environmental conditions. However, they don't allow defining "thresholds" values of a good or bad water quality status. Rather, they quickly and reliably reflect the gradual changes occurring in the environment. In a dynamic system such as the Wadden Sea, the assessment of water quality should focus on such temporal trends, which can only be analysed with continued monitoring programs creating their own baselines for comparison. These programs should include not only regular and consistent phytoplankton sampling but also measurement of cell size (biovolume) and carbon content.

Conclusion

The Wadden Sea has been changing dramatically over the last 50 or more years captured by the RWS and NLWKN monitoring. An order of magnitude change in nutrient concentrations, exceptionally strong warming and massive changes in phytoplankton biomass and diversity coincide. The phytoplankton change is not characterized by a reaction and recovery response to the peak eutrophication in the 80's, but reflects continuous drifts to new assemblages, which mirrors the continuous addition of new taxa and changes in dominance.

Recommendations for improvement

We include a few recommendations to further develop the assessment

1) Include light measurements: In the highly turbid waters of the Wadden Sea, light will potentially be frequently limiting algal growth and nutrient uptake. Including this information would strongly strengthen our ability to discuss limitation, understanding compositional change and derive mechanistic understanding of system behaviour. Light could be measured by optical sensors and should include surface values as well as values at 2-3 depth (e.g. 0.5, 1 and 2 m).
2) Align zooplankton measurements with phytoplankton measurements: NLWKN started 5 years ago to amend the phytoplankton time series with a zooplankton analysis. This is highly important given that without such information no change in top-down control of algal biomass can be detected. We thus recommend extending the analysis of zooplankton also to the Dutch stations. Moreover, we observed that zooplankton sampling occurred at different stations and different days than the sampling of the phytoplankton. This discrepancy should be remedied.
3) Publish harmonized data: This project has made major efforts to harmonize the data sets, which has resulted in the first cross-country high-resolution data set. We strongly advise to publish this status, preferably in a data repository which allows version control and thus continuous updating of the data. This has advantages for both

assessment and science. As new data analysis methods emerge much faster than the agencies can adapt their approaches, open data access will allow leveraging the efforts of scientists worldwide that will use these data to calibrate their methods and propose advances. The assessment can directly profit from these insights as the data set is cited and its use can be tracked. For science, this does not only open a great data resource, but also avoids reinventing the wheel by starting from disparate excel files.

4) Cross-check taxonomy: We were unable to resolve some discrepancies in the composition of the NL and DE data. We recommend to actually exchanging a few samples between laboratories in order to check their take on the species inventory. This will potentially enable further harmonization as the current small overlap in the taxon lists indicates some major discrepancies between the monitoring programs.
5) Bioassays: We recommend a pilot project with simple nutrient bioassays to check for the preponderance of nutrient limitation. In its easiest version, it would require additions of N, P and N+P to phytoplankton samples obtained during the normal monitoring campaigns for at least a subset of stations, measuring Chl a after 24 hr. More comprising manipulations (including Si and light) are possible.
6) Measuring cell size from samples: Phytoplankton cell size is an important trait that can provide insights on different morphological and physiological aspects of species, and can be related to environmental changes and grazing (Hillebrand et al. 2022). Cell size analysis of the German Wadden Sea phytoplankton revealed that species are 30% smaller now than 15 years ago (Hillebrand et al., 2021). This and further analyses can only be done when cell sizes are measured per sample. Based on these findings, we highlight the importance of measuring the cells from/in the samples instead of using standardized literature values, which are often overestimated and do not capture temporal changes.
7) Particulate organic carbon and pigments: We recommend amending the current sampling with two additional analyses. From the same sample that serves as basis for counting and chlorophyll, two further subsamples shall be taken for a relevant number of stations and times. It would be sufficient to use 2-3 stations each in NL and DE for ca 1 year. The first subsample shall be filtered on GF/F filters for measurement in a CN analyser (the additional measurement of N is included and helpful for the limitation question) giving an independent total C measurement. These C-values can be compared to Chl and microscopy-based C estimates to identify congruence and discrepancies. The second subsample shall be used for a newly developed inexpensive way of estimating several pigments using photometers (Thrane et al. 2015). These could potentially suffice to identify major algal taxa, which could be compared to the counted data. The method is also comparable to the pigment based analyses taken by ferry boxes and other high frequency sampling.
8) Continuation of the phytoplankton monitoring program: Our analysis suggests that the temporal trends of biotic and abiotic factors provide important information about the Wadden Sea ecosystem. Therefore, a continuous and consistent monitoring program is beneficial for comprehending changes in environmental conditions and designing better water management plans.

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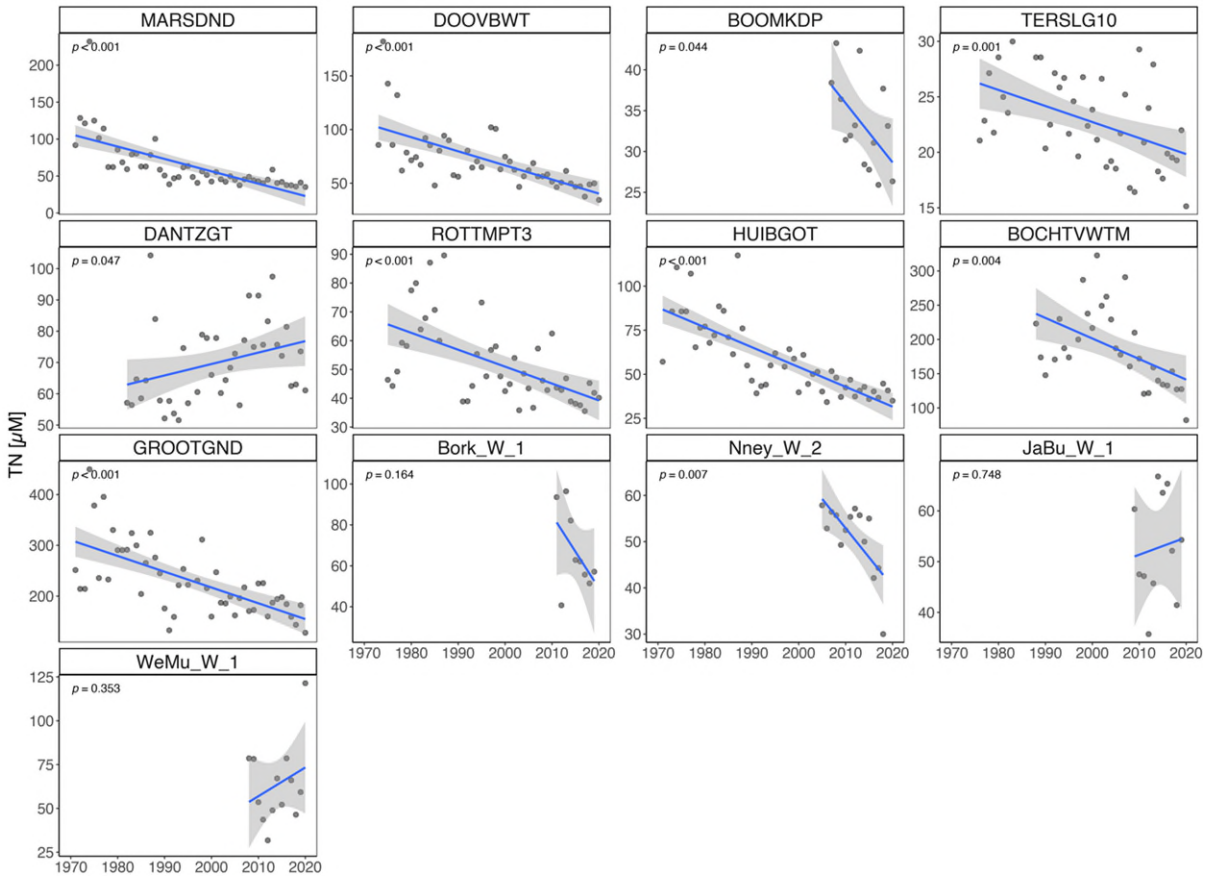
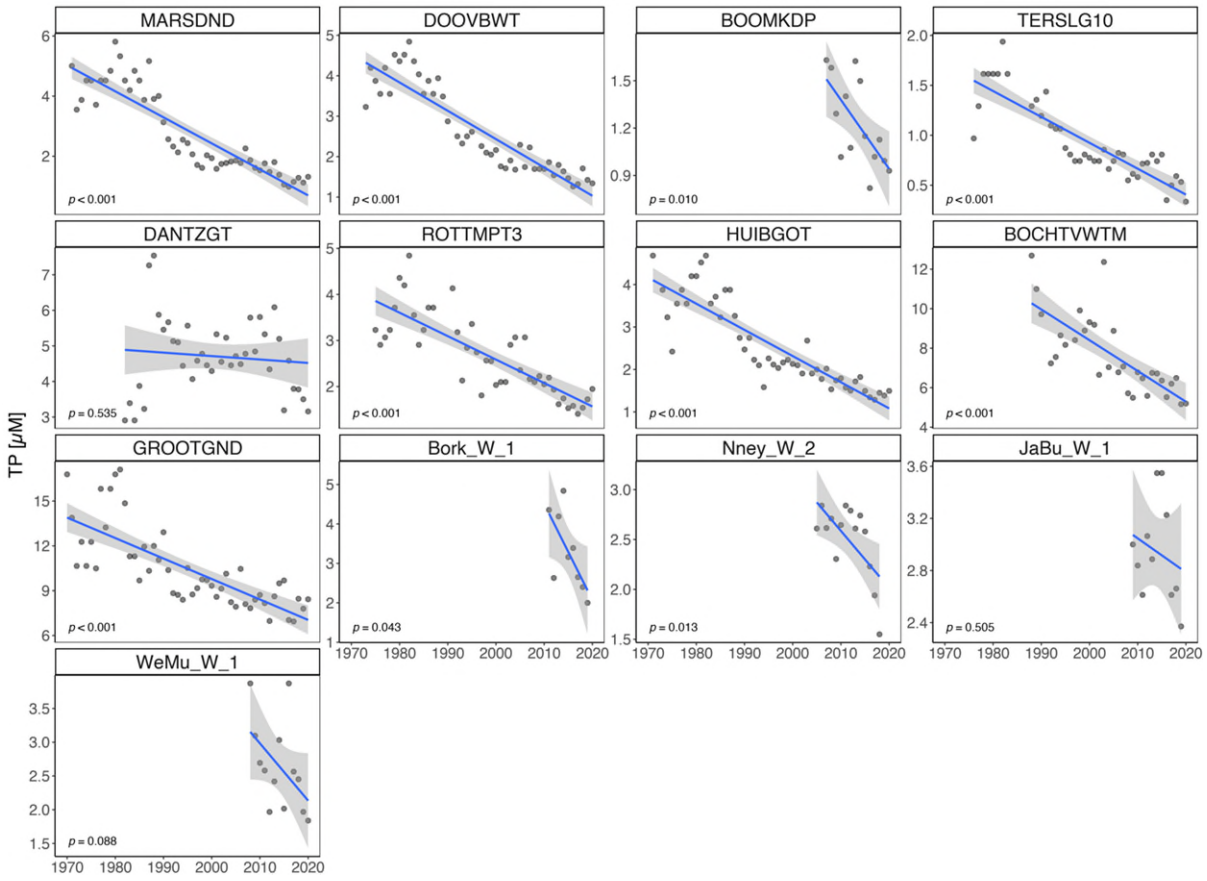
Appendices


TN [µM]
MARSDND
p < 0.001
DOOVBWT
p < 0.001
BOOMKDP
p = 0.044
TERSLG10
p = 0.001
DANTZGT
p = 0.047
ROTTMPT3
p < 0.001
HUIBGOT
p < 0.001
BOCHTVWTM
p = 0.004
GROOTGND
p < 0.001
Bork_W_1
p = 0.164
Nney_W_2
p = 0.007
JaBu_W_1
p = 0.748
WeMu_W_1
p = 0.353


TP [µM]
MARSDND
p < 0.001
DOOVBWT
p < 0.001
BOOMKDP
p = 0.010
TERSLG10
p < 0.001
DANTZGT
p = 0.535
ROTTMPT3
p < 0.001
HUIBGOT
p < 0.001
BOCHTVWTM
p < 0.001
GROOTGND
p < 0.001
Bork_W_1
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Nney_W_2
p = 0.013
JaBu_W_1
p = 0.505
WeMu_W_1
p = 0.088

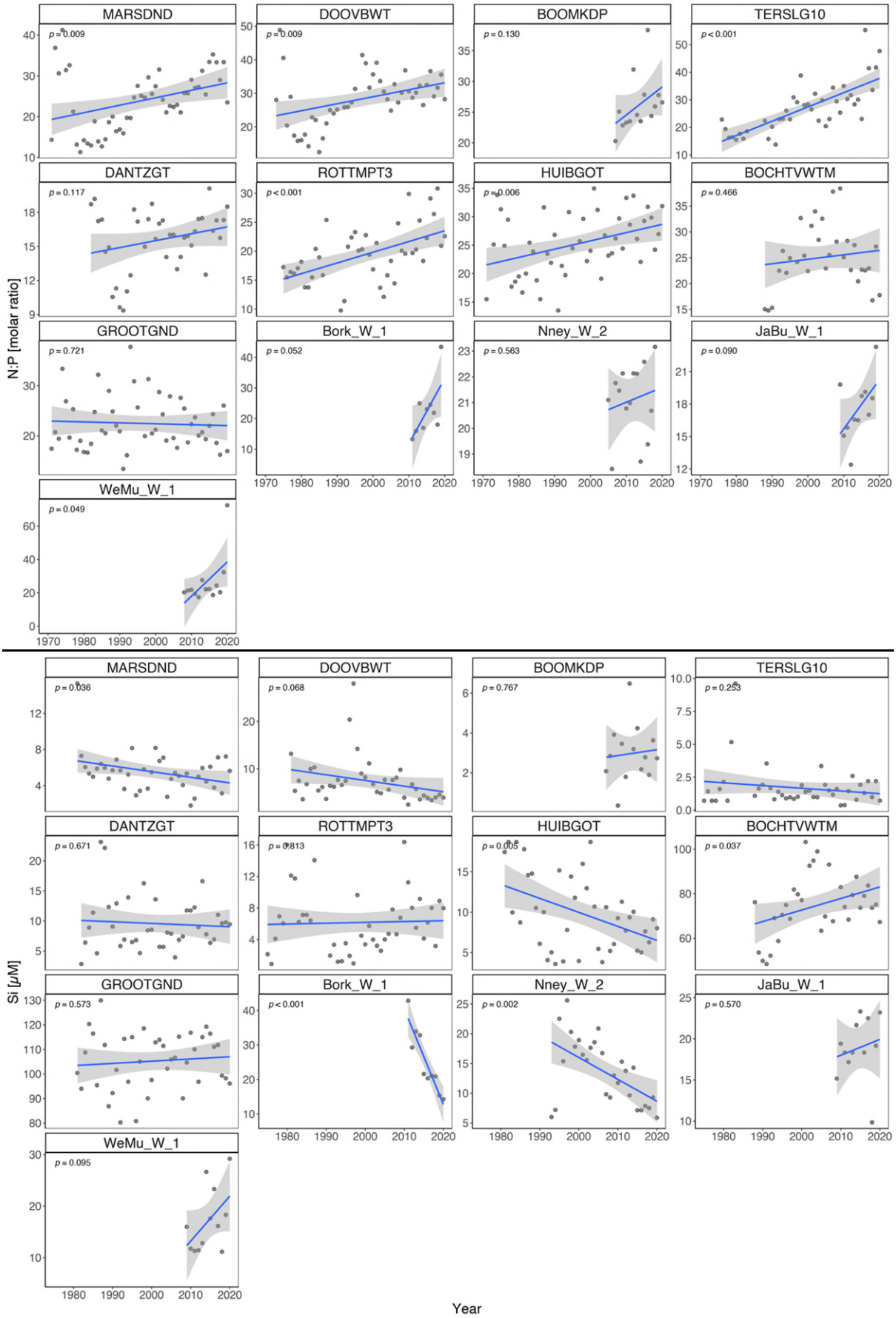
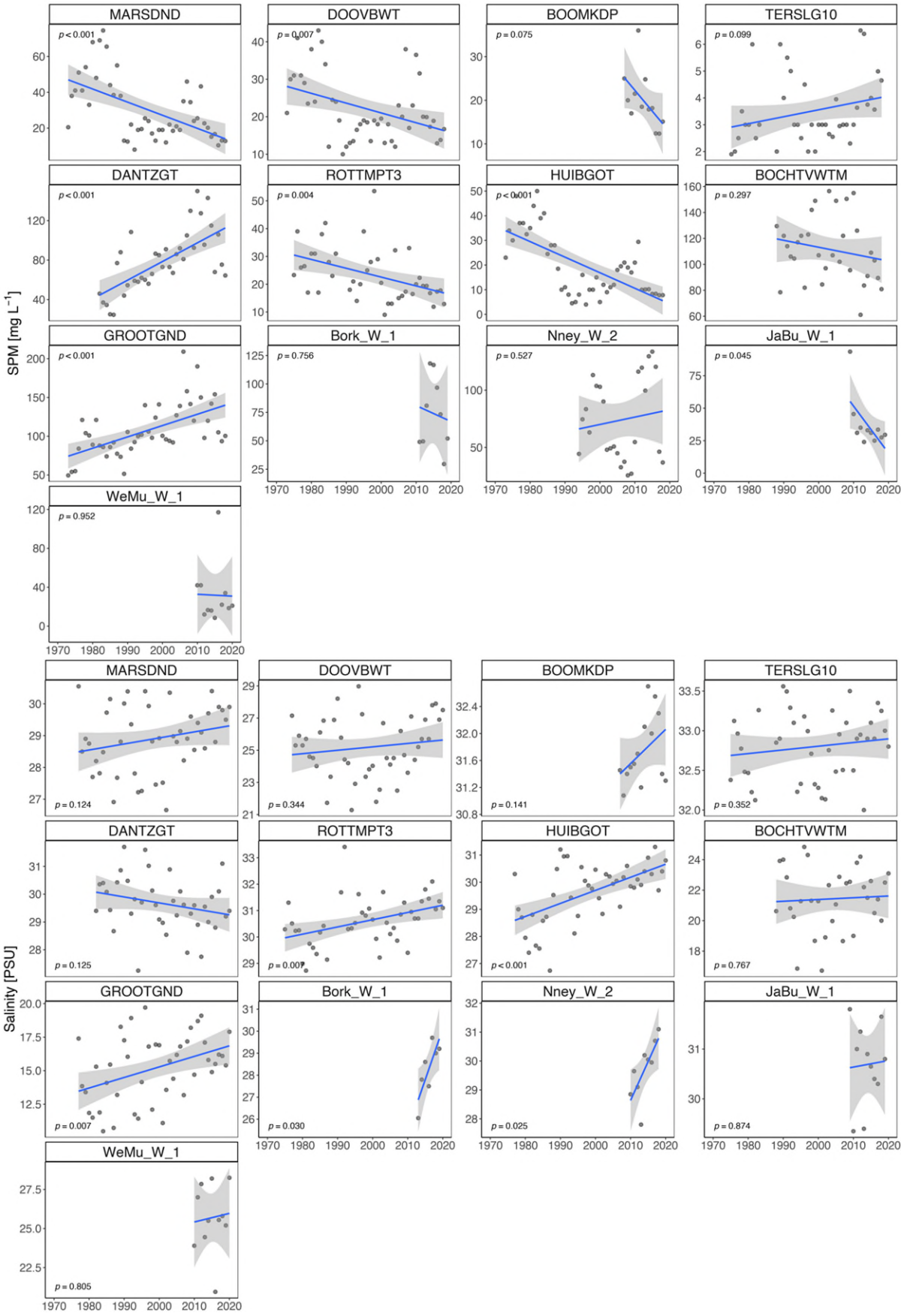


Fig. S1. Temporal trend of nutrient concentrations at the Wadden Sea coastal stations. *Data input: annual median*


SPM [mg L^{-1}]
MARSDND
p < 0.001
DOOVBWT
p = 0.007
BOOMKDP
p = 0.075
TERSLG10
p = 0.099
DANTZGT
p < 0.001
ROTTMPT3
p = 0.004
HUIBGOT
p < 0.001
BOCHTVWTM
p = 0.297
GROOTGND
p < 0.001
Bork_W_1
p = 0.756
Nney_W_2
p = 0.527
JaBu_W_1
p = 0.045
WeMu_W_1
p = 0.952
Salinity [PSU]
MARSDND
p = 0.124
DOOVBWT
p = 0.344
BOOMKDP
p = 0.141
TERSLG10
p = 0.352
DANTZGT
p = 0.125
ROTTMPT3
p = 0.007
HUIBGOT
p < 0.001
BOCHTVWTM
p = 0.767
GROOTGND
p = 0.007
Bork_W_1
p = 0.030
Nney_W_2
p = 0.025
JaBu_W_1
p = 0.874
WeMu_W_1
p = 0.805
1970 1980 1990 2000 2010 2020

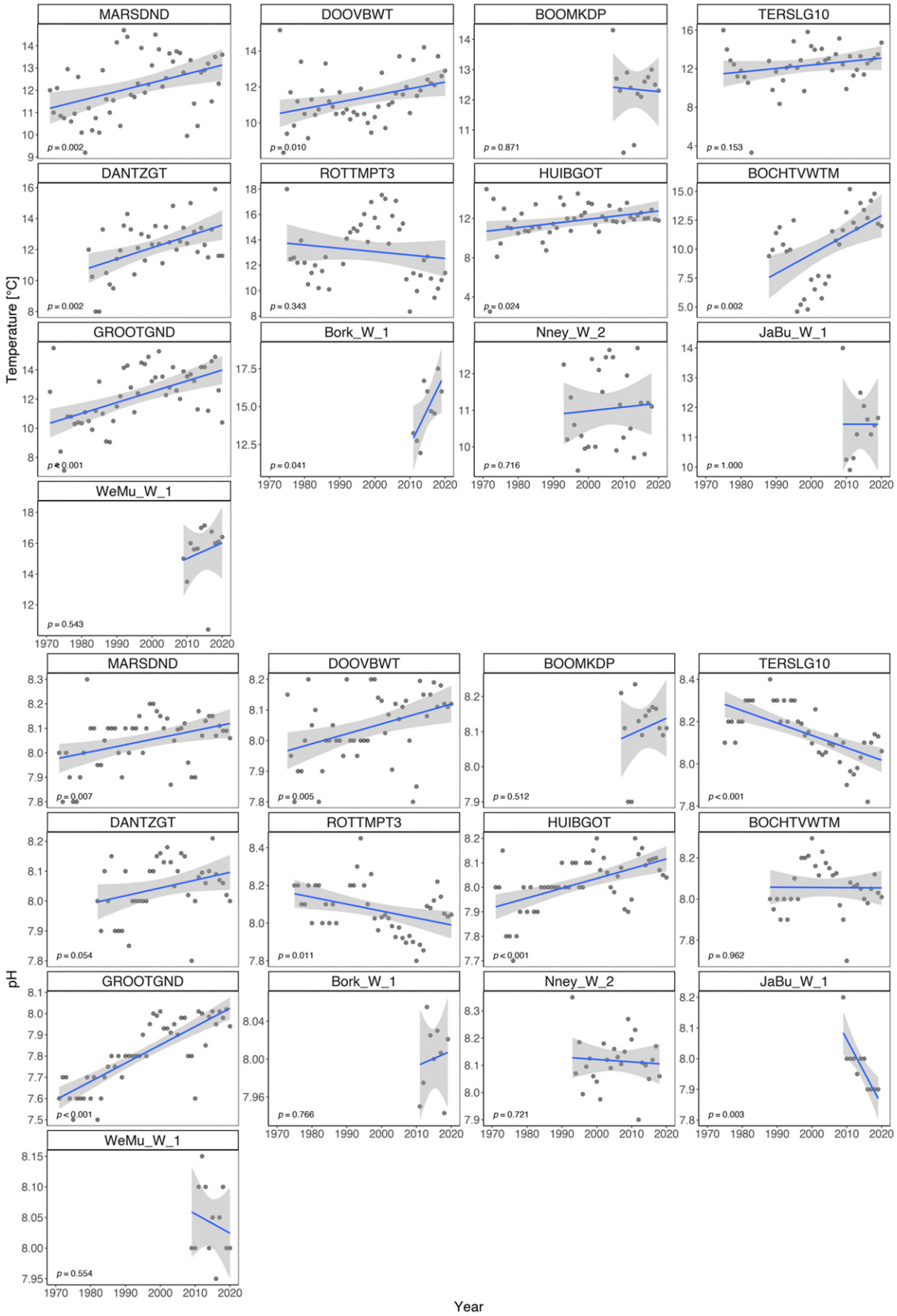
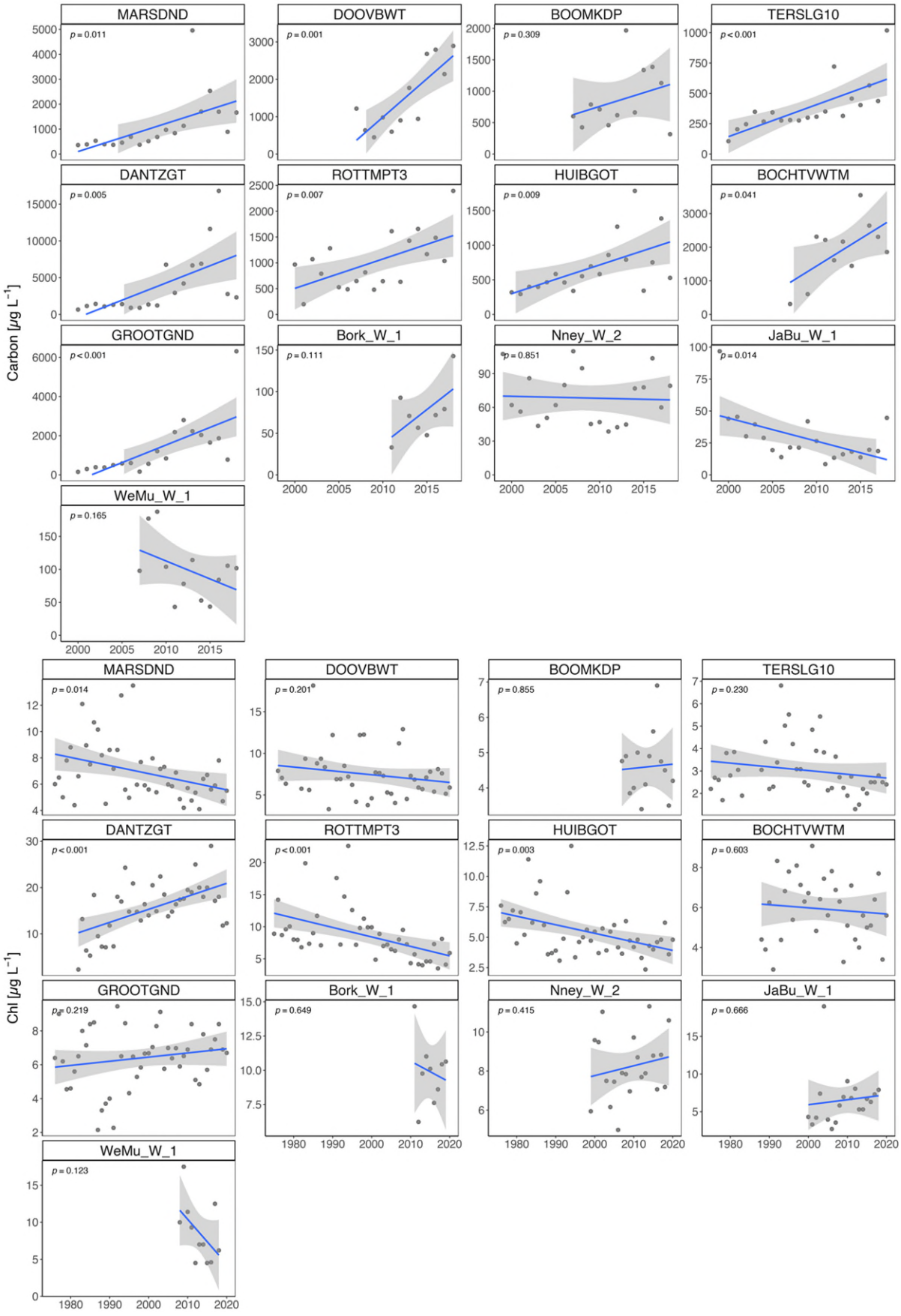


Fig. S2. Temporal trend of environmental factors at the Wadden Sea coastal stations. *Data input: annual median*


Carbon [µg L⁻¹]
MARSDND
p = 0.011
DOOVBWT
p = 0.001
BOOMKDP
p = 0.309
TERSLG10
p < 0.001
DANTZGT
p = 0.005
ROTTMPT3
p = 0.007
HUIBGOT
p = 0.009
BOCHTVWTM
p = 0.041
GROOTGND
p < 0.001
Bork_W_1
p = 0.111
Nney_W_2
p = 0.851
JaBu_W_1
p = 0.014
WeMu_W_1
p = 0.165
Chl [µg L⁻¹]
MARSDND
p = 0.014
DOOVBWT
p = 0.201
BOOMKDP
p = 0.855
TERSLG10
p = 0.230
DANTZGT
p < 0.001
ROTTMPT3
p < 0.001
HUIBGOT
p = 0.003
BOCHTVWTM
p = 0.603
GROOTGND
p = 0.219
Bork_W_1
p = 0.649
Nney_W_2
p = 0.415
JaBu_W_1
p = 0.666
WeMu_W_1
p = 0.123

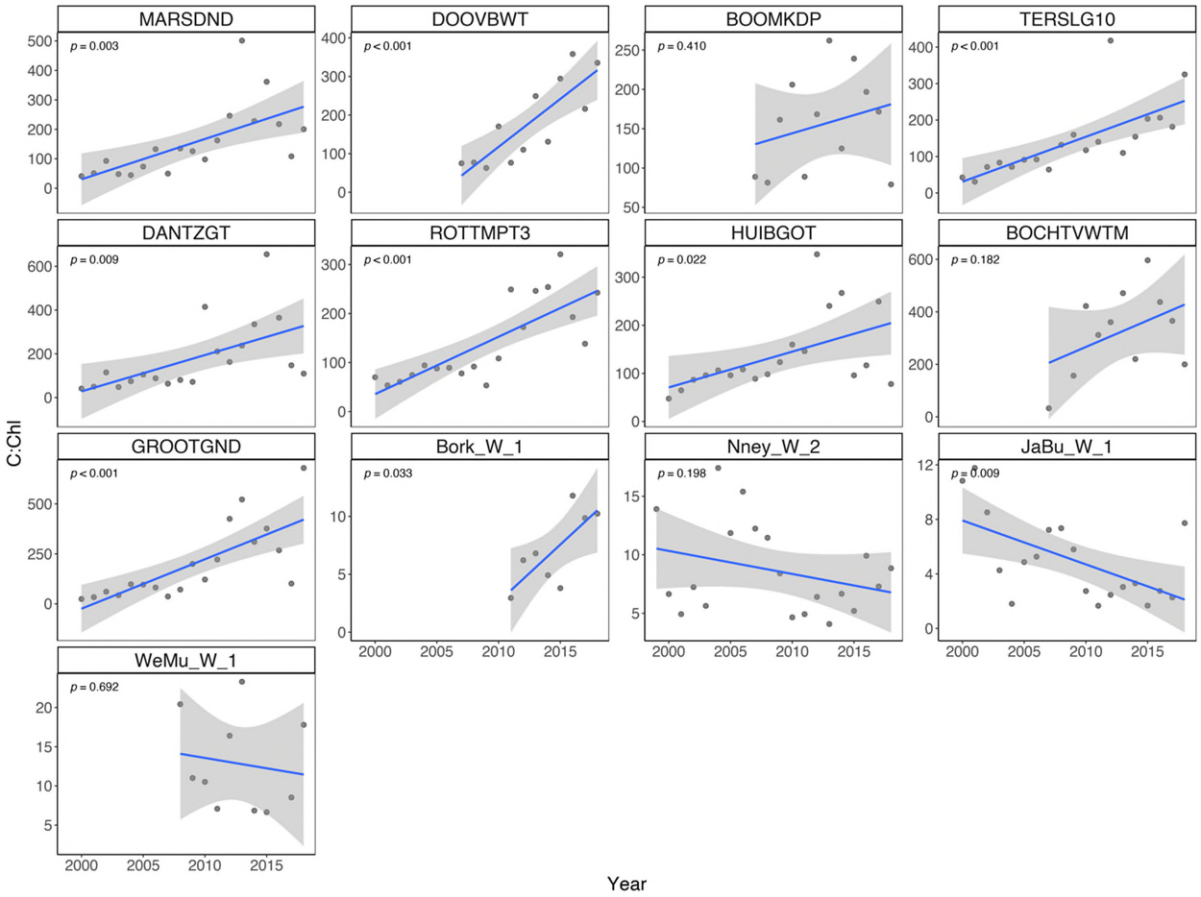
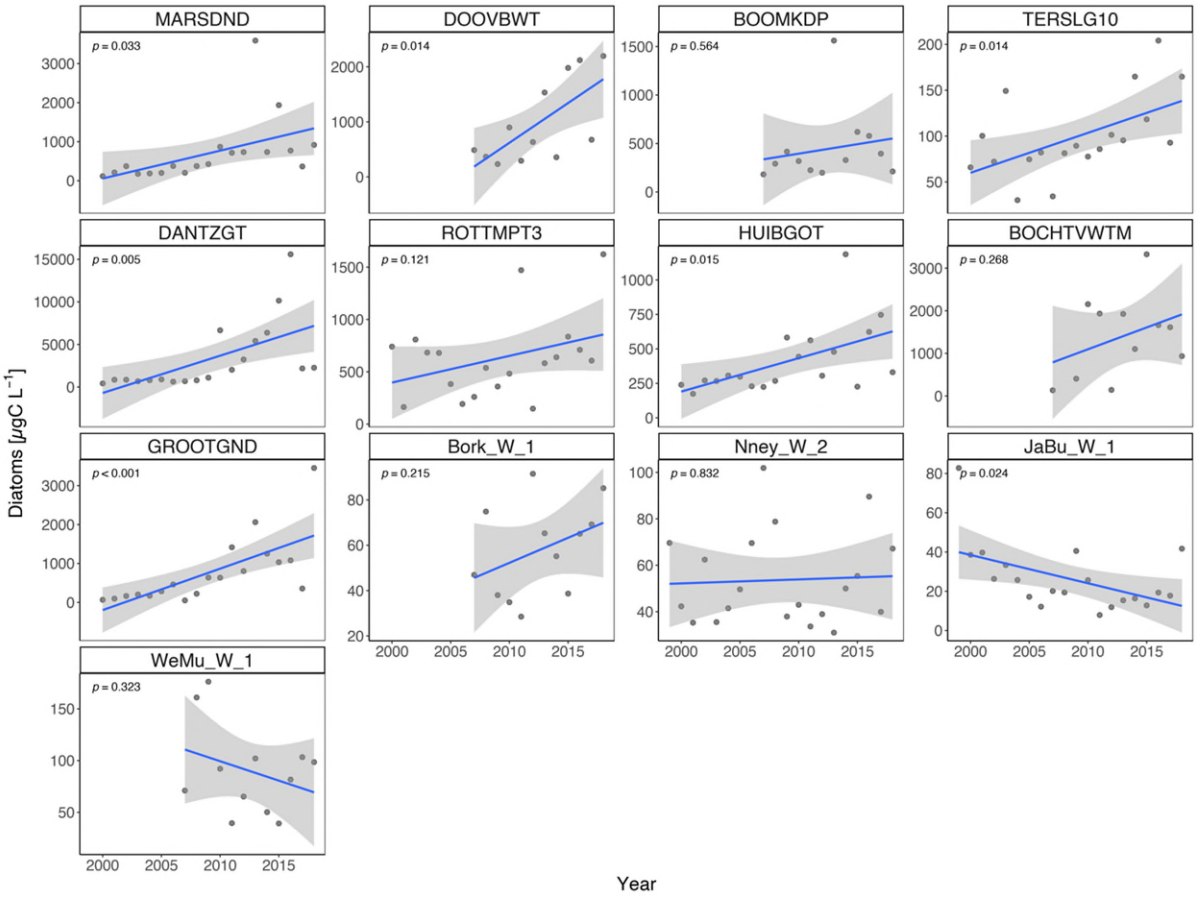
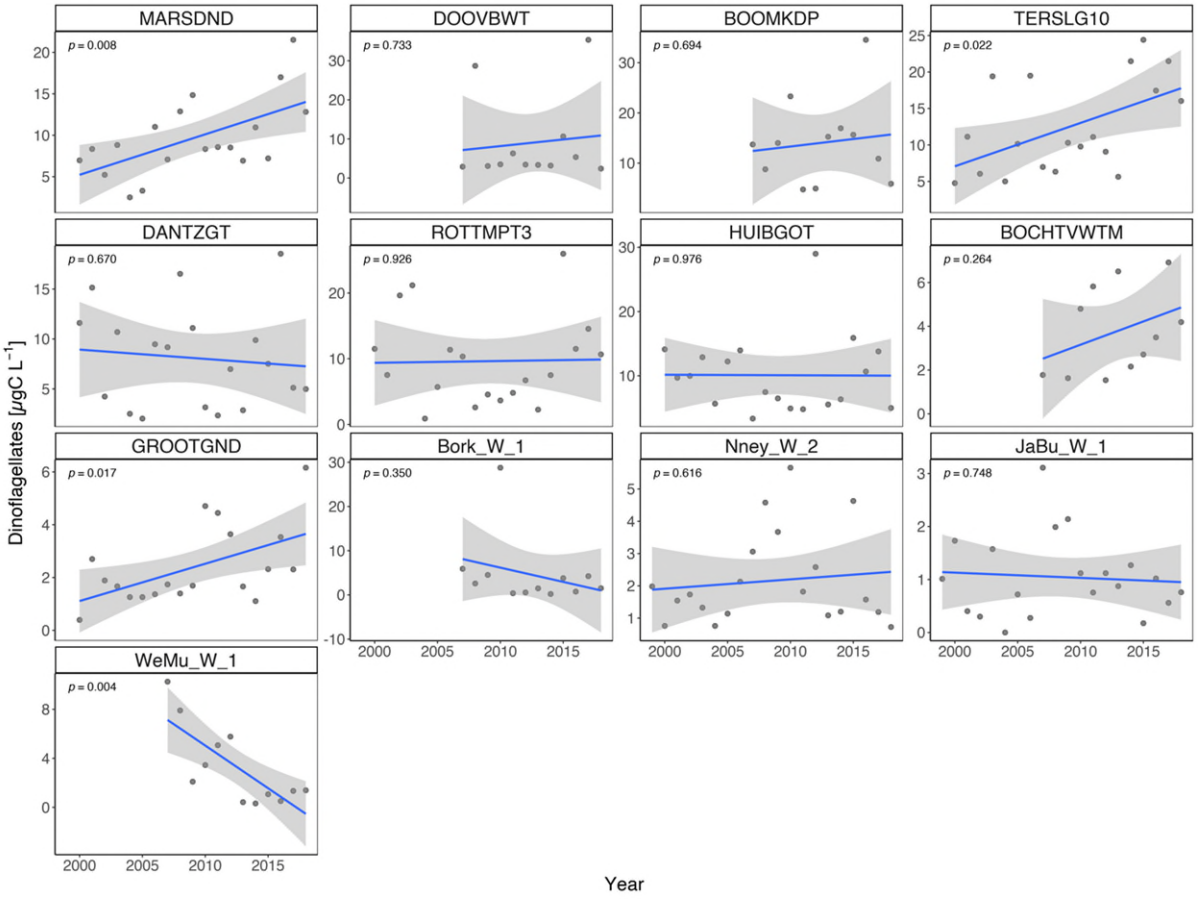
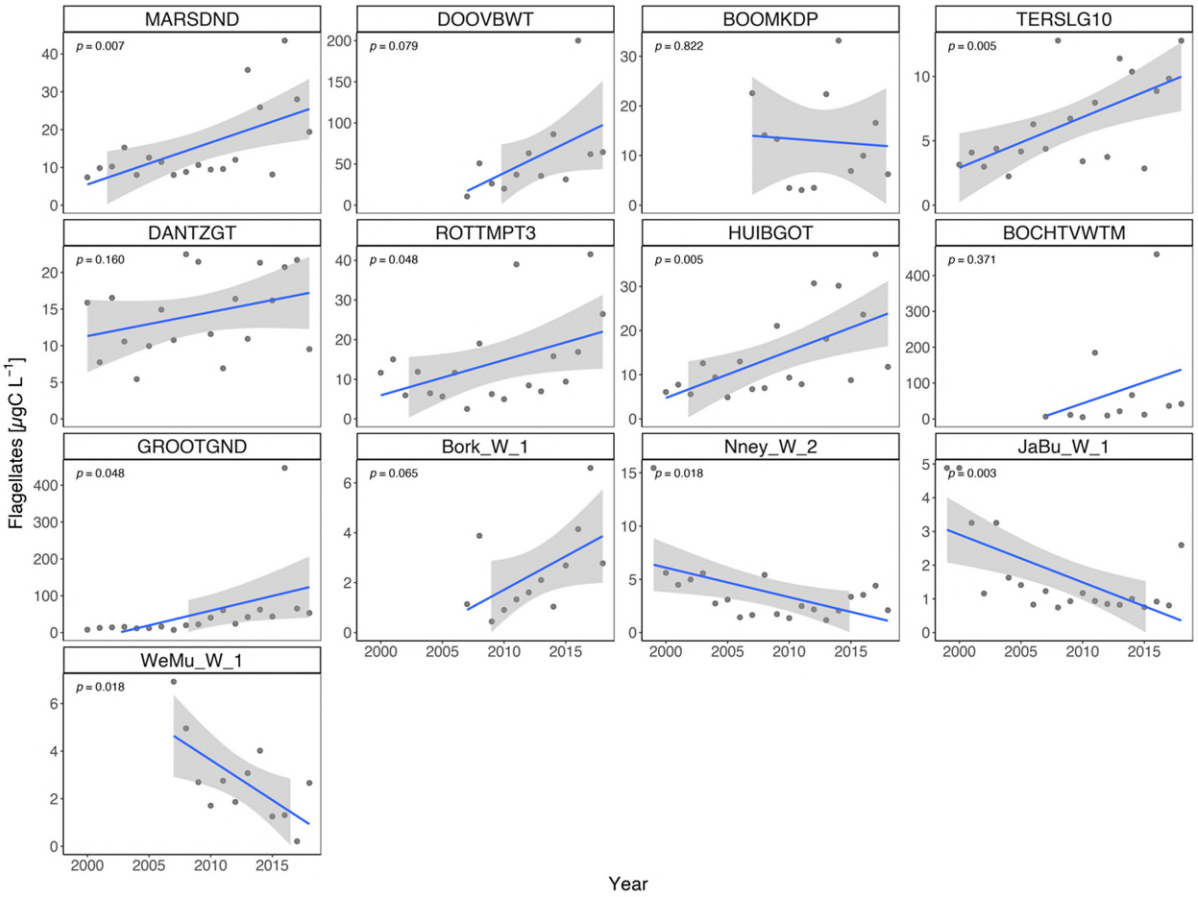
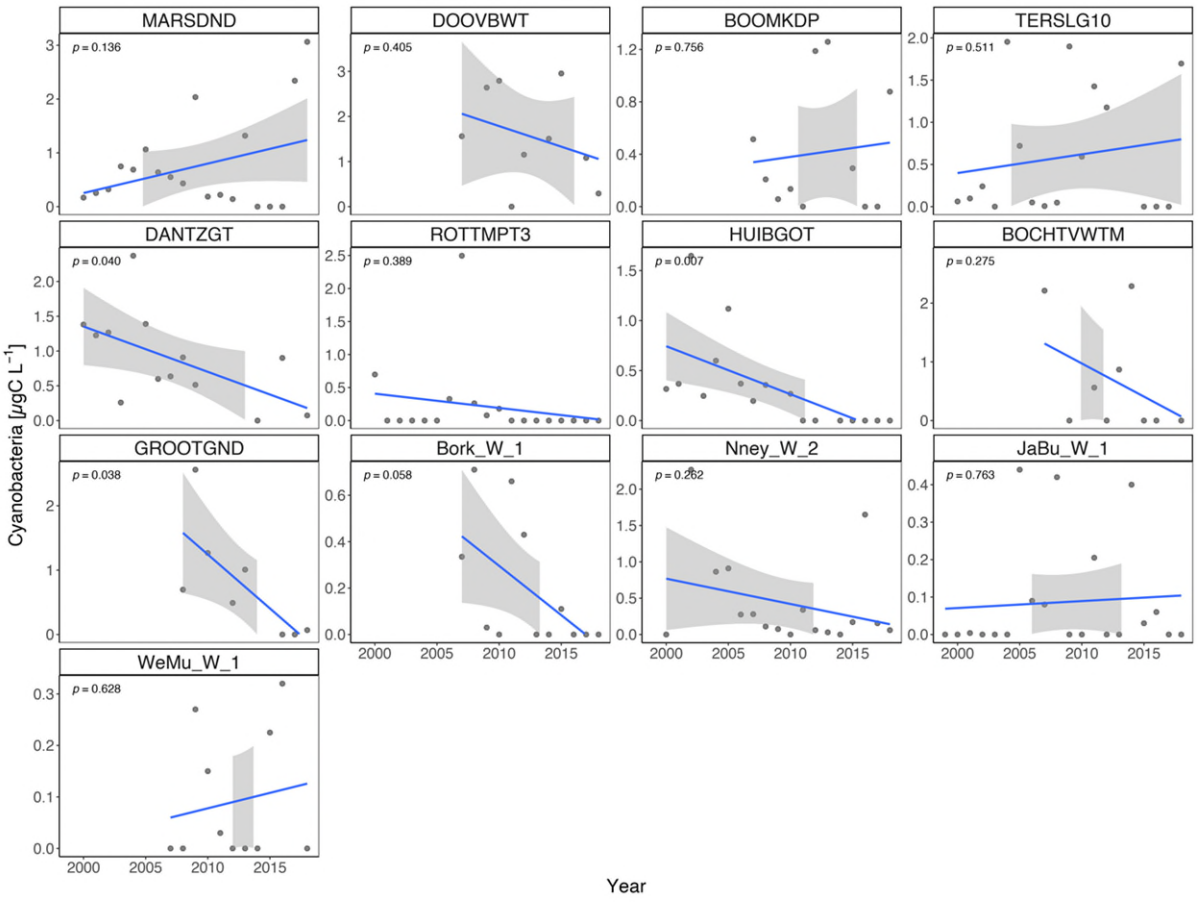


Fig. S3. Temporal trend of the phytoplankton biomass measured as carbon, chlorophyll *a* and the C:Chl ratio at the Wadden Sea coastal stations. *Data input: annual median*


MARSDND
p = 0.033
DOOVBWT
p = 0.014
BOOMKDP
p = 0.564
TERSLG10
p = 0.014
DANTZGT
p = 0.005
ROTTMPT3
p = 0.121
HUIBGOT
p = 0.015
BOCHTVWTM
p = 0.268
GROOTGND
p < 0.001
Bork_W_1
p = 0.215
Nney_W_2
p = 0.832
JaBu_W_1
p = 0.024
WeMu_W_1
p = 0.323
Diatoms [µgC L⁻¹]
Year


MARSDND
p = 0.008
DOOVBWT
p = 0.733
BOOMKDP
p = 0.694
TERSLG10
p = 0.022
DANTZGT
p = 0.670
ROTTMPT3
p = 0.926
HUIBGOT
p = 0.976
BOCHTVWTM
p = 0.264
GROOTGND
p = 0.017
Bork_W_1
p = 0.350
Nney_W_2
p = 0.616
JaBu_W_1
p = 0.748
WeMu_W_1
p = 0.004
Dinoflagellates [µgC L⁻¹]
Year


MARSDND
p = 0.007
DOOVBWT
p = 0.079
BOOMKDP
p = 0.822
TERSLG10
p = 0.005
DANTZGT
p = 0.160
ROTTMPT3
p = 0.048
HUIBGOT
p = 0.005
BOCHTVWTM
p = 0.371
GROOTGND
p = 0.048
Bork_W_1
p = 0.065
Nney_W_2
p = 0.018
JaBu_W_1
p = 0.003
WeMu_W_1
p = 0.018
Flagellates [µgC L⁻¹]
Year


MARSDND
p = 0.136
DOOVBWT
p = 0.405
BOOMKDP
p = 0.756
TERSLG10
p = 0.511
DANTZGT
p = 0.040
ROTTMPT3
p = 0.389
HUIBGOT
p = 0.007
BOCHTVWTM
p = 0.275
GROOTGND
p = 0.038
Bork_W_1
p = 0.058
Nney_W_2
p = 0.262
JaBu_W_1
p = 0.763
WeMu_W_1
p = 0.628
Cyanobacteria [µgC L⁻¹]
Year

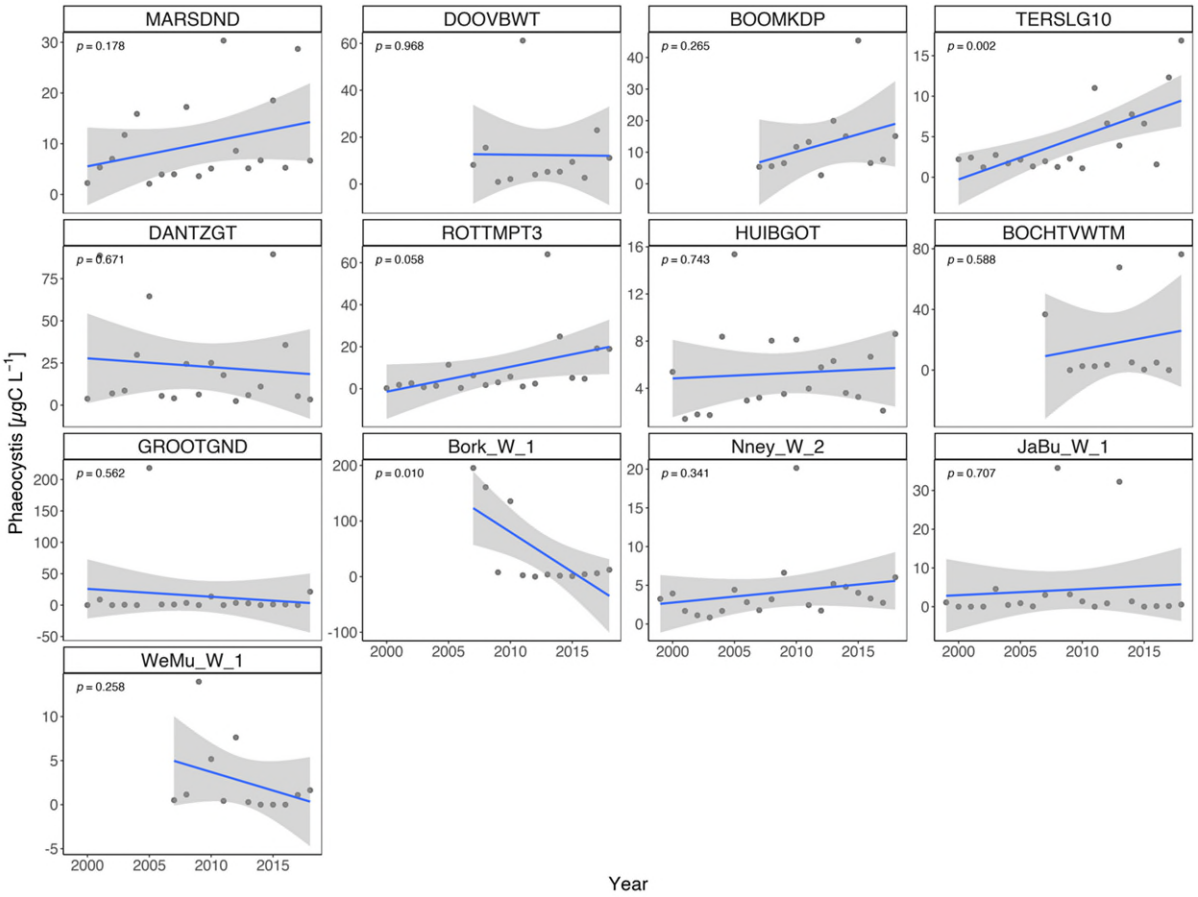


Fig. S4. Temporal trend of phytoplankton functional groups measured as the yearly biomass median of Diatoms, Dinoflagellates, Flagellates, Cyanobacteria and *Phaeocystis* at the Wadden Sea coastal stations. *Data input: annual median*

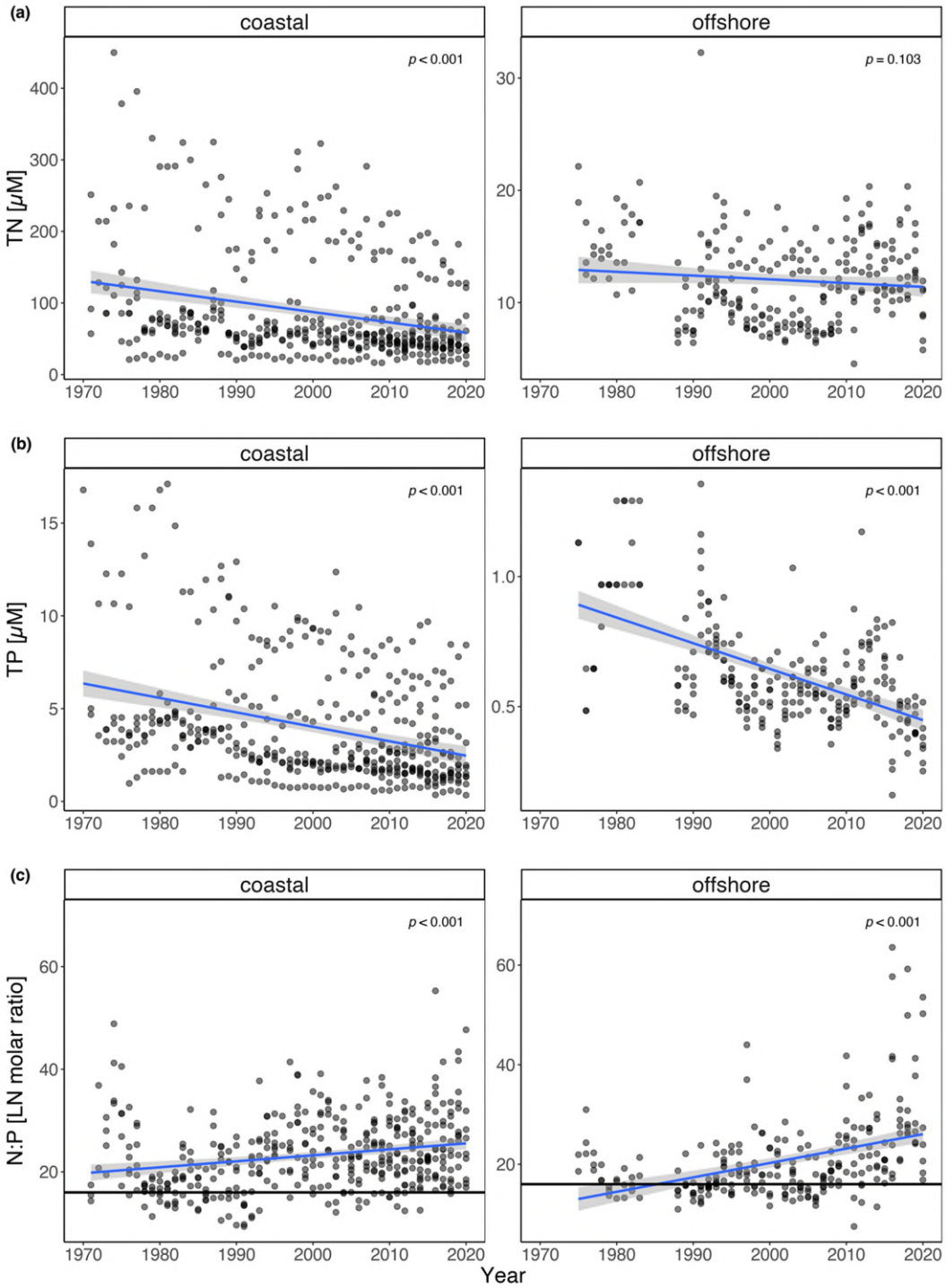


Fig. S5. Temporal trend of total nutrients at the Wadden Sea (coastal) and North Sea (offshore) stations. Each dot represents the annual median in one station. Black horizointal line in (c) represents the Redfield ratio of N:P = 16. Overlaying oints result in darker shades of greay.

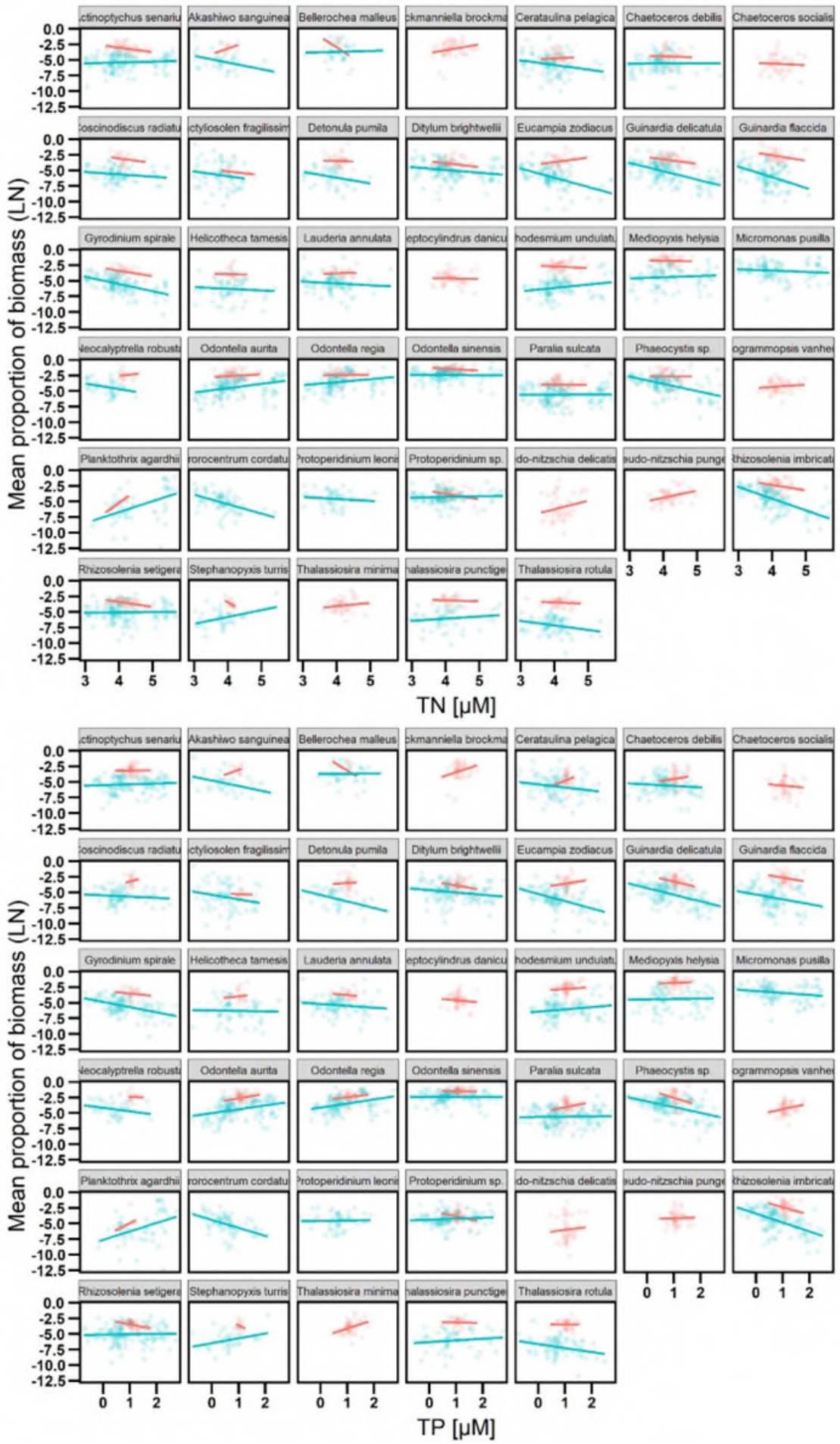


Fig. S6: Proportion of dominant species per year in relation to TN and TP for NL (blue) and DE (red) stations.

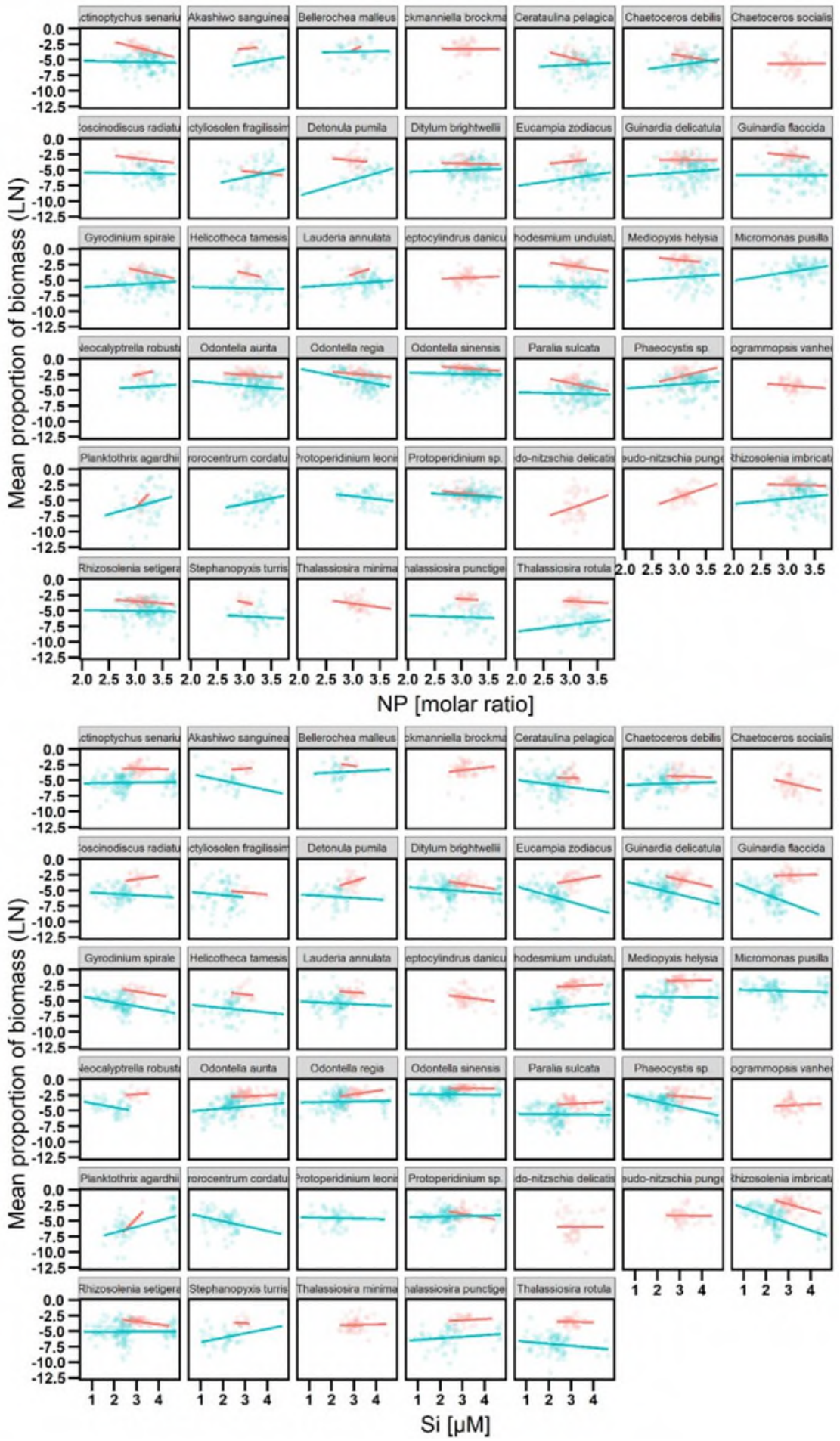


Fig. S7: Proportion of dominant species per year in relation to NP ratios and Si concentration for NL (blue) and DE (red) stations.

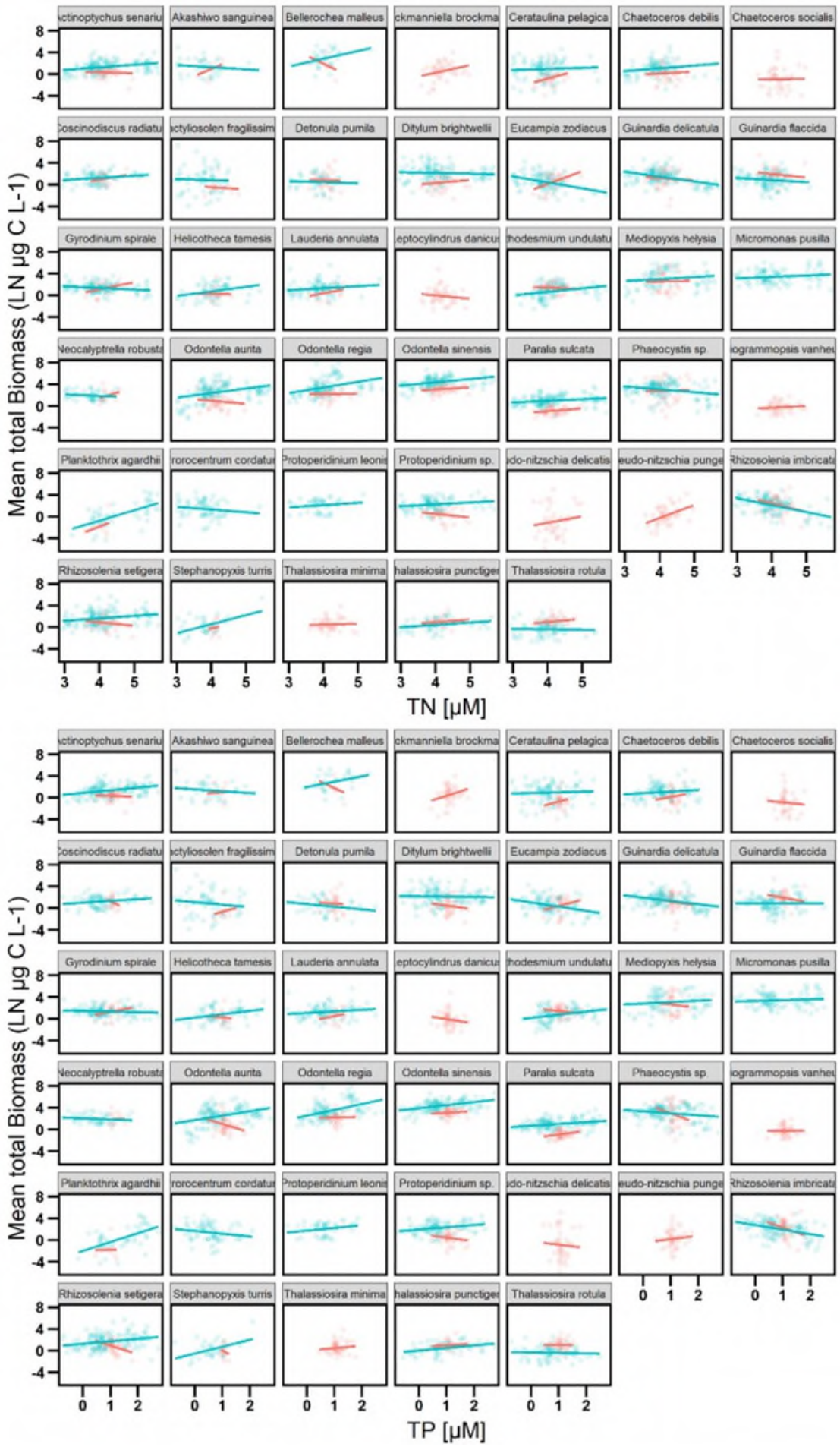


Fig. S8: Biomass of dominant species per year in relation to TN and TP for NL (blue) and DE (red) stations.

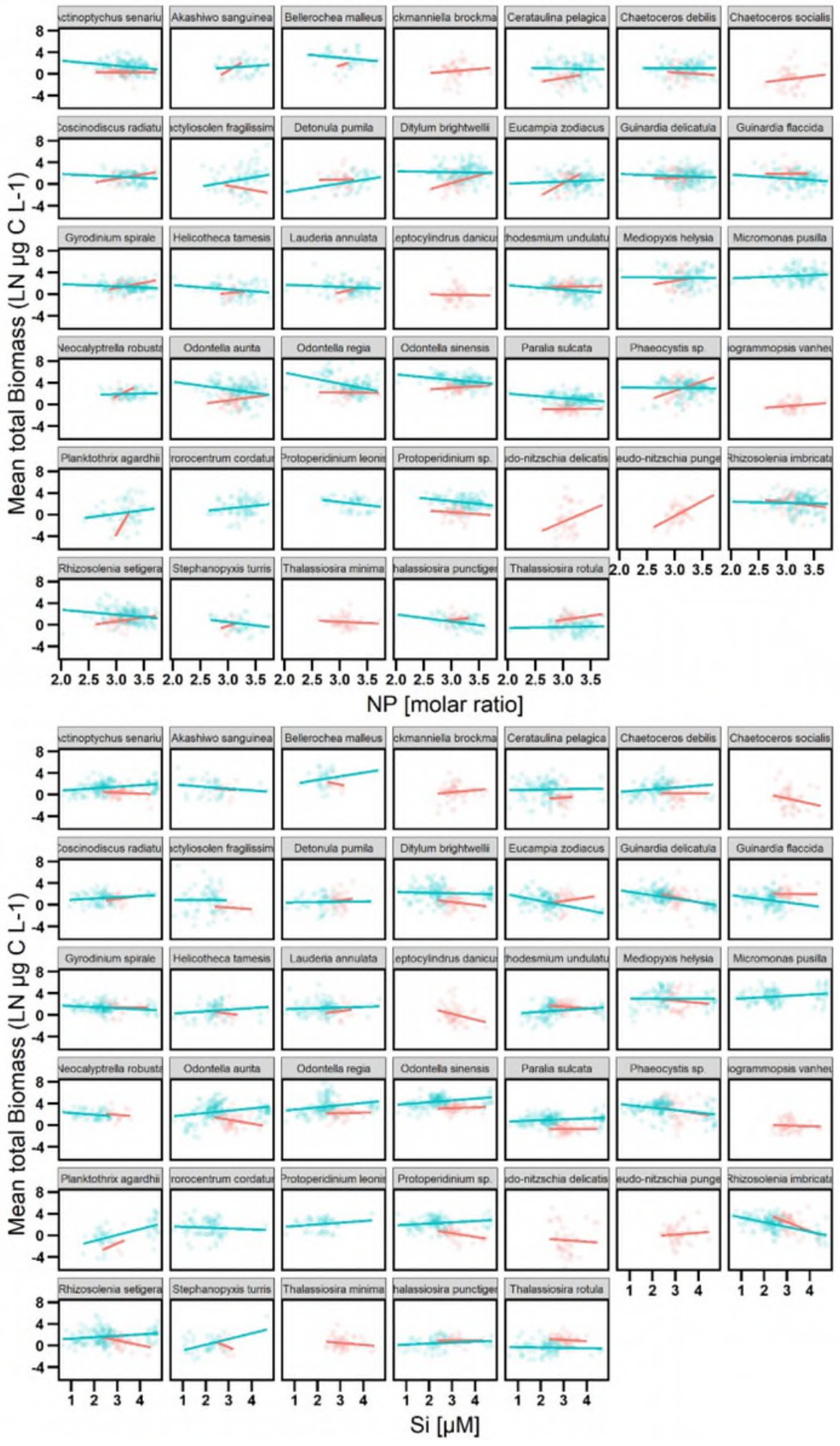


Fig. S9: Biomass of dominant species per year in relation to N:P ratio and Si concentration for NL (blue) and DE (red) stations.

Table S1. Results of the linear mixed effect model, analyzing the effects of environmental factors on phytoplankton biomass (carbon and chlorophyll *a*), considering "station" as a random effect. The different outputs between NL and DE have been highlighted in gray. Data input: *annual median*

	Carbon (LN µgL^{-1})						**Chl (LN µgL^{-1})**					
	all		**NL**		**DE**		**all**		**NL**		**DE**	
Predictors	*Estimates*	*p*	*Estimates*	*p*	*Estimates*	*p*	*Estimates*	*p*	*Estimates*	*p*	*Estimates*	*p*
(Intercept)	-1.782	0.721	-6.522	0.239	10.353	0.167	-5.381	**<0.001**	-6.044	**<0.001**	1.644	0.706
LN TN	-0.264	0.501	0.089	0.843	-0.122	0.832	0.462	**<0.001**	0.517	**<0.001**	0.002	0.995
LN NP	0.448	0.173	0.216	0.547	0.366	0.587	-0.121	0.072	-0.116	0.100	0.167	0.519
LN Si	-0.004	0.981	0.180	0.278	-0.912	**0.023**	-0.157	**<0.001**	-0.162	**<0.001**	-0.033	0.862
LN SPM	0.235	0.114	0.519	**0.003**	0.133	0.376	0.117	**0.003**	0.110	**0.013**	0.163	**0.025**
Salinity	0.128	**0.009**	0.105	**0.015**	-0.137	**0.020**	0.007	0.560	0.008	0.508	0.014	0.462
Temperature	-0.046	0.206	-0.043	0.347	0.116	**0.016**	0.050	**<0.001**	0.052	**<0.001**	0.031	0.138
pH	0.449	0.392	0.967	0.103	-0.323	0.713	0.632	**<0.001**	0.671	**<0.001**	-0.163	0.752
Random Effects												
σ^2	0.41		0.50		0.13		0.07		0.08		0.05	
τ_{00}	3.16 $_{StationID}$		0.09 $_{StationID}$		0.12 $_{StationID}$		0.05 $_{StationID}$		0.07 $_{StationID}$		0.00 $_{StationID}$	
ICC	0.88		0.16		0.49		0.43		0.47			
N	13 $_{StationID}$		9 $_{StationID}$		4 $_{StationID}$		13 $_{StationID}$		9 $_{StationID}$		4 $_{StationID}$	
Observations	182		148		34		338		303		35	
Marginal R^2 / Conditional R^2	0.117 / 0.897		0.265 / 0.382		0.560 / 0.775		0.309 / 0.605		0.321 / 0.640		0.244 / NA	

Table S2. Results of the linear mixed effect model, analyzing the effects of environmental factors on phytoplankton standing diversity (annual richness and ENS), considering "station" as a random effect. The different outputs between NL and DE have been highlighted in gray. Data input: *annual median (scaled values)*

	Annual Richness						**Annual ENS**					
	all		**NL**		**DE**		**all**		**NL**		**DE**	
Predictors	*Estimates*	*p*	*Estimates*	*p*	*Estimates*	*p*	*Estimates*	*p*	*Estimates*	*p*	*Estimates*	*p*
(Intercept)	0.148	0.515	0.412	0.055	-0.482	0.178	0.124	0.580	-0.137	0.434	0.678	0.339
TN	0.450	0.120	0.386	0.228	1.220	0.126	0.553	0.133	0.737	**0.038**	1.298	0.553
NP	-0.274	**0.001**	-0.300	**0.001**	-0.089	0.692	-0.064	0.536	-0.090	0.358	0.292	0.641
Si	-0.875	**0.004**	-0.898	**0.003**	-1.634	**0.042**	-1.068	**0.002**	-0.751	**0.010**	-2.398	0.262
SPM	-0.242	**0.017**	-0.339	**0.015**	-0.032	0.756	0.040	0.753	-0.351	**0.020**	0.446	0.108
Salinity	-0.220	0.340	-0.391	0.156	0.834	**0.021**	-0.464	0.101	-0.355	0.206	0.264	0.705
Temperature	0.018	0.798	-0.006	0.940	-0.049	0.633	0.166	0.060	0.165	0.085	0.194	0.399
pH	-0.178	**0.011**	-0.183	**0.023**	-0.299	**0.017**	0.119	0.184	0.067	0.451	0.013	0.969
Random Effects												
σ^2	0.36		0.40		0.11		0.60		0.51		0.92	
τ_{00}	0.56 $_{StationID}$		0.33 $_{StationID}$		0.30 $_{StationID}$		0.47 $_{StationID}$		0.17 $_{StationID}$		0.45 $_{StationID}$	
ICC	0.61		0.45		0.73		0.44		0.25		0.33	
N	13 $_{StationID}$		9 $_{StationID}$		4 $_{StationID}$		13 $_{StationID}$		9 $_{StationID}$		4 $_{StationID}$	
Observations	182		148		34		182		148		34	
Marginal R^2 / Conditional R^2	0.258 / 0.713		0.346 / 0.639		0.341 / 0.825		0.152 / 0.528		0.169 / 0.380		0.222 / 0.476	

Table S3: Results of the linear mixed effect model analyzing the effects of nutrients (TN, TP and N:P ratio, see term) on the absolute and relative biomass (see biomass) for each of the dominant species in the data set, considering “station” as a random effect. The table gives the estimate for the slope, it’s standard error and significance level.

Morphotype_harmonized	biomass	term	estimate	std.error	p.value
Actinoptychus senarius	relative	TN	0.927	0.380	0.018
Akashiwo sanguinea	relative	TN	-0.830	0.584	0.171
Bellerochea malleus	relative	TN	0.250	0.728	0.734
Brockmanniella brockmannii	relative	TN	2.537	0.671	0.000
Cerataulina pelagica	relative	TN	-0.432	0.375	0.261
Chaetoceros debilis	relative	TN	-0.074	0.400	0.855
Chaetoceros socialis	relative	TN	0.647	0.827	0.440
Coscinodiscus radiatus	relative	TN	-0.275	0.508	0.592
Dactyliosolen fragilissimus	relative	TN	-0.533	0.514	0.303
Detonula pumila	relative	TN	0.031	0.724	0.966
Ditylum brightwellii	relative	TN	-0.399	0.275	0.166
Eucampia zodiacus	relative	TN	-0.535	0.512	0.301
Guinardia delicatula	relative	TN	-0.255	0.417	0.544
Guinardia flaccida	relative	TN	-0.583	0.665	0.383
Gyrodinium spirale	relative	TN	-0.382	0.399	0.343
Helicotheca tamesis	relative	TN	-0.096	0.658	0.885
Lauderia annulata	relative	TN	0.196	0.432	0.655
Leptocylindrus danicus	relative	TN	0.323	0.570	0.574
Lithodesmium undulatum	relative	TN	0.443	0.516	0.394
Mediopyxis helysia	relative	TN	0.706	0.544	0.204
Micromonas pusilla	relative	TN	-0.471	0.318	0.182
Neocalyptrella robusta	relative	TN	0.689	0.692	0.334
Odontella aurita	relative	TN	1.126	0.378	0.005
Odontella regia	relative	TN	0.498	0.342	0.154
Odontella sinensis	relative	TN	0.040	0.232	0.864
Paralia sulcata	relative	TN	1.522	0.421	0.001
Phaeocystis sp.	relative	TN	-0.787	0.341	0.030
Plagiogrammopsis vanheurckii	relative	TN	1.668	0.531	0.003
Planktothrix agardhii	relative	TN	1.601	0.406	0.029

Morphotype_harmonized	biomass	term	estimate	std.error	p.value
Prorocentrum cordatum	relative	TN	-1.499	0.357	0.001
Protoperidinium leonis	relative	TN	-0.312	0.460	0.501
Protoperidinium sp.	relative	TN	0.071	0.173	0.690
Pseudo-nitzschia delicatissima	relative	TN	2.671	1.128	0.023
Pseudo-nitzschia pungens	relative	TN	1.339	0.580	0.029
Rhizosolenia imbricata	relative	TN	-0.430	0.436	0.327
Rhizosolenia setigera	relative	TN	-0.074	0.331	0.824
Stephanopyxis turris	relative	TN	1.664	0.653	0.019
Thalassiosira minima	relative	TN	0.536	0.502	0.291
Thalassiosira punctigera	relative	TN	-0.189	0.679	0.782
Thalassiosira rotula	relative	TN	-0.439	0.603	0.469
Actinoptychus senarius	absolute	TN	0.422	0.243	0.096
Akashiwo sanguinea	absolute	TN	-0.322	0.455	0.483
Bellerochea malleus	absolute	TN	1.033	0.766	0.191
Brockmanniella brockmannii	absolute	TN	2.730	0.816	0.002
Cerataulina pelagica	absolute	TN	0.074	0.493	0.881
Chaetoceros debilis	absolute	TN	-0.206	0.408	0.617
Chaetoceros socialis	absolute	TN	1.658	1.068	0.129
Coscinodiscus radiatus	absolute	TN	0.388	0.217	0.077
Dactyliosolen fragilissimus	absolute	TN	-0.771	0.690	0.304
Detonula pumila	absolute	TN	-0.042	0.447	0.925
Ditylum brightwellii	absolute	TN	-0.083	0.377	0.827
Eucampia zodiacus	absolute	TN	-0.800	0.346	0.031
Guinardia delicatula	absolute	TN	-0.623	0.265	0.032
Guinardia flaccida	absolute	TN	-0.302	0.439	0.496
Gyrodinium spirale	absolute	TN	-0.138	0.188	0.476
Helicotheca tamesis	absolute	TN	0.482	0.404	0.243
Lauderia annulata	absolute	TN	0.277	0.258	0.285
Leptocylindrus danicus	absolute	TN	0.545	0.785	0.491

Morphotype_harmonized	biomass	term	estimate	std.error	p.value
Lithodesmium undulatum	absolute	TN	0.276	0.289	0.349
Mediopyxis helysia	absolute	TN	0.600	0.423	0.173
Micromonas pusilla	absolute	TN	0.114	0.230	0.647
Neocalyptrella robusta	absolute	TN	-0.134	0.321	0.690
Odontella aurita	absolute	TN	0.081	0.414	0.846
Odontella regia	absolute	TN	-0.066	0.402	0.870
Odontella sinensis	absolute	TN	0.303	0.259	0.248
Paralia sulcata	absolute	TN	0.756	0.281	0.008
Phaeocystis sp.	absolute	TN	-0.480	0.336	0.170
Plagiogrammopsis vanheurckii	absolute	TN	0.606	0.559	0.285
Planktothrix agardhii	absolute	TN	2.095	0.433	0.003
Prorocentrum cordatum	absolute	TN	-0.595	0.458	0.214
Protoperidinium leonis	absolute	TN	0.425	0.425	0.322
Protoperidinium sp.	absolute	TN	-0.607	0.367	0.105
Pseudo-nitzschia delicatissima	absolute	TN	2.190	1.479	0.147
Pseudo-nitzschia pungens	absolute	TN	2.749	0.828	0.002
Rhizosolenia imbricata	absolute	TN	-1.205	0.289	0.000
Rhizosolenia setigera	absolute	TN	0.043	0.283	0.881
Stephanopyxis turris	absolute	TN	1.658	0.514	0.007
Thalassiosira minima	absolute	TN	0.147	0.534	0.784
Thalassiosira punctigera	absolute	TN	0.381	0.277	0.196
Thalassiosira rotula	absolute	TN	0.072	0.405	0.859
Actinoptychus senarius	relative	TP	1.036	0.305	0.001
Akashiwo sanguinea	relative	TP	-0.517	0.467	0.283
Bellerochea malleus	relative	TP	0.000	0.544	1.000
Brockmanniella brockmannii	relative	TP	2.748	0.761	0.001
Cerataulina pelagica	relative	TP	-0.177	0.298	0.559
Chaetoceros debilis	relative	TP	-0.369	0.342	0.289
Chaetoceros socialis	relative	TP	0.285	0.934	0.762

Morphotype_harmonized	biomass	term	estimate	std.error	p.value
Coscinodiscus radiatus	relative	TP	0.178	0.377	0.639
Dactyliosolen fragilissimus	relative	TP	-0.561	0.367	0.131
Detonula pumila	relative	TP	-0.956	0.528	0.076
Ditylum brightwellii	relative	TP	-0.183	0.246	0.467
Eucampia zodiacus	relative	TP	-0.244	0.404	0.548
Guinardia delicatula	relative	TP	0.027	0.345	0.937
Guinardia flaccida	relative	TP	0.567	0.479	0.239
Gyrodinium spirale	relative	TP	0.176	0.304	0.565
Helicotheca tamesis	relative	TP	0.305	0.499	0.546
Lauderia annulata	relative	TP	0.005	0.332	0.987
Leptocylindrus danicus	relative	TP	0.147	0.650	0.823
Lithodesmium undulatum	relative	TP	0.391	0.410	0.343
Mediopyxis helysia	relative	TP	0.381	0.446	0.399
Micromonas pusilla	relative	TP	-0.410	0.236	0.114
Neocalyptrella robusta	relative	TP	0.333	0.464	0.480
Odontella aurita	relative	TP	0.663	0.298	0.030
Odontella regia	relative	TP	0.650	0.263	0.018
Odontella sinensis	relative	TP	0.284	0.192	0.146
Paralia sulcata	relative	TP	1.515	0.330	0.000
Phaeocystis sp.	relative	TP	-0.850	0.284	0.005
Plagiogrammopsis vanheurckii	relative	TP	1.380	0.587	0.023
Planktothrix agardhii	relative	TP	1.167	0.467	0.053
Prorocentrum cordatum	relative	TP	-1.277	0.261	0.001
Protoperidinium leonis	relative	TP	0.069	0.339	0.844
Protoperidinium sp.	relative	TP	0.175	0.145	0.252
Pseudo-nitzschia delicatissima	relative	TP	1.601	1.378	0.252
Pseudo-nitzschia pungens	relative	TP	0.293	0.706	0.681
Rhizosolenia imbricata	relative	TP	-0.669	0.346	0.055
Rhizosolenia setigera	relative	TP	0.058	0.273	0.834

Morphotype_harmonized	biomass	term	estimate	std.error	p.value
Stephanopyxis turris	relative	TP	1.309	0.527	0.022
Thalassiosira minima	relative	TP	1.390	0.575	0.020
Thalassiosira punctigera	relative	TP	-0.224	0.528	0.672
Thalassiosira rotula	relative	TP	-0.629	0.441	0.157
Actinoptychus senarius	absolute	TP	0.409	0.207	0.057
Akashiwo sanguinea	absolute	TP	-0.371	0.347	0.291
Bellerochea malleus	absolute	TP	0.193	0.585	0.745
Brockmanniella brockmannii	absolute	TP	3.126	0.916	0.001
Cerataulina pelagica	absolute	TP	-0.178	0.386	0.649
Chaetoceros debilis	absolute	TP	-0.467	0.343	0.181
Chaetoceros socialis	absolute	TP	0.988	1.216	0.421
Coscinodiscus radiatus	absolute	TP	0.256	0.170	0.135
Dactyliosolen fragilissimus	absolute	TP	-0.920	0.517	0.120
Detonula pumila	absolute	TP	-0.292	0.303	0.338
Ditylum brightwellii	absolute	TP	-0.224	0.313	0.479
Eucampia zodiacus	absolute	TP	-0.665	0.288	0.029
Guinardia delicatula	absolute	TP	-0.250	0.249	0.326
Guinardia flaccida	absolute	TP	0.230	0.310	0.463
Gyrodinium spirale	absolute	TP	-0.029	0.160	0.860
Helicotheca tamesis	absolute	TP	0.321	0.305	0.303
Lauderia annulata	absolute	TP	0.195	0.190	0.306
Leptocylindrus danicus	absolute	TP	0.886	0.875	0.317
Lithodesmium undulatum	absolute	TP	0.179	0.242	0.465
Mediopyxis helysia	absolute	TP	0.212	0.351	0.552
Micromonas pusilla	absolute	TP	-0.032	0.212	0.886
Neocalyptrella robusta	absolute	TP	-0.166	0.235	0.499
Odontella aurita	absolute	TP	-0.274	0.341	0.423
Odontella regia	absolute	TP	-0.260	0.322	0.422
Odontella sinensis	absolute	TP	0.223	0.209	0.290
Paralia sulcata	absolute	TP	0.854	0.220	0.000

Morphotype_harmonized	biomass	term	estimate	std.error	p.value
Phaeocystis sp.	absolute	TP	-0.668	0.285	0.027
Plagiogrammopsis vanheurckii	absolute	TP	0.137	0.609	0.823
Planktothrix agardhii	absolute	TP	1.385	0.507	0.029
Prorocentrum cordatum	absolute	TP	-0.604	0.363	0.120
Protoperidinium leonis	absolute	TP	0.501	0.305	0.107
Protoperidinium sp.	absolute	TP	-0.124	0.301	0.683
Pseudo-nitzschia delicatissima	absolute	TP	0.453	1.766	0.799
Pseudo-nitzschia pungens	absolute	TP	1.129	1.030	0.279
Rhizosolenia imbricata	absolute	TP	-0.934	0.278	0.002
Rhizosolenia setigera	absolute	TP	-0.056	0.243	0.819
Stephanopyxis turris	absolute	TP	1.107	0.416	0.037
Thalassiosira minima	absolute	TP	0.455	0.639	0.480
Thalassiosira punctigera	absolute	TP	0.463	0.197	0.049
Thalassiosira rotula	absolute	TP	-0.251	0.326	0.447
Actinoptychus senarius	relative	NP	-0.855	0.434	0.051
Akashiwo sanguinea	relative	NP	-0.038	1.085	0.972
Bellerochea malleus	relative	NP	0.466	1.013	0.650
Brockmanniella brockmannii	relative	NP	0.594	1.186	0.619
Cerataulina pelagica	relative	NP	-0.316	0.611	0.608
Chaetoceros debilis	relative	NP	0.862	0.570	0.134
Chaetoceros socialis	relative	NP	0.740	1.274	0.565
Coscinodiscus radiatus	relative	NP	-0.910	0.618	0.144
Dactyliosolen fragilissimus	relative	NP	1.415	0.816	0.087
Detonula pumila	relative	NP	2.292	0.783	0.005
Ditylum brightwellii	relative	NP	-0.412	0.477	0.389
Eucampia zodiacus	relative	NP	-0.163	0.622	0.794
Guinardia delicatula	relative	NP	-0.278	0.497	0.577
Guinardia flaccida	relative	NP	-1.736	0.666	0.010
Gyrodinium spirale	relative	NP	-0.707	0.439	0.109

Morphotype_harmonized	biomass	term	estimate	std.error	p.value
Helicotheca tamesis	relative	NP	-1.152	0.894	0.202
Lauderia annulata	relative	NP	0.347	0.590	0.558
Leptocylindrus danicus	relative	NP	0.457	0.902	0.615
Lithodesmium undulatum	relative	NP	-0.228	0.597	0.703
Mediopyxis helysia	relative	NP	0.307	0.824	0.710
Micromonas pusilla	relative	NP	1.175	0.471	0.025
Neocalyptrella robusta	relative	NP	-0.065	0.813	0.936
Odontella aurita	relative	NP	0.194	0.461	0.675
Odontella regia	relative	NP	-0.981	0.451	0.031
Odontella sinensis	relative	NP	-0.647	0.301	0.033
Paralia sulcata	relative	NP	-1.034	0.462	0.026
Phaeocystis sp.	relative	NP	0.975	0.464	0.037
Plagiogrammopsis vanheurckii	relative	NP	0.754	0.854	0.382
Planktothrix agardhii	relative	NP	2.842	1.318	0.036
Prorocentrum cordatum	relative	NP	1.335	0.755	0.084
Protoperidinium leonis	relative	NP	-1.124	0.718	0.135
Protoperidinium sp.	relative	NP	-0.675	0.328	0.042
Pseudo-nitzschia delicatissima	relative	NP	3.718	1.839	0.050
Pseudo-nitzschia pungens	relative	NP	2.990	0.785	0.000
Rhizosolenia imbricata	relative	NP	0.815	0.491	0.099
Rhizosolenia setigera	relative	NP	-0.321	0.456	0.482
Stephanopyxis turris	relative	NP	-0.698	1.016	0.495
Thalassiosira minima	relative	NP	-1.215	0.839	0.154
Thalassiosira punctigera	relative	NP	0.221	0.787	0.780
Thalassiosira rotula	relative	NP	0.741	0.609	0.226
Actinoptychus senarius	absolute	NP	-0.315	0.364	0.388
Akashiwo sanguinea	absolute	NP	1.106	0.854	0.202
Bellerochea malleus	absolute	NP	1.589	1.023	0.128
Brockmanniella brockmannii	absolute	NP	0.719	1.425	0.617

Morphotype_harmonized	biomass	term	estimate	std.error	p.value
Cerataulina pelagica	absolute	NP	0.787	0.716	0.276
Chaetoceros debilis	absolute	NP	0.838	0.553	0.132
Chaetoceros socialis	absolute	NP	1.798	1.642	0.280
Coscinodiscus radiatus	absolute	NP	-0.090	0.381	0.815
Dactyliosolen fragilissimus	absolute	NP	1.836	0.958	0.074
Detonula pumila	absolute	NP	1.084	0.595	0.073
Ditylum brightwellii	absolute	NP	0.459	0.518	0.378
Eucampia zodiacus	absolute	NP	0.356	0.556	0.523
Guinardia delicatula	absolute	NP	-0.291	0.427	0.497
Guinardia flaccida	absolute	NP	-1.034	0.499	0.042
Gyrodinium spirale	absolute	NP	-0.206	0.318	0.520
Helicotheca tamesis	absolute	NP	-0.123	0.610	0.841
Lauderia annulata	absolute	NP	-0.184	0.405	0.663
Leptocylindrus danicus	absolute	NP	-0.333	1.238	0.789
Lithodesmium undulatum	absolute	NP	0.097	0.436	0.824
Mediopyxis helysia	absolute	NP	1.018	0.781	0.197
Micromonas pusilla	absolute	NP	0.318	0.507	0.535
Neocalyptrella robusta	absolute	NP	0.577	0.562	0.311
Odontella aurita	absolute	NP	0.578	0.478	0.229
Odontella regia	absolute	NP	0.402	0.478	0.402
Odontella sinensis	absolute	NP	-0.061	0.319	0.849
Paralia sulcata	absolute	NP	-0.629	0.296	0.035
Phaeocystis sp.	absolute	NP	1.152	0.518	0.028
Plagiogrammopsis vanheurckii	absolute	NP	1.065	0.826	0.204
Planktothrix agardhii	absolute	NP	2.115	1.042	0.047
Prorocentrum cordatum	absolute	NP	1.043	0.770	0.185
Protoperidinium leonis	absolute	NP	-1.204	0.632	0.063
Protoperidinium sp.	absolute	NP	-0.649	0.479	0.177
Pseudo-nitzschia delicatissima	absolute	NP	4.786	2.325	0.046

Morphotype_harmonized	biomass	term	estimate	std.error	p.value
Pseudo-nitzschia pungens	absolute	NP	4.967	1.224	0.000
Rhizosolenia imbricata	absolute	NP	0.509	0.490	0.301
Rhizosolenia setigera	absolute	NP	0.217	0.432	0.616
Stephanopyxis turris	absolute	NP	-0.131	1.050	0.901
Thalassiosira minima	absolute	NP	-0.480	0.900	0.597
Thalassiosira punctigera	absolute	NP	-1.018	0.474	0.041
Thalassiosira rotula	absolute	NP	0.768	0.526	0.148
Actinoptychus senarius	relative	Si	0.285	0.228	0.216
Akashiwo sanguinea	relative	Si	-0.370	0.367	0.326
Bellerochea malleus	relative	Si	0.412	0.414	0.328
Brockmanniella brockmannii	relative	Si	1.365	0.495	0.008
Cerataulina pelagica	relative	Si	-0.075	0.245	0.763
Chaetoceros debilis	relative	Si	0.264	0.238	0.277
Chaetoceros socialis	relative	Si	-0.383	0.565	0.504
Coscinodiscus radiatus	relative	Si	0.120	0.302	0.694
Dactyliosolen fragilissimus	relative	Si	-0.128	0.351	0.717
Detonula pumila	relative	Si	0.828	0.402	0.047
Ditylum brightwellii	relative	Si	-0.207	0.180	0.266
Eucampia zodiacus	relative	Si	-0.518	0.316	0.105
Guinardia delicatula	relative	Si	-0.344	0.254	0.183
Guinardia flaccida	relative	Si	-0.027	0.384	0.943
Gyrodinium spirale	relative	Si	-0.384	0.231	0.103
Helicotheca tamesis	relative	Si	-0.034	0.388	0.932
Lauderia annulata	relative	Si	0.124	0.260	0.636
Leptocylindrus danicus	relative	Si	0.079	0.455	0.864
Lithodesmium undulatum	relative	Si	0.528	0.318	0.102
Mediopyxis helysia	relative	Si	0.315	0.352	0.378
Micromonas pusilla	relative	Si	-0.240	0.200	0.267
Neocalyptrella robusta	relative	Si	0.625	0.381	0.117
Odontella aurita	relative	Si	0.256	0.227	0.267

Morphotype_harmonized	biomass	term	estimate	std.error	p.value
Odontella regia	relative	Si	0.326	0.205	0.120
Odontella sinensis	relative	Si	0.088	0.143	0.544
Paralia sulcata	relative	Si	0.381	0.233	0.111
Phaeocystis sp.	relative	Si	-0.561	0.219	0.016
Plagiogrammopsis vanheurckii	relative	Si	1.101	0.436	0.015
Planktothrix agardhii	relative	Si	0.961	0.264	0.031
Prorocentrum cordatum	relative	Si	-0.646	0.268	0.025
Protoperidinium leonis	relative	Si	-0.074	0.311	0.812
Protoperidinium sp.	relative	Si	0.093	0.105	0.374
Pseudo-nitzschia delicatissima	relative	Si	0.824	0.896	0.365
Pseudo-nitzschia pungens	relative	Si	-0.085	0.447	0.853
Rhizosolenia imbricata	relative	Si	-0.489	0.266	0.070
Rhizosolenia setigera	relative	Si	-0.085	0.212	0.690
Stephanopyxis turris	relative	Si	1.160	0.350	0.004
Thalassiosira minima	relative	Si	0.068	0.332	0.838
Thalassiosira punctigera	relative	Si	0.339	0.394	0.394
Thalassiosira rotula	relative	Si	0.104	0.358	0.772
Actinoptychus senarius	absolute	Si	0.268	0.162	0.110
Akashiwo sanguinea	absolute	Si	-0.356	0.275	0.202
Bellerochea malleus	absolute	Si	0.712	0.441	0.118
Brockmanniella brockmannii	absolute	Si	1.064	0.601	0.083
Cerataulina pelagica	absolute	Si	-0.162	0.286	0.578
Chaetoceros debilis	absolute	Si	0.082	0.250	0.746
Chaetoceros socialis	absolute	Si	-0.145	0.719	0.841
Coscinodiscus radiatus	absolute	Si	0.184	0.132	0.168
Dactyliosolen fragilissimus	absolute	Si	-0.457	0.419	0.304
Detonula pumila	absolute	Si	0.210	0.249	0.401
Ditylum brightwellii	absolute	Si	-0.335	0.222	0.145
Eucampia zodiacus	absolute	Si	-0.612	0.214	0.008

Morphotype_harmonized	biomass	term	estimate	std.error	p.value
Guinardia delicatula	absolute	Si	-0.527	0.146	0.002
Guinardia flaccida	absolute	Si	0.031	0.254	0.905
Gyrodinium spirale	absolute	Si	-0.194	0.112	0.106
Helicotheca tamesis	absolute	Si	0.146	0.249	0.561
Lauderia annulata	absolute	Si	-0.053	0.174	0.767
Leptocylindrus danicus	absolute	Si	0.011	0.599	0.986
Lithodesmium undulatum	absolute	Si	0.349	0.179	0.062
Mediopyxis helysia	absolute	Si	-0.004	0.271	0.988
Micromonas pusilla	absolute	Si	0.218	0.129	0.131
Neocalyptrella robusta	absolute	Si	-0.227	0.199	0.288
Odontella aurita	absolute	Si	-0.008	0.259	0.976
Odontella regia	absolute	Si	0.197	0.235	0.406
Odontella sinensis	absolute	Si	0.141	0.163	0.389
Paralia sulcata	absolute	Si	0.541	0.180	0.003
Phaeocystis sp.	absolute	Si	-0.479	0.197	0.026
Plagiogrammopsis vanheurckii	absolute	Si	0.207	0.439	0.640
Planktothrix agardhii	absolute	Si	1.147	0.368	0.017
Prorocentrum cordatum	absolute	Si	-0.111	0.294	0.710
Protoperidinium leonis	absolute	Si	0.314	0.285	0.277
Protoperidinium sp.	absolute	Si	-0.407	0.224	0.076
Pseudo-nitzschia delicatissima	absolute	Si	0.548	1.145	0.635
Pseudo-nitzschia pungens	absolute	Si	0.416	0.688	0.550
Rhizosolenia imbricata	absolute	Si	-0.738	0.183	0.000
Rhizosolenia setigera	absolute	Si	-0.140	0.182	0.450
Stephanopyxis turris	absolute	Si	0.663	0.350	0.104
Thalassiosira minima	absolute	Si	-0.363	0.350	0.304
Thalassiosira punctigera	absolute	Si	0.174	0.160	0.296
Thalassiosira rotula	absolute	Si	0.134	0.230	0.564