Oikos

OIK-05673

Limberger, R., Birtel, J., Peter, H., Catalán, N., da Silva Farias, D., Best, R. J., Brodersen, J., Bürgmann, H. and Matthews, B. 2018. Predator-induced changes in dissolved organic carbon dynamics. – Oikos doi: 10.1111/oik.05673

Appendix 1

Table A1. DOC parameters (partly from (Helms et al. 2008, Fellman et al. 2010)). References for characterization of the PARAFAC components are provided in the main text.

Parameter	Calculation	Description
Diagnostic indices		
SR (Slope ratio)	$slope_{275\text{-}295nm}$ / $slope_{350\text{-}400nm}$	Higher SR indicates higher proportion of low-molecular weight DOC compounds
FI (Fluorescence index)	Em_{450nm}/Em_{500nm} (Ex: 370nm)	Higher FI indicates higher proportion of microbially (rather than terrestrially) derived DOC.
HIX (Humification index)	$Em_{435\text{-}480nm}/Em_{300\text{-}445nm}$ (Ex: 254nm)	Higher HIX indicates higher proportion of humic substances.
BIX (Freshness index)	Em_{380nm}/Em_{430nm} (Ex: 310nm)	Higher BIX indicates higher proportion of recently produced DOC.
DOC components		
C1	PARAFAC	Humic-like, terrestrial origin
C2	PARAFAC	Protein-like, microbial origin, related with bioavailable compounds and algal sources
C3	PARAFAC	Humic-like, terrestrial origin
C4	PARAFAC	Protein-like, microbial origin
C5	PARAFAC	Humic-like, microbial origin

Table A2. Results of linear mixed effects models testing for effects of fish on biotic and abiotic ecosystem properties. Data are from mid-experiment (week 7 for periphyton, week 11 for net ecosystem productivity (NEP) and respiration, week 13 for sedimentation, week 9 for all other parameters) and from the end of the experiment (week 17 for abundance of bacteria, week 19 for all other parameters). Abundance of bacteria and biomass of phyto- and zooplankton were log-transformed prior to statistical analyses. DOC parameters include total DOC concentration (DOC), slope ratio (SR), fluorescence index (FI), freshness index (BIX), humification index (HIX), and five DOC components (C1-C5) identified with PARAFAC modelling from emission-excitation matrices. Tests on C1-C5 are based on absolute values normalized by DOC concentration, and on relative proportions, respectively. Bold font denotes results with $P < 0.05$. Effect of fish specified as positive (+), negative (-), or not significant (n.s.).

	Mid-experiment			End of experiment		
	F-value	p-value	Effect	F-value	p-value	Effect
Biomass/Abundance						
Bacteria	27.731	**< 0.001**	+	49.993	**< 0.001**	+
Phytoplankton	252.97	**< 0.001**	+	9.968	**0.005**	+
Zooplankton				31.04	**< 0.001**	-
Periphyton	17.374	**< 0.001**	-	1.221	0.283	n.s.
Diversity						
Bacteria				28.49	**< 0.001**	-
Phytoplankton				3.392	0.081	n.s.
Zooplankton				36.59	**< 0.001**	-
Ecosystem functions						
NEP	14.426	**0.001**	+	5.314	**0.033**	+
Respiration	5.612	**0.029**	+	1.302	0.268	n.s.
Sedimentation	0.882	0.36	n.s.	0.181	0.676	n.s.
DOC parameters						
DOC	0.009	0.925	n.s.	0	0.988	n.s.
SR	20.257	**< 0.001**	+	1.778	0.198	n.s.
FI	2.877	0.107	n.s.	1.701	0.208	n.s.
BIX	20.141	**< 0.001**	+	0.097	0.759	n.s.
HIX	8.562	**0.009**	-	0.02	0.89	n.s.
C1 (absolute)	0.087	0.772	n.s.	0.107	0.748	n.s.
C2 (absolute)	16.22	**< 0.001**	+	2.663	0.119	n.s.
C3 (absolute)	0.795	0.384	n.s.	0.089	0.768	n.s.
C4 (absolute)	4.575	**0.046**	+	1.257	0.276	n.s.
C5 (absolute)	1.522	0.234	n.s.	1.645	0.215	n.s.
C1 (relative)	22.542	**< 0.001**	-	2.527	0.128	n.s.
C2 (relative)	2.139	0.161	n.s.	0.423	0.523	n.s.
C3 (relative)	3.601	0.074	n.s.	0.024	0.877	n.s.
C4 (relative)	2.516	0.130	n.s.	5.029	**0.037**	+
C5 (relative)	1.233	0.281	n.s.	1.661	0.213	n.s.

Table A3. Results of linear mixed effects models testing for effects of fish and time on abundances of bacteria (Bac), phytoplankton biomass measured as chlorophyll-a (Phyto), DOC concentration, and slope ratio (SR). Abundance of bacteria and biomass of phytoplankton were log-transformed prior to statistical analyses. Bold font denotes results with $P < 0.05$.

	Fish		Week		Fish × Week	
	F-value	p-value	F-value	p-value	F-value	p-value
Bac	41.359	**< 0.001**	19.047	**< 0.001**	10.556	**< 0.001**
Phyto	175.656	**< 0.001**	29.956	**< 0.001**	7.525	**< 0.001**
DOC	5.147	**0.035**	261.611	**< 0.001**	7.792	**< 0.001**
SR	14.852	**0.001**	12.085	**< 0.001**	4.048	**< 0.001**

Table A4. Results of db-RDA testing for the effects of fish on the composition of bacteria, phyto- and zooplankton. Abundances were Hellinger-transformed prior to analyses. p-values were calculated using 10 000 permutations. Bold font denotes results with $p < 0.05$.

Group	F-value	p-value
Bacteria	3.557	**< 0.001**
Phytoplankton	9.02	**< 0.001**
Zooplankton	11.909	**< 0.001**

Table A5. Results of db-RDA testing for the effect of fish on the composition of the DOC pool. Absolute values of the five PARAFAC components were used as response variables. P-values were calculated using 10 000 permutations. Bold font denotes results with $p < 0.05$.

Group	F-value	p-value
Week 1	0.300	0.785
Week 9	7.096	**0.007**
Week 19	1.964	0.112

References

Fellman, J. B., E. Hood, and R. G. M. Spencer. 2010. Fluorescence spectroscopy opens new windows into dissolved organic matter dynamics in freshwater ecosystems: A review. Limnology and Oceanography 55:2452-2462.

Helms, J. R., A. Stubbins, J. D. Ritchie, E. C. Minor, D. J. Kieber, and K. Mopper. 2008. Absorption spectral slopes and slope ratios as indicators of molecular weight, source, and photobleaching of chromophoric dissolved organic matter. Limnology and Oceanography 53:955-969.

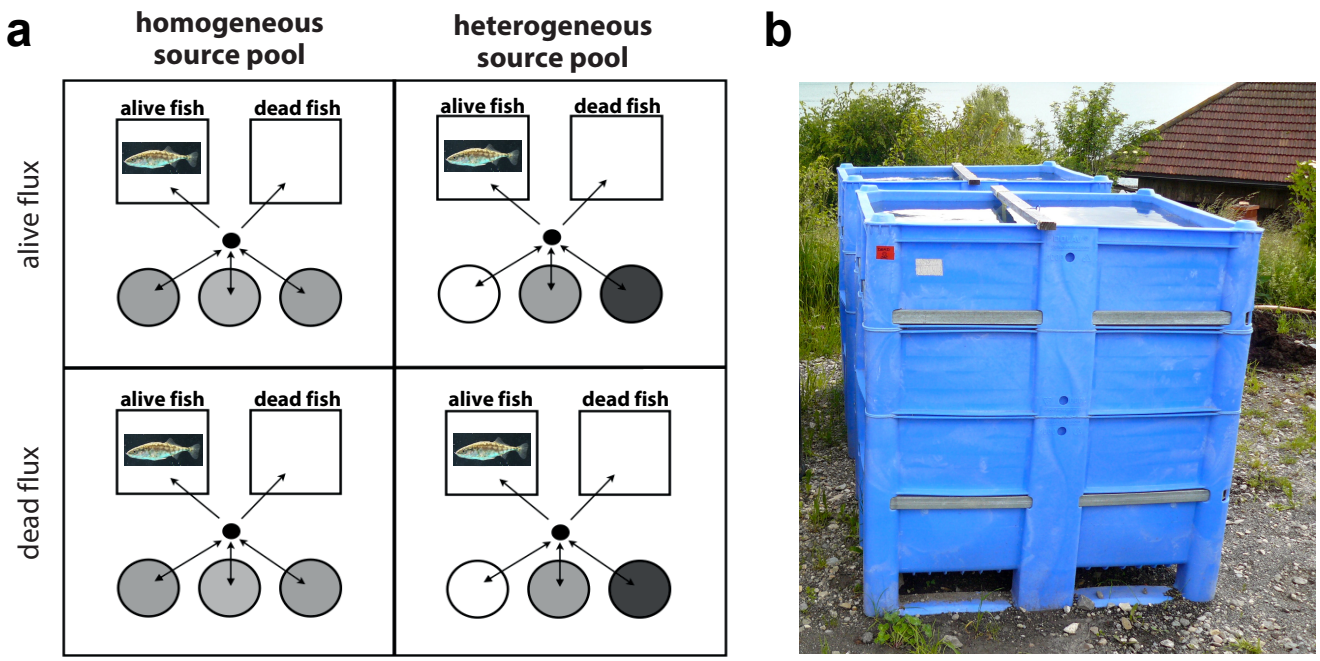

Fig. A1. Full experimental design (a) and picture of mesocosms (b). The experiment that we present in the main text was part of a larger mesocosm experiment about effects of ecosystem flux and environmental heterogeneity on aquatic ecosystems. We here describe the full design of the experiment, but note that (i) the results presented in the main text only include data from those mesocosms that are depicted as squares in the scheme, and (ii) the analyses in the main text focus on analyses of the fish contrast, irrespective of the flux and heterogeneity treatment.
We set up 20 pairs of mesocosms of 1000 L volume (squares). One mesocosm of each pair was stocked with six living stickleback, the other one with six dead stickleback. Once every other week, each pair of tanks received water from three donor ecosystems (i.e. three mesocosms of 300 L water volume; circles in the scheme). To this end, water from each of the three donor ecosystems was sampled, mixed in a barrel (small circle in the scheme), and 1 L was added to each of the two large tanks. The three small tanks were connected with each other through bi-weekly exchanges of 300 mL water, but did not receive any input from the large tanks. In a 2 x 2 design, we manipulated the nature of this ecosystem flux (alive or dead) and the heterogeneity of the source pool (homogeneous or heterogeneous). In the treatment with dead flux, the organisms in the water were killed by autoclaving prior to addition to the large tanks and exchange among the small tanks, respectively. The three donor ecosystems were either identical in environmental conditions (homogenous source pool) or differed in nutrient and DOC loading (heterogeneous source pool). In the heterogeneous treatment, one of the three donor ecosystems received high nutrient input, one received DOC additions, and one received low input of nutrients and DOC. In the homogeneous treatment, all three donor ecosystems received intermediate levels of nutrient and DOC input (see Limberger et al. (2017) for further details on the three donor ecosystems). The two large tanks did not receive additions of nutrients and DOC other than through addition of water from the three donor ecosystems. We here focus our analyses on the fish contrast, irrespective of the flux and heterogeneity treatment. Almost none of the response variables were affected by the manipulation of flux and heterogeneity of the donor ecosystems, possibly reflecting the low amount of water added (0.1% of the water volume every other week). However, to take into account the non-independence of the two tanks of a pair, because of spatial proximity and connectivity to the same donor ecosystem, we included pair of tank (i.e. same donor ecosystem) as a random factor in our statistical analyses.

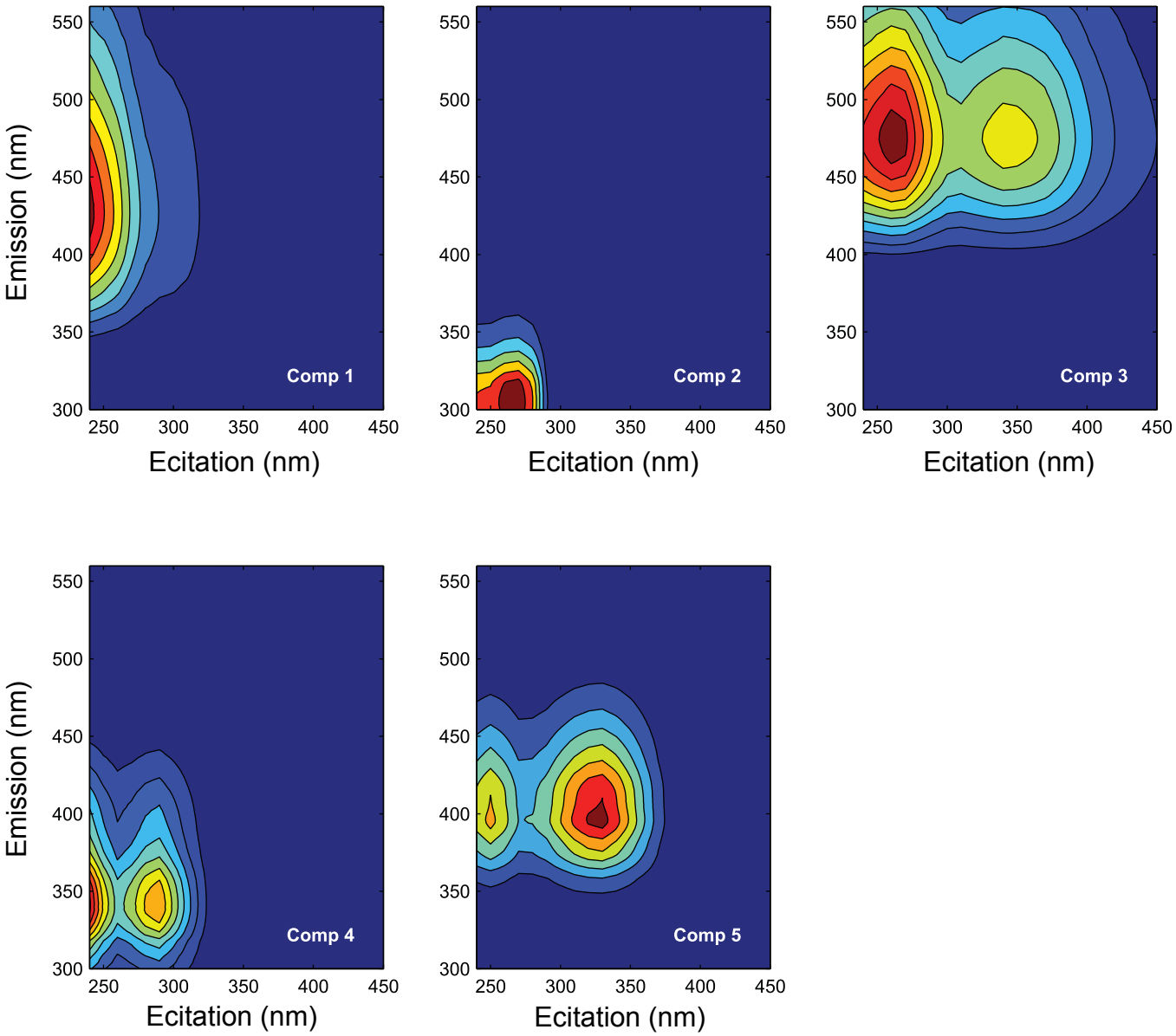

Fig. A2. Five DOC components identified from excitation-emission matrices with PARAFAC modelling.

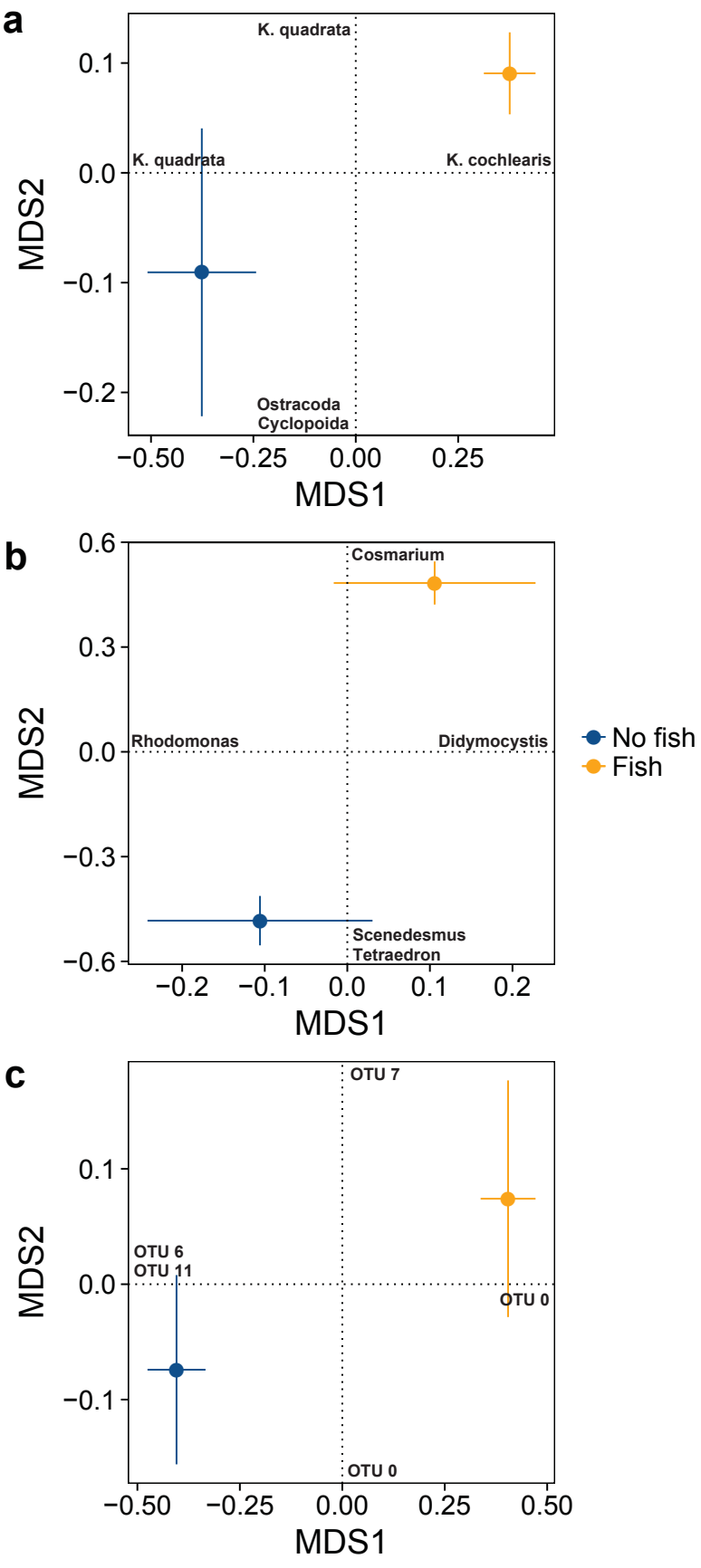

Fig. A3. Principle coordinates analysis of Hellinger-transformed abundance data. (a) Zooplankton (i.e. Crustacea and rotifers), (b) phytoplankton, and (c) bacteria. Taxa with highest loadings on the first two axes are plotted in the graph. Zooplankton taxa with high loadings were the rotifers *Keratella quadrata* and *Keratella cochlearis*, and the crustacean groups Ostracoda and Cyclopoida. Phytoplankton taxa with high loadings were *Scenedesmus*, *Tetraedron*, and *Didymocystis* (all Chlorophyta), *Cosmarium* (Charophyta), and *Rhodomonas* (Cryptophyta). Bacterial OTUs with high loadings were OTU 0 (Family Oxalobacteraceae, Class Betaproteo-bacteria), OTU 6 (Family Rhodocyclaceae, Class Betaproteobacteria), OTU 7 (Family Hyphomicrobiaceae, Class Alphaproteobacteria), and OTU 11 (Family Comamonadaceae, Class Betaproteobacteria).

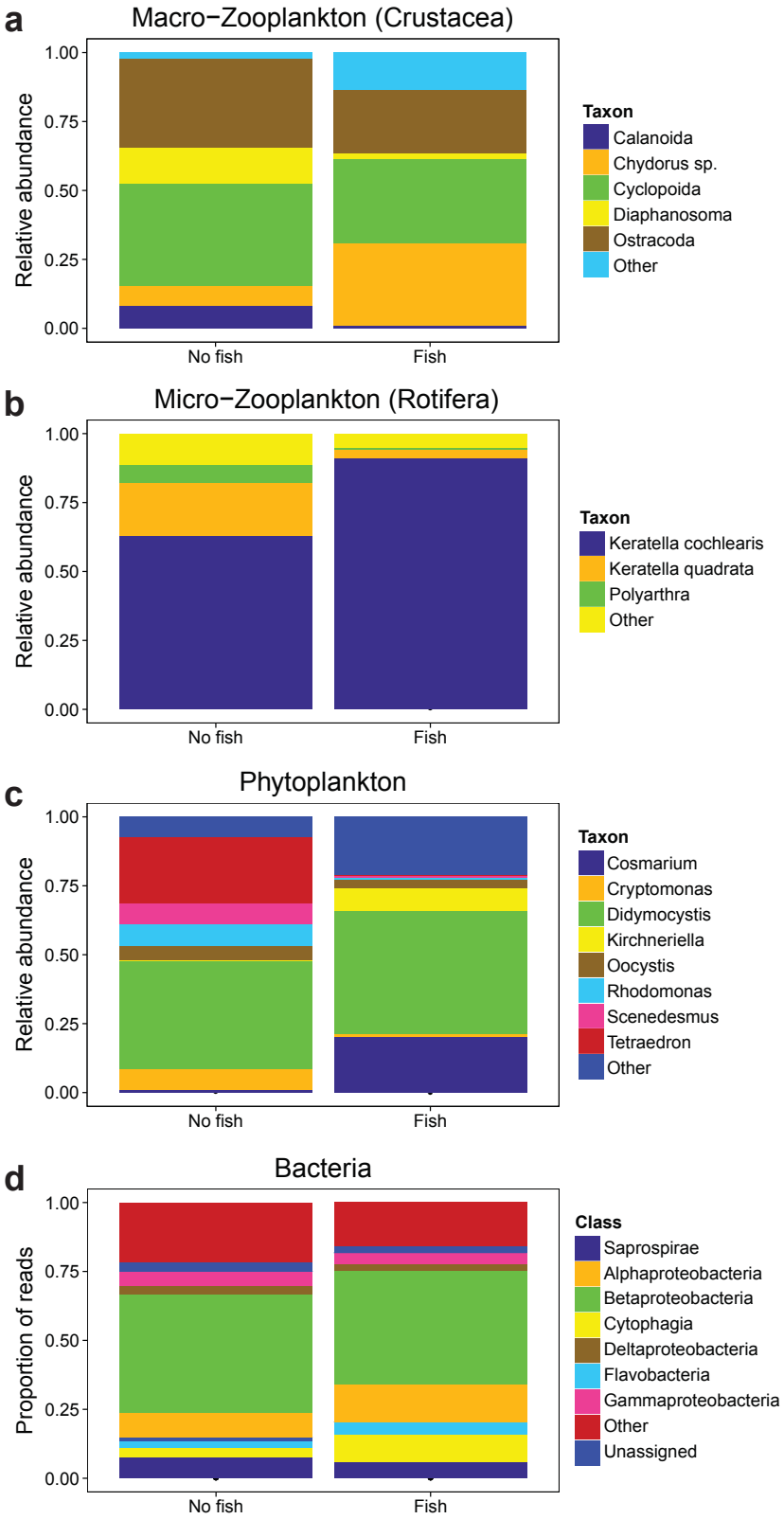

Fig. A4. Effect of fish on the composition of the plankton. Relative abundances of (a) taxa of macro-zooplankton (i.e. Crustacea), (b) taxa of micro-zooplankton (i.e. rotifers), (c) taxa of phytoplankton, and (d) proportion of reads of bacterial classes. Rare taxa were lumped together as "other". Values are averages across tanks, n = 20.

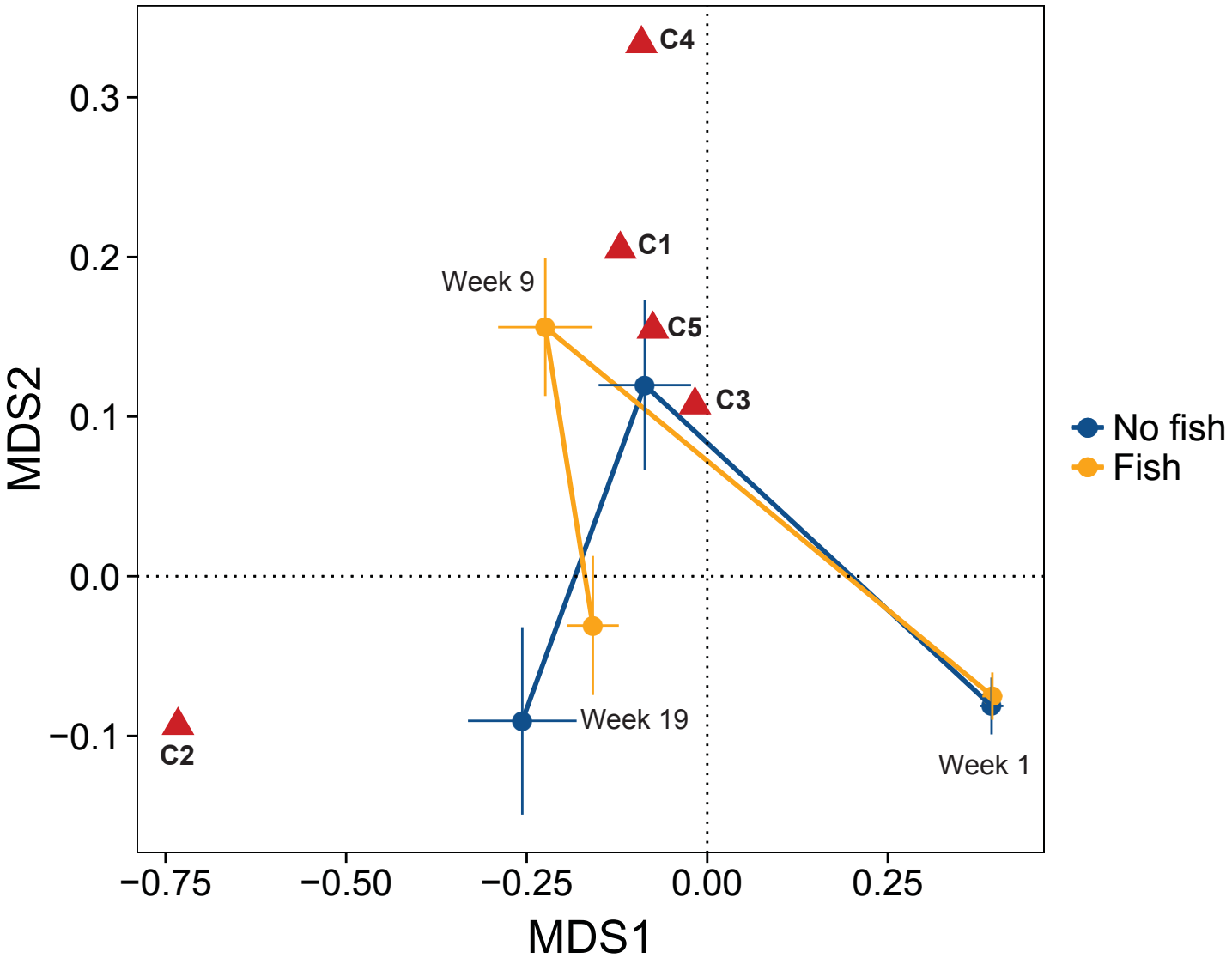

Fig. A5. Principle coordinates analysis of the five PARAFAC components. PCoA was computed using the absolute values of the five PARAFAC components from weeks 1, 9, and 19. Values were not normalized by DOC concentration prior to analysis. Scores of the five PARAFAC components were scaled by a factor of 5 prior to plotting and are shown with red triangles.

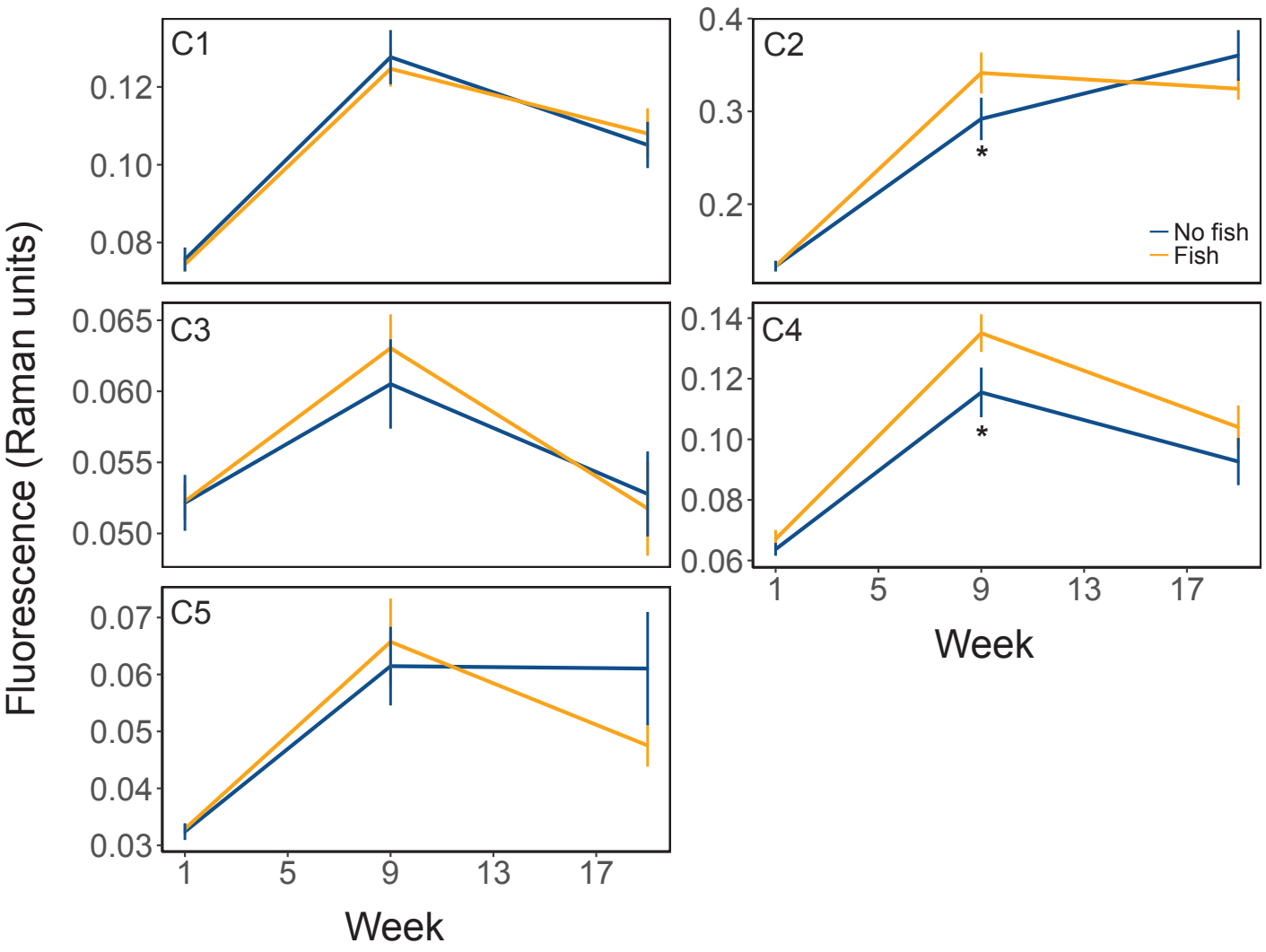

Figure A6. Fluorescence of the five PARAFAC components over the course of the experiment. Significant effects of the fish treatment at individual weeks are denoted with * ($p < 0.05$). Values are mean ± SE, n = 20.

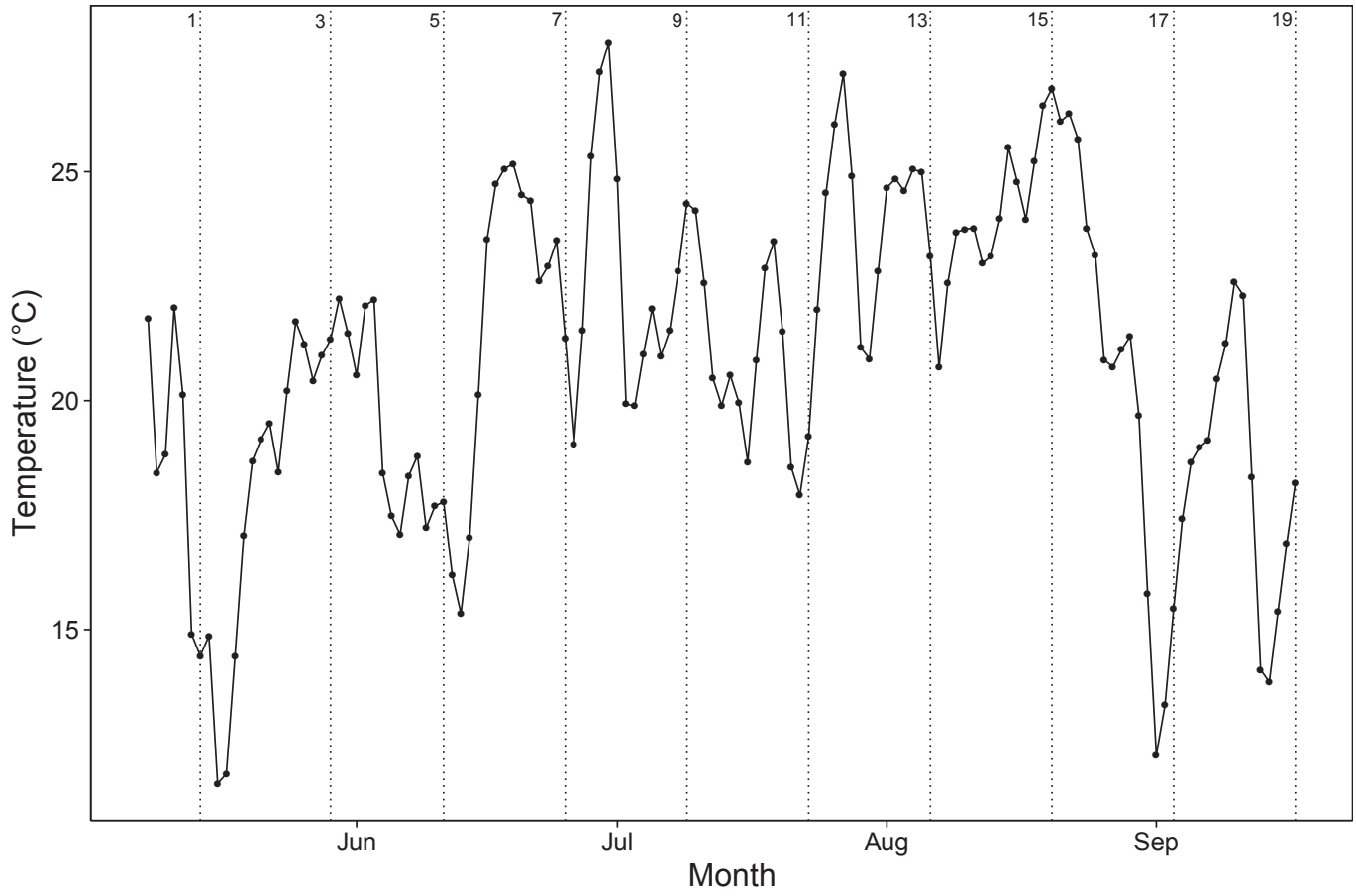

Fig. A7. Water temperature in the mesocosms. Temperature was measured with data loggers in nine mesocosms in 15 minute intervals. Values are daily averages of the nine tanks. Vertical dotted lines mark the ten sampling dates (Week 1 to 19).