

## Final report of the two phases of the project DYNATLOSS I (2014-2107) and II(2018-2021) within the DynaTrait priority programme:

The main objectives/ goals of DYNATLOSS I were:

A1) Ecologically relevant, **dynamic** and **functional traits** affecting plankton dynamics in “wild nature” are e.g. the taxon-specific pigment composition of phytoplankton as a determinant of primary production and the taxon-specific composition of essential lipids (in particular fatty acids) in the phytoplankton community that determine the trophic transfer efficiency to zooplankton.

B1) The taxon and thereby **functional trait diversity of natural phytoplankton** (pigments, fatty acids) is **correlated with the nutritional complementarity** of this community for herbivorous zooplankton. Since the importance of essential fatty acids for zooplankton nutrition has been demonstrated in multiple systems, it is reasonable that a **loss of trait diversity can affect** the supply of essential fatty acids and thereby **zooplankton productivity**.

C1) Different zooplankton groups (e.g. different *Daphnia* genotypes) differ in their susceptibility to limitations by the availability of essential fatty acids. A loss of trait diversity (fatty acids) can thus affect competitive interactions between zooplankton. This can in turn affect the genetic and/or taxonomic composition of the zooplankton community resulting on **dynamic feedback effects on phytoplankton community composition and the corresponding functional traits**.

### Main outcome DYNATLOSS I:

Establishing diversity gradients in phytoplankton communities is a challenge compared to classic removal experiments in terrestrial primary producer communities. Two techniques, dilution and disturbance, were employed to establish diversity gradients within natural phytoplankton communities from ponds differing in nutrient availability. Both methods were effective in creating diversity gradients, with dilution resulting in species loss ranging from around 40% to 65%, and disturbance inducing losses from 33% to 45%. The impact of these manipulations varied between oligotrophic and eutrophic lake systems, with dilution affecting oligotrophic communities more significantly, while disturbance showed the opposite pattern. Disturbance effects aligned with the intermediate disturbance hypothesis, though responses varied between systems (Fig 1 and 2; published in Hammerstein et al. 2017). Community similarities varied with manipulation levels, indicating varying responses to stressors. Despite initial challenges and the need for time to establish diversity gradients (Fig 2; Hammerstein et al. 2017), these methods offer valuable insights into biodiversity ecosystem functioning relationships, complementing studies on artificially composed phytoplankton communities.

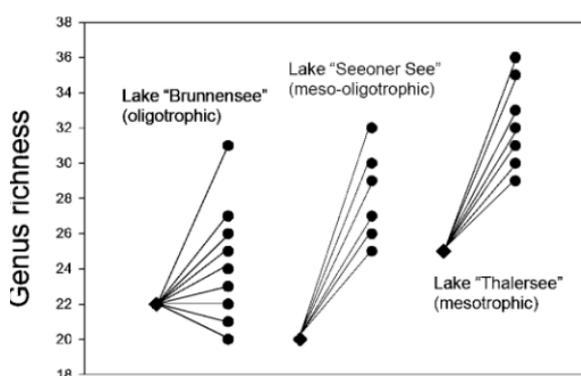


Fig 1: Changes in genus richness in three different lakes with different nutrient status after applying a gradient of disturbance.

understanding of biodiversity dynamics and ecosystem functioning (BEF), although careful consideration of experimental design constraints and selection of appropriate manipulation methods are necessary. Overall, dilution and disturbance emerge as feasible approaches for manipulating diversity in natural phytoplankton communities, offering opportunities for detailed investigations into BEF relationships.

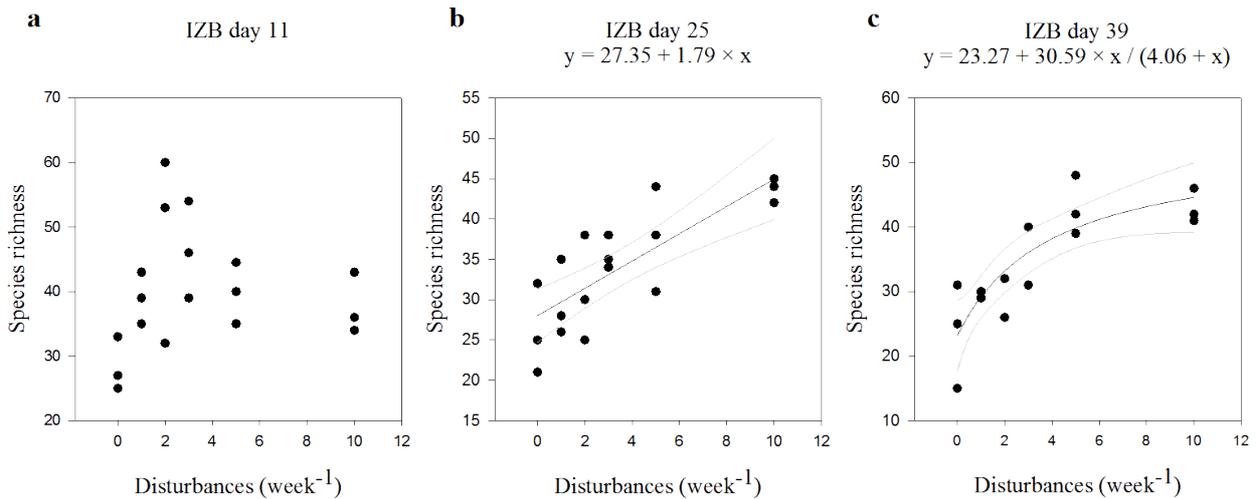


Fig 2: Effects of applying disturbance on species richness after different time intervals.

By artificially manipulating diversity, we aimed to understand whether short-term responses to stressors mirrored longer-term natural diversity differences between water bodies. Nutrient enrichment indeed increased biomass production across all lakes but resulted in varying community stability with increasing diversity loss. Short- and long-term responses diverged, indicating a complex interplay between diversity and nutrient inputs, influenced by lake-specific conditions and yearly variations. While species richness alone does not fully capture ecosystem functioning, analyses of community integrity alongside species richness provided a more comprehensive understanding. Higher genus richness correlated with increased stability during nutrient addition, highlighting the importance of functional diversity (Hammerstein 2019; dissertation thesis). However, community responses varied, emphasizing the need for holistic approaches in understanding ecosystem responses to stressors. Evolutionary adaptation and ecological responses likely act on similar time scales, potentially influencing community stability. Our results underscore the complexity of ecological stability and the necessity for multifaceted approaches to predict ecosystem responses accurately.

We then explored the relationship between functional diversity and ecosystem functioning in phytoplankton, focusing on their spectral light use efficiency. Both laboratory cultures and natural communities were investigated, with absorbance spectra used to assess the link between diversity and light use efficiency. Functional diversity, characterized by the presence of different phytoplankton groups, showed a significant positive relationship with light use efficiency, particularly in the green, yellow, and orange spectral ranges. This suggests that diverse communities are more efficient in utilizing light for photosynthesis, especially in spectral regions not readily absorbed by chlorophyll-a. Accessory pigments within diverse communities widen the range of light wavelengths available for photosynthesis. Interestingly, functional diversity exerted a stronger influence on light use

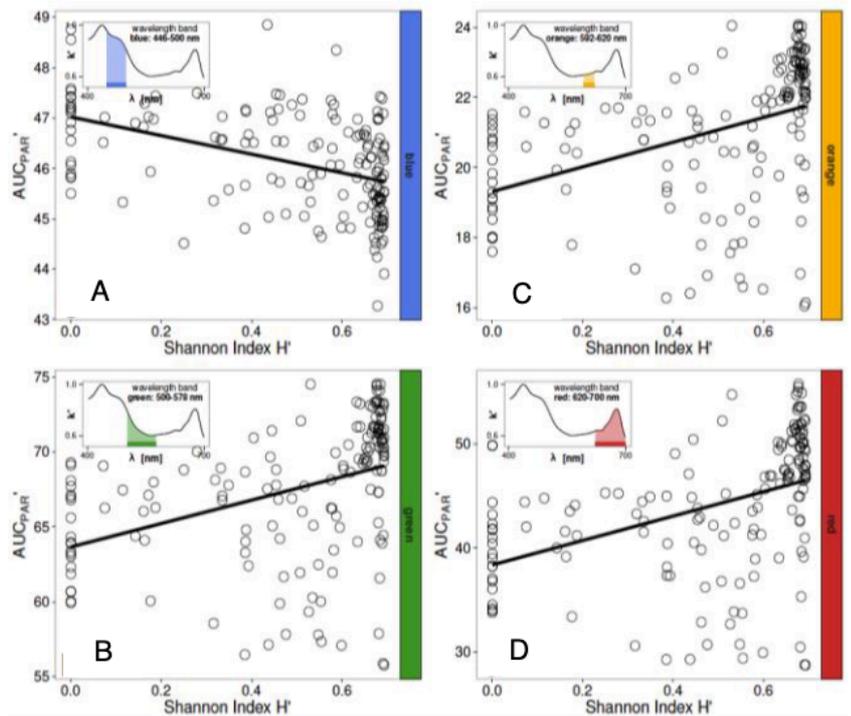
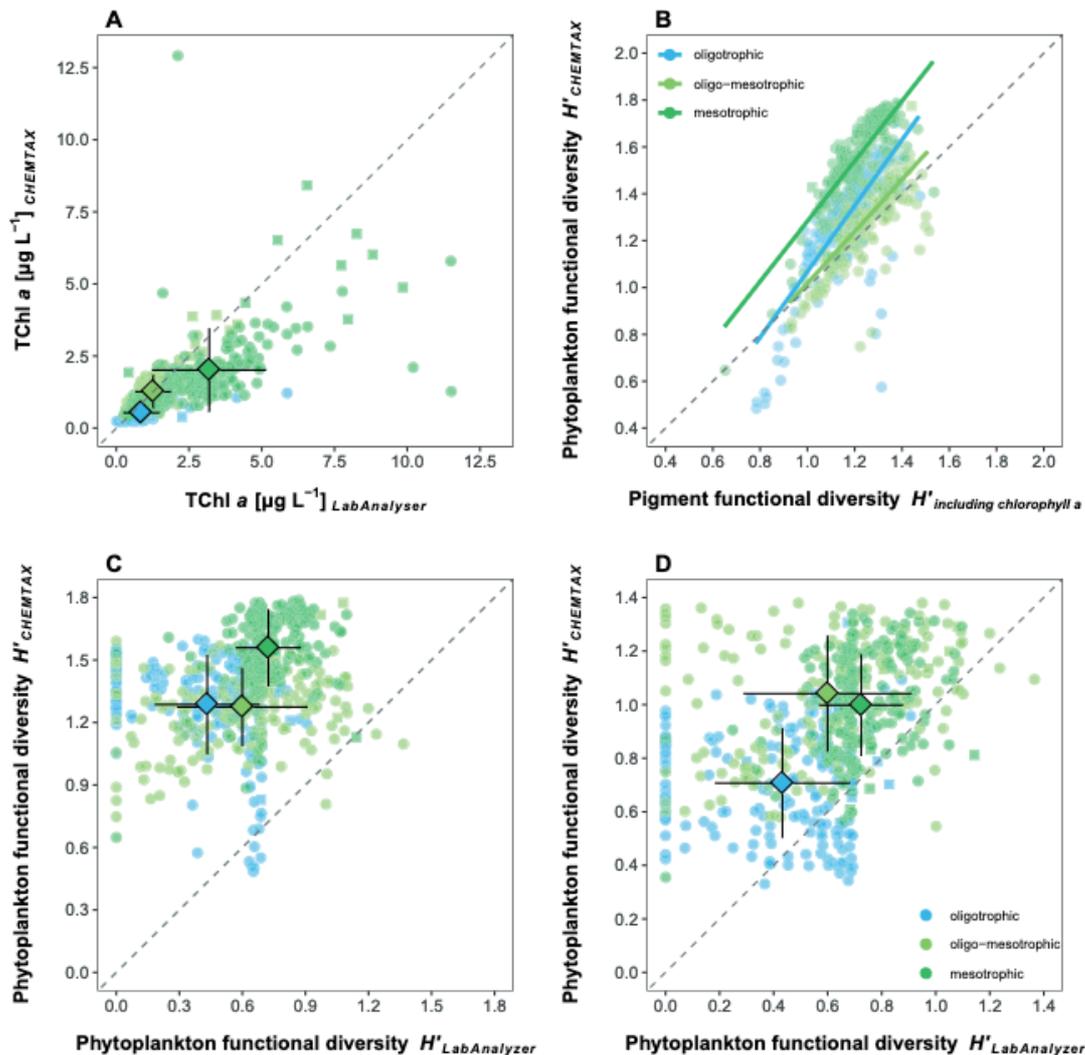


Fig. 3 A-D: Normalized area under the curve (AUC') nm), **A**: within the blue part (446 – 500 nm)  $p < 0.001$ ,  $R^2 = 0.2$ , **B**: within the green part (500 – 578 nm)  $p < 0.001$ ,  $R^2 = 0.16$ , **C**: within the orange part (592 – 620 nm)  $p < 0.001$ ,  $R^2 = 0.17$  and **D**: within the red part (620 – 700 nm)  $p < 0.001$ ,  $R^2 = 0.17$  of the PAR spectrum versus the Shannon Index  $H'$  of functional group diversity in natural phytoplankton communities with manipulated diversity through disturbance from lake Brunnensee with linear regression (solid line).

efficiency than species richness. Field experiments further supported these findings, demonstrating increased light use efficiency with higher functional diversity across freshwater communities (Fig 3; Gollnisch et al. 2024, for submission). Overall, our results highlight the importance of functional diversity in enhancing ecosystem functioning in phytoplankton communities (Stockenreiter & Litchman 2019).

The range of available techniques for quantifying and distinguishing various phytoplankton groups has expanded in recent years. For instance, the composition of phytoplankton communities can be indirectly assessed using CHEMTAX, a matrix factorization program. CHEMTAX calculates the contribution of different phytoplankton taxa to total chlorophyll-a based on pigment concentrations analyzed via liquid chromatography. A more direct, *in vivo* assessment can be achieved with instruments like the Algae Lab Analyser, which uses spectral fluorometry to differentiate four phytoplankton groups based on their fluorescence signatures. We compared both methods by analyzing phytoplankton biomass and functional diversity in communities from three lakes with varying trophic states. A subset of biomass and diversity estimates obtained from microscopic counts served as a reference. We observed significant differences in biomass estimates for all phytoplankton groups, particularly with cyanobacteria being notably underestimated by the Algae Lab Analyser. Additionally, we found that the agreement between the methods varied depending on the trophic state of the lake. We conclude that both methods are suitable for estimating phytoplankton functional diversity, each with its own advantages and disadvantages (Fig 4; Ilić et al. 2023).



**Fig. 4** (A) Total chlorophyll-a concentration ( $\mu\text{g L}^{-1}$ ) and (C, D) phytoplankton functional diversity  $H'$  determined spectrofluorometrically *in vivo* with Algae Lab Analyser (y-axis) and chromatographically *in vitro* via HPLC and CHEMTAX (x-axis). (B) Pigment functional diversity  $H'$  including chlorophyll-a (x-axis) and phytoplankton functional diversity  $H'$  CHEMTAX (y-axis), determined via HPLC and CHEMTAX. Data from all 9 sampling events (July to September 2014) is shown. Coloured lines in (B) represent the linear regression for each lake. Colour of the symbols represents the trophic state of the lakes, blue: oligotrophic (lake Brunensee,  $n=186$ ); light green: oligo-mesotrophic (lake Klostersee,  $n=187$ ); dark green: mesotrophic (lake Thaler See,  $n=189$ );  $n$  in parentheses indicates the number of water samples per lake analysed within this study. Data originating from enclosures are depicted as circles, while data originating directly from the lakes are depicted as squares. Diamonds in panels (A, C, D) represent the mean values, while horizontal and vertical error bars represent the standard deviation (based on all data points per lake).

We further demonstrated that our experimental shifts in the diversity of functional traits of natural phytoplankton (in our case pigments and fatty acids) had consequences for zooplankton composition: Lower content of phytoplankton diversity related PUFAs was linked to lower growth of cladocerans, resulting in shifts of the copepod : cladocera ratio in the zooplankton. These shifts were only correlated with the nutritional complementarity of phytoplankton communities for zooplankton and independent of changes in phytoplankton biomass (Fig 5; unpublished, data analyses ongoing). This had so far only been found for laboratory studies with either single species or very low diversity communities). The resulting zooplankton (consumer) community shifts could be observed in zooplankton biomass, abundance, composition and diversity.

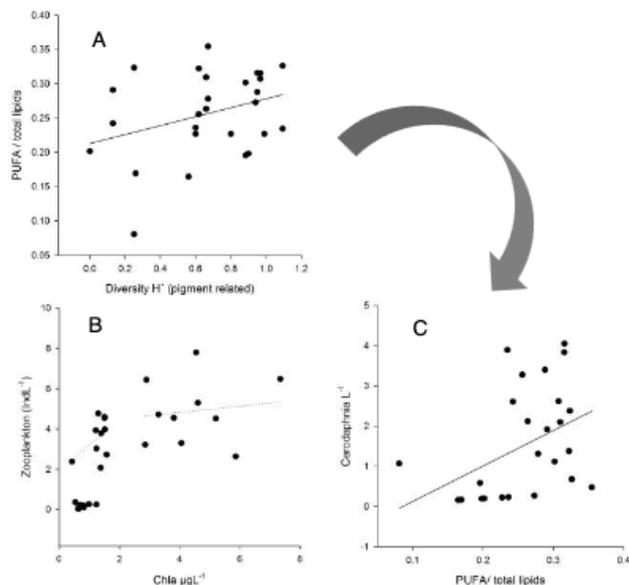


Fig. 5 A) PUFA content on total lipids as a function of diversity;  $R^2=0.15$ ;  $p=0.03$  B) Zooplankton ( $\text{Ind L}^{-1}$ ) as a function of Chl-a in all three lakes (BS:  $R^2=0.03$ ;  $p=0.6$ ; KS:  $R^2=0.01$ ;  $p=0.9$ ; TS:  $R^2=0.01$ ;  $p=0.7$ ). C) Overall *Ceriodaphnia* sp.  $\text{L}^{-1}$  as a function of the PUFA content on total lipids. ( $R^2=0.17$ ;  $p=0.03$ ).

Contrary to  $\omega 3$ -PUFAs,  $\omega 6$ -PUFAs have historically been seen less ecologically relevant, although the role of the  $\omega 6$ -PUFA arachidonic acid (ARA, 20:4 $\omega 6$ ) remains contentious. To explore its potential significance, we conducted dose-response growth experiments with two *Daphnia* species, *D. pulex* and *D. magna*, supplemented with either EPA or ARA. This enabled us to determine EPA and ARA saturation thresholds for both *Daphnia* species' growth and reproduction. Our findings demonstrate that not only the presence of  $\omega 3$ -PUFAs but also that of the  $\omega 6$ -PUFA ARA can equally restrict the growth and reproduction of *Daphnia* spp. The saturation thresholds for growth and reproduction were consistently higher for EPA than for ARA in both *Daphnia* species (Fig 6; Ilić et al. 2019). As shifts in phytoplankton community composition can lead to variations in the dietary availability of  $\omega 3$ - and  $\omega 6$ -PUFAs, our results represent a significant advancement in understanding the repercussions of ongoing global biodiversity loss on trophic transfer efficiency at the phytoplankton-zooplankton interface.

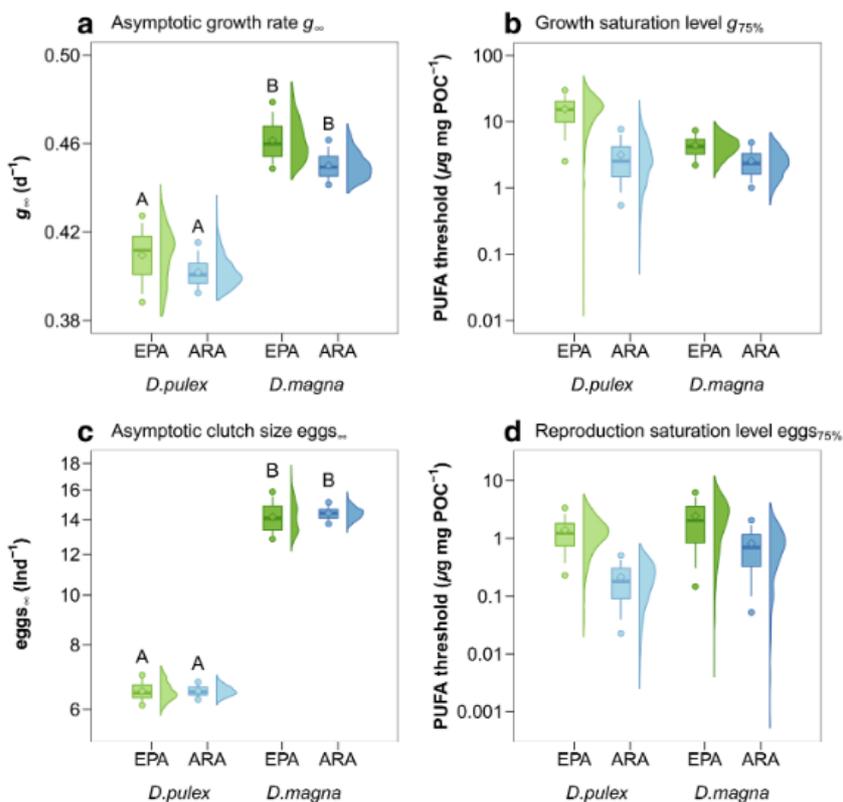


Fig. 6 (a) Asymptotic growth rate  $g_{\infty}$  ( $\text{d}^{-1}$ ), (b) PUFA threshold concentration  $S_{75\%}$  ( $\text{in } \mu\text{g PUFA mg POC}^{-1}$ ) for growth saturation level  $g_{75\%}$  (corresponding to reduction of  $g_{\infty}$  by 25% relative to the baseline  $g_{\infty}$ ), (c) asymptotic clutch size  $\text{eggs}_{\infty}$  (number of eggs per individual) and (d) PUFA threshold concentration  $S_{75\%}$  ( $\text{in } \mu\text{g PUFA mg POC}^{-1}$ ) for reproduction saturation level  $\text{eggs}_{75\%}$  (corresponding to reduction of  $\text{eggs}_{\infty}$  by 25% relative to the baseline  $\text{eggs}_{\infty}$ ) for egg-bearing *D. pulex* and *D. magna* grown on *A. obliquus* supplemented with either EPA (green) or ARA (blue). In all panels, the flat violin plot represents the distribution (density) of the data, while the boxplots represent the median (horizontal lines within each box), 25% and 75% quartiles (box), 10% and 90% percentiles (whiskers), 95% confidence interval (circles), and the mean value (diamond). In panels (a, b), the asymptotic growth rate and the PUFA threshold concentration were derived from nonlinear least-square fits through bootstrapped growth rate data (see "Materials and methods" section). In panels (c, d), the asymptotic clutch size and the PUFA threshold concentration were derived from nonlinear least-square fits through bootstrapped reproduction data (clutch size, i.e., number of eggs per individual; see "Materials and methods" section). Note that the y-axis in the panels (b, c, d) has a logarithmic scale. Summary of the plots (sample size, median, mean  $\pm$  standard deviation) and statistical analyses can be found in Tables 2–3, respectively. Different letters indicate significantly different groups (two-way ANOVA).

The main objectives/ goals of DYNATLOSS II were:

A2) **Phytoplankton diversity** manipulations resulting in zooplankton community shifts have the **feedback potential to alter phytoplankton size distribution**. We hypothesize that this will affect **size dependent resource uptake** and thereby growth and quality of phytoplankton.

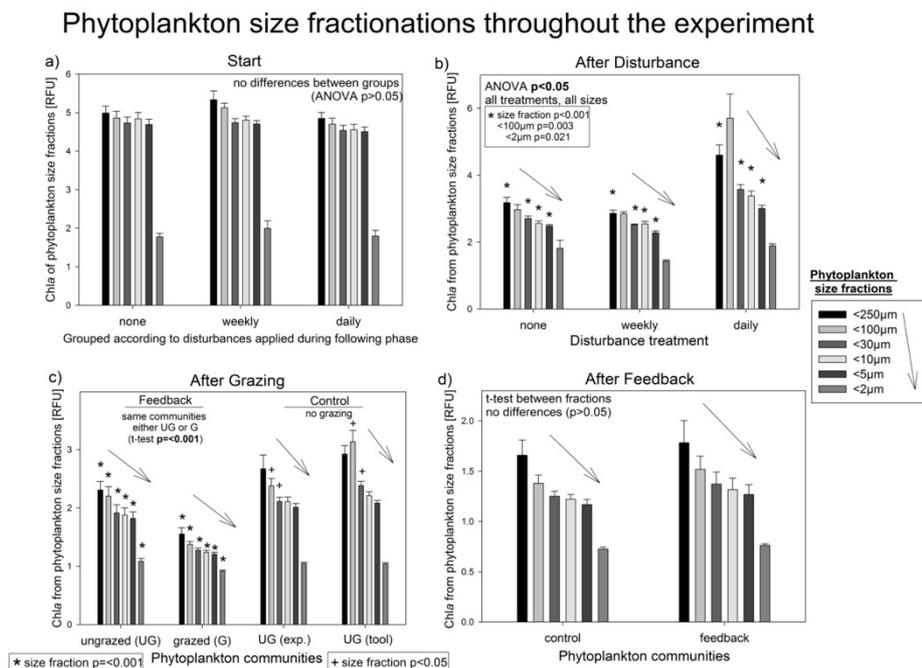
B2) **Phytoplankton diversity** manipulations resulting in zooplankton community shifts have the **feedback potential to alter nutrient regeneration**: We hypothesize that the **stoichiometry** of the nutrient regeneration differs between relatively **phosphorus rich (cladocera-dominated)** and relatively **nitrogen rich (copepod-dominated)** zooplankton resulting in different phytoplankton growth dynamics.

C2) **Phytoplankton diversity** manipulations resulting in zooplankton community shifts have the **feedback potential to alter composition of essential PUFAs** in natural phytoplankton by selective feeding and nutrient recycling: We hypothesize that **selective feeders** with chemosensory abilities (such as herbivorous copepods) actively **select for high PUFA algae** in order to meet their PUFA demand (which is impossible for unselective filter-feeding *Daphnia* sp.). The **phytoplankton composition may change further towards a dominance of poor-quality species** with low PUFA content. This may (further) **decrease** the zooplankton community's **trophic transfer efficiency** and limit its ability to control phytoplankton biomass development due to food quality constraints. Additionally, **different recycling of nutrients can also directly affect fatty acid production** of natural phytoplankton

Main outcome:

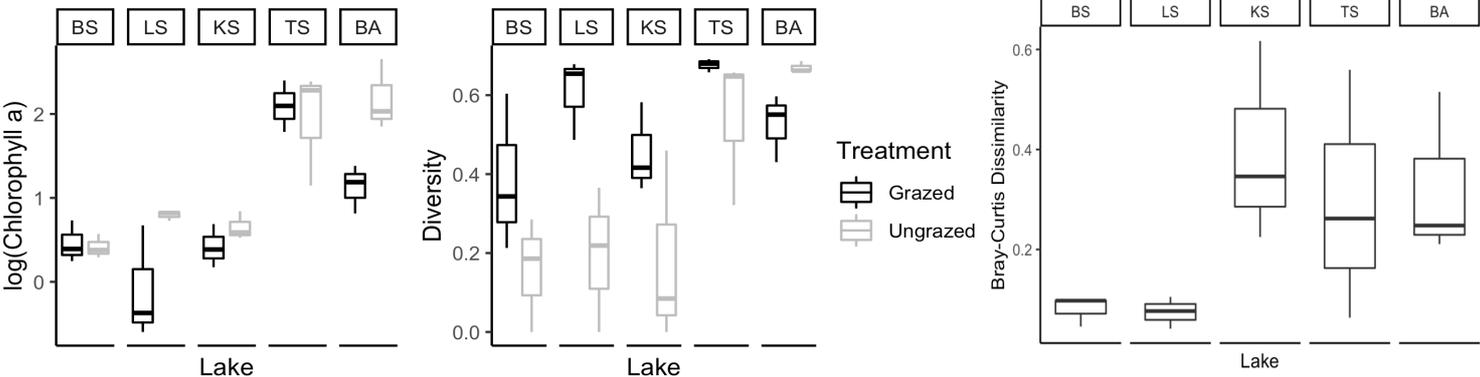
To investigate if the disturbance treatment to create different diverse communities had an effect on the size distribution of phytoplankton in the mesocosms, we measured Chl-a contribution of phytoplankton size fraction to total Chl-a. It can be seen that in all disturbance treatments size fraction distribution shows a very similar pattern but differences in total chl-a (Fig. 7; unpublished; data analysis ongoing).

The crucial interactions between primary producers and consumers are well acknowledged in ecological studies, with a potential for dynamic interplay between these trophic levels. However, to date, investigations into the effects of phytoplankton on zooplankton and vice versa have largely been conducted in isolation and within simplified aquatic ecosystems. We therefore used natural phytoplankton and mesozooplankton communities to explore the feedback effect of primary producer communities on themselves through the altered composition and feeding behavior of consumers. Experiments were carried out in the laboratory and in the field using mesocosms. Our findings reveal that diverse

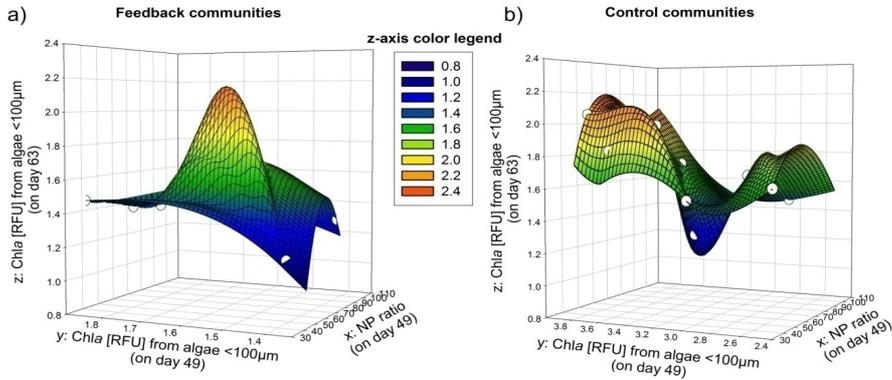


**Fig. 7: Results for size fractionations at the start (a) of the field experiment and after main experimental phases (b for disturbance, c for grazing and d for feedback and end of experiment).** Six phytoplankton size fractions were investigated (see legend). Error bars depict standard errors. Statistical tests and p-values are given in the figure and in the text. a) Starting communities grouped according to the subsequent disturbance intensities during the disturbance phase, every bar with n=6. b) Diversity manipulated communities after disturbance phase grouped according to the disturbance intensities applied, every bar with n=6. c) Phytoplankton communities after grazing treatment with natural zooplankton grazers. Phytoplankton communities are grouped according to whether they were grazed (G) or not grazed (UG) and to whether they were controls or feedback communities. d) Diversity manipulated phytoplankton communities at the end of the field experiment grouped according to feedback treatment or control. Each bar with n=9. Statistical significance with p < 0.001 is given as asterisk, with p < 0.05 as plus.

phytoplankton communities influence the composition of zooplankton. Moreover, our results indicate that zooplankton grazing reduces phytoplankton biomass while simultaneously enhancing phytoplankton diversity (Fig 8; Lyberger et al., for submission).



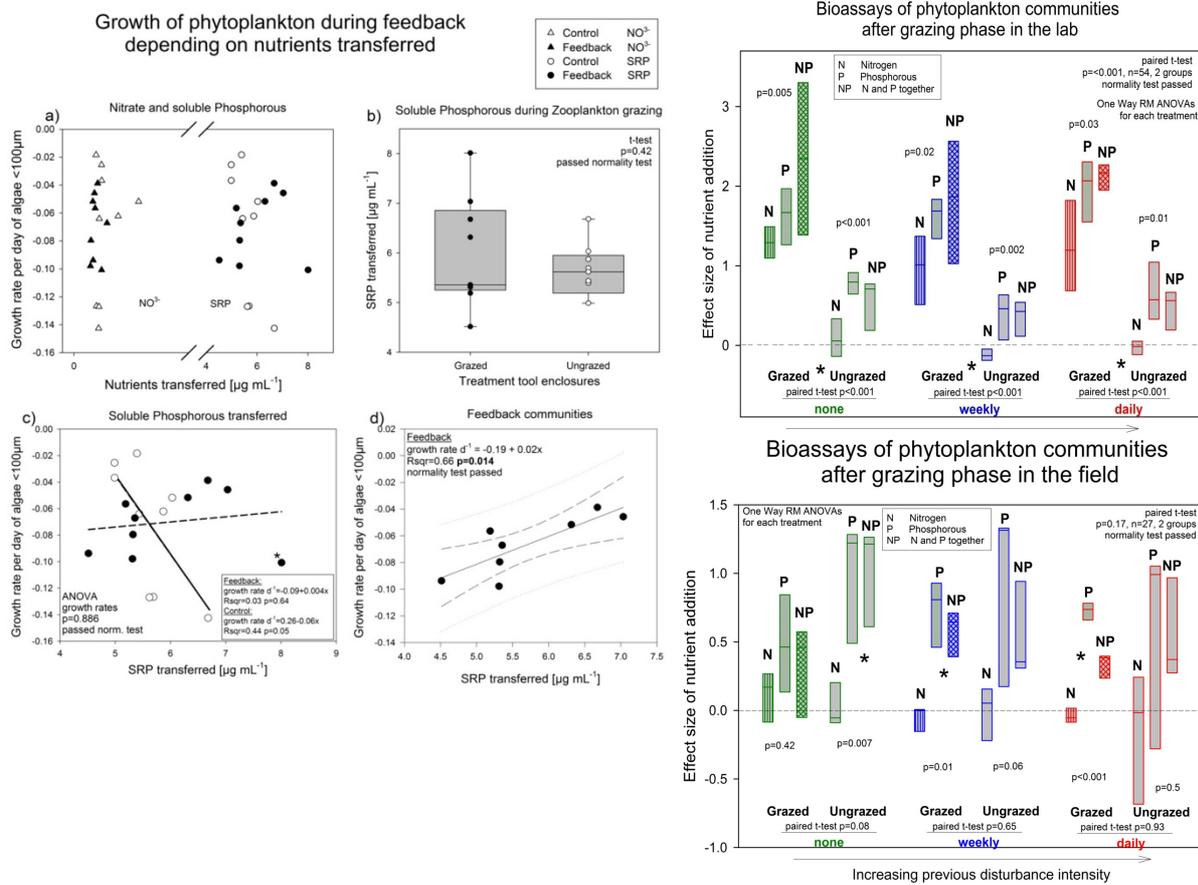
Potential effects on nutrient cycling from feedback transfers of algae



**Fig. 8:** Boxplot showing grazing effects on (a) log-transformed phytoplankton biomass and (b) phytoplankton functional group diversity measured using the Shannon Index. Samples with zooplankton are in black and samples without zooplankton are in gray. (c) Boxplot showing the Bray-Curtis dissimilarity between phytoplankton communities one week after receiving grazed water versus ungrazed water.

**Below:** 3D representation of potential effects on nutrient cycling from algae transferred during feedback allowing for time effects of feedback dynamics. The x and y axis represent a time point of algae transferred after zooplankton grazing, while the z-axis the resulting potential effects on algae smaller than 100µm two weeks later. Feedback communities (a) and control communities (b) are shown; legend for the z-axis in the middle. X-axes represent phytoplankton NP ratios in the tool communities after one week zooplankton grazing. Each x-axis forms a functional relationship with the respective y-axis, which shows the Chlorophyll a (Chla) concentration from the algae size fraction <100 µm available after one week zooplankton grazing. These initial starting conditions of transferred algae are plotted against the response Chla concentrations from the algae size fraction <100 µm in experimental enclosures two weeks later (z-axis). Control communities are given for the same sampling time points. Data points are shown as white circles.

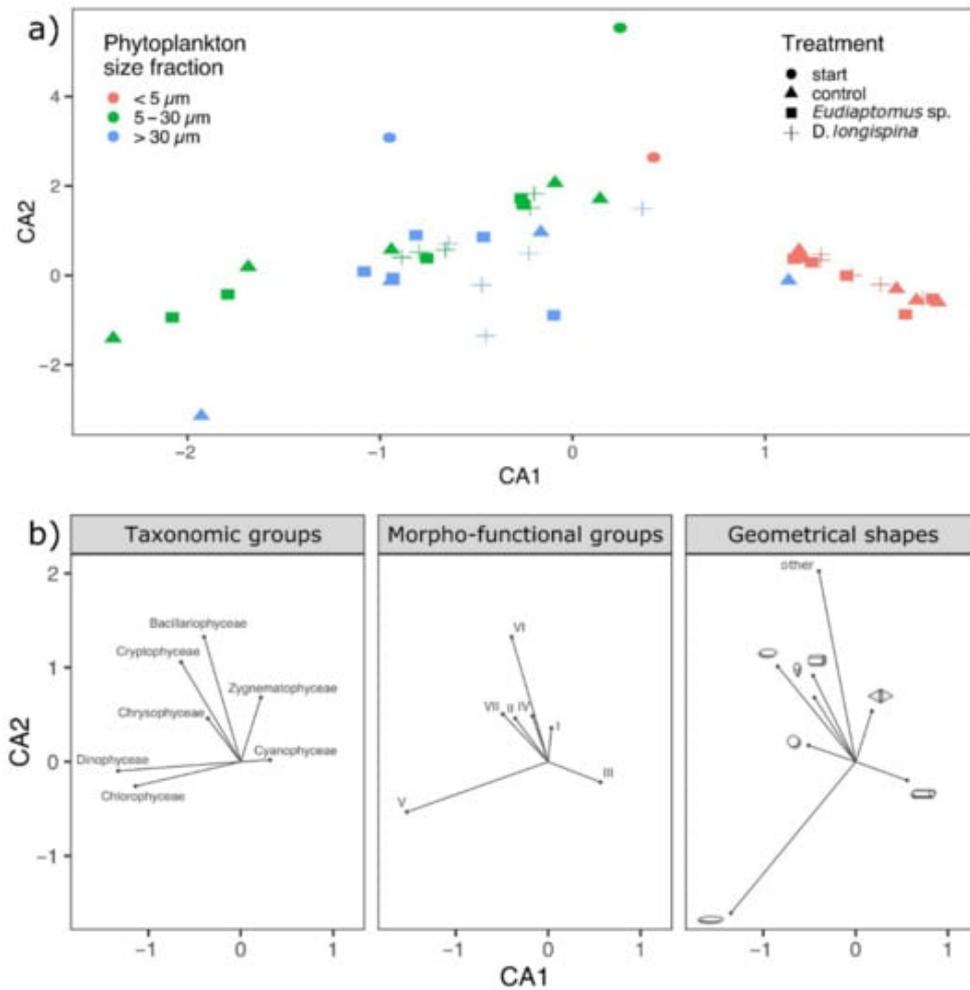
This phenomenon is partly driven by the recycling of elemental nutrients by consumers (Fig 9), whereby recycled nutrients exert feedback effects on phytoplankton. Overall, our approach illuminates how phytoplankton dynamics can be influenced by the changes they induce in zooplankton communities.



**Fig. 9: Left graph panel: Analyses after the first transfer of soluble nutrients during the feedback phase of growth rates of phytoplankton species smaller than  $100\mu\text{m}$ .** a) Soluble nutrients transferred (nitrate,  $\text{NO}_3^-$  and soluble reactive phosphorous, SRP) were measured before being transferred from grazed (black) or ungrazed (white) communities into experimental phytoplankton communities. b) Box plots of SRP concentrations showing differences between grazed and ungrazed tool communities (t-test  $p=0.42$ , passed normality test). c) Relationships between the SRP transferred and phytoplankton growth rate. Growth rates were not significantly different (ANOVA, passed normality test,  $p=89$ ). Linear regressions (lines, passed normality tests) were statistically non-significant (black circles, feedback:  $y=-0.09+0.004x$ ,  $\text{Rsqr}=0.03$ ,  $p=0.64$ ) and significant (white circles, controls:  $y=0.26-0.06x$ ,  $\text{Rsqr}=0.44$ ,  $p=0.05$ ). An intuitive outlier (asterisk) for feedback communities was discarded for the linear regression analysis in graph d) Linear regression of SRP transferred from grazed communities into feedback communities and growth rate dependency for algae  $<100\mu\text{m}$ . Regression significantly positive:  $y=-0.19+0.02x$ ,  $\text{Rsqr}=0.66$ ,  $p=0.014$ . 95% confidence (dashed line) and prediction (dotted line) interval lines are shown. **Right graph panel: top: Effect sizes of nutrient additions in bioassays performed after grazing phase under laboratory settings. Same phytoplankton communities were either grazed or ungrazed.** Effect sizes are color coded with respect to previous disturbance. Statistical analyses are shown with significance depicted by asterisks. **Bottom: Effect sizes of nutrient additions in bioassays performed after grazing phase under field settings. Same phytoplankton communities were either grazed or ungrazed.** Effect sizes are color coded with respect to previous disturbance. Statistical analyses are shown with significance depicted by asterisks.

The recent adoption of functional trait-based methodologies enables a more thorough assessment of functional roles and interactions within communities. Given that phytoplankton size and shape significantly influence their susceptibility to herbivores, alterations or losses in certain morpho-functional traits of phytoplankton are likely to impact zooplankton grazing, fitness, and population dynamics. We explored in further laboratory experiments the response of modified phytoplankton morpho-functional trait distributions to grazing pressure from zooplankton exhibiting diverse food size preferences and feeding behaviors. To investigate this, we conducted feeding trials in laboratory microcosms using size-fractionated freshwater phytoplankton (divided into three size classes:  $>30\mu\text{m}$ ,  $5-30\mu\text{m}$ , and  $<5\mu\text{m}$ ) and two distinct consumer types: the cladoceran *Daphnia longispina* (a generalist unselective filter feeder) and the calanoid copepod *Eudiaptomus* sp. (a selective feeder). Surprisingly, we found no significant changes in traits and composition between the control and grazed phytoplankton communities. However, there were considerable variations in community composition and structure between the small and large size fractions, underscoring the pivotal role of size in shaping natural phytoplankton communities (Fig. 10: Titocci et al. 2022).

Our results underscore the importance of integrating taxonomy and trait-based morpho-functional approaches when investigating ecological dynamics in phytoplankton-zooplankton interactions.



**Fig. 10:** Ordination plot resulting from a correspondence analysis (CA), (a) main biplot with the mean phytoplankton trait abundance composition according to treatments and size fractions represented by different symbols and colors respectively, (b) plots for taxonomic, morpho-functional and geometrical shape

Publications (Phase 1 and 2):

Titocci J, Fink P (2024) Disturbance alters phytoplankton functional traits and consequently drives changes in zooplankton life-history traits and lipid composition. *Hydrobiologia* 851: 161-180. doi: 10.1007/s10750-023-05323-2.

Stibor H, and Stockenreiter M, (2023) Let there be light to interact. *Front. Photobiol.*, 07 November 2023 Sec. Photoecology and Environmental Photobiology. Volume 1 - 2023 | <https://doi.org/10.3389/fphbi.2023.1284620>

Ilić M, Walden S, Hammerstein SK, Stockenreiter M, Stibor H, Fink P (2023) Pigment and fluorescence proxies to estimate functional diversity of phytoplankton communities. *Fundamental and Applied Limnology* 196 (3-4): 229 - 249. doi: 10.1127/fal/2023/1466. .

Titocci J, Bon M, Fink P (2022) Morpho-functional traits reveal differences in size fractionated phytoplankton communities but do not significantly affect zooplankton grazing. *Microorganisms* 10 (1): 182. doi: 10.3390/microorganisms10010182..

Titocci J, Fink P (2022) Food quality impacts on reproductive traits, development and fatty acid composition of the freshwater calanoid copepod *Eudiaptomus* sp. *Journal of Plankton Research* 44 (4): 528–541. doi: 10.1093/plankt/fbac030..

Ilić M, Cordellier M, Fink P (2021) Intrapopulation variability in a functional trait: Susceptibility of *Daphnia* to limitation by dietary fatty acids. *Freshwater Biology* 66 (1): 130-141. doi: 10.1111/fwb.13623.

Stockenreiter M, Isanta Navarro J, Buchberger F, Stibor H (2021) Community shifts from eukaryote to cyanobacteria dominated phytoplankton: The role of mixing depth and light quality. *Freshwater Biology* 66(11): 2145-2157.

Drugă B, Ramm E, Szekeres E, Chiriac C, Hegedüs A, Stockenreiter M (2021) Long-term acclimation might enhance the growth and competitive ability of *Microcystis aeruginosa* in warm environments. *Freshwater Biology* 67(4): 589-602, <https://doi.org/10.1111/fwb.13865>

Buchberger F, Stibor H, Neusius D, Nickelsen J, Stockenreiter M (2020) Transgenic and cell wall-deficient *Chlamydomonas reinhardtii* food affects life history of *Daphnia magna*. *Journal of Applied Phycology* 32 (1): 319-328.

Ilić M, Werner C, Fink P (2019) Equal relevance of omega-3 and omega-6 polyunsaturated fatty acids for the fitness of *Daphnia* spp. *Limnology and Oceanography* 64 (6): 2512-2525. doi: 10.1002/lno.11201.

Stockenreiter M, Litchman E (2019) Nitrogen-fixer enhances lipid yields in algal polycultures, *Algal Research* 44.

Trommer G, Lorenz P, Lentz A, Fink P, Stibor H (2019) Nitrogen enrichment leads to changing fatty acid composition in phytoplankton and negatively affects zooplankton in a lake community, *Scientific Reports* 9: 16805.

Windisch HS, Fink P (2019) Transcriptome sequencing of a keystone aquatic herbivore yields insights on the temperature-dependent metabolism of essential lipids, *BMC Genomics* 20: 894.

Fink P, Windisch HS (2018) The essential omega-3 fatty acid EPA affects expression of genes involved in the metabolism of omega-6 derived eicosanoids in *Daphnia magna*. *Hydrobiologia* 846 (1): 5-16. doi: 10.1007/s10750-018-3675-z.

Hammerstein S, Stockenreiter M, Stibor H (2017) Directed diversity manipulations within natural phytoplankton communities. *Limnology and Oceanography: Methods* 15 (7) 653-662.