The importance of ecotype diversity on duckweed growth with and without salt stress

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SUPPLEMENTARY MATERIAL to:

The importance of ecotype diversity on duckweed growth with and without salt stress

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Table S1: Overview sampling locations. Distance to nearest road refers to paved roads with traffic (cars, buses) that likely experience significant de-icing in winter.

Ecotype number	Location	Coordinates	Description	Conductivity (µs)	Distance to road (m)
1	Bönibach, Thalwil	47°17'04.1"N 8°34'02.0"E	Small stream, flowing water, urban, open canopy	377	77
2	Neeracher Ried, Niederglatt	47°29'55.3"N 8°29'23.2"E	Medium-sized pond (small lake) near the road and agricultural fields, near protected area, sunny.	513	85
3	Stocklen, Fällanden	47°22'14.0"N 8°39'04.8"E	Water inflow into Greifensee, very shaded, near protected are, restoration project. Agricultural input from nearby fields	371	470
4	Rehalp, Zürich	47°21'08.4"N 8°34'58.2"E	Very small (3 m diameter) artificial pond in the forest, plastic sheet visible. Near houses. No agricultural input.	552	58
5	Irchelpark, Zürich	47°23'55.2"N 8°32'38.6"E	Medium-sized, pond/outflow in the park surrounding Irchel campus. Shady. Urban.	295	117
6	Seebach, Zürich	47°25'15.1"N 8°32'21.8"E	Small artificial pond/stream in a private garden. No agricultural input. Some chemicals added to manage algae. Little shade. Urban.	183	32
7	Stettbach, Dübendorf	47°23'37.1"N 8°35'38.9"E	Small, artificial garden pond, shady. Close to houses and road.	NA	12

Table S2: Conductivity of water from sampling locations. Conductivity (in µS/cm) was measured with a handheld probe (Hannah instruments). The three replicates for tap water to which subsequently both fertilizer and salt was added were independent and come from three different containers. Conductivity for the waters source of ecotype 7 was not measured.

Water sample	Details	Conductivity [µS/cm]
Source water ecotype 1	Bönibach, Thalwil	377
Source water ecotype 2	Neeracher Ried, Niederglatt	513
Source water ecotype 3	Stocklen, Fällanden	371
Source water ecotype 4	Rehalp, Zürich	552
Source water ecotype 5	Irchelpark, Zürich	295
Source water ecotype 6	Seebach, Zürich	183
Source water ecotype 7	Stettbach, Dübendorf	N/A
tap water_ctrl_1	Tap water 1	306
tap water_ctrl_2	Tap water 2	322
tap water_ctrl_3	Tap water 3	327
tap water_0mM_1	with 18 ml Hoaglands 1	310
tap water_0mM_2	with 18 ml Hoaglands 2	320
tap water_0mM_3	with 18 ml Hoaglands 3	327
tap water_50mM_1	plus 17.53 g NaCl 1 (per 6 L)	6020
tap water_50mM_2	plus 17.53 g NaCl 2 (per 6L)	5910
tap water_50mM_3	plus 17.53 g NaCl 3 (per 6 L)	5900

Table S3: Overview experimental design. Imbalances are highlighted in bold.

	Number of compositions in			Number of containers in		
Ecotype	Monoculture	2-ecotype polyculture	4-ecotype polyculture	Monoculture	2-ecotype polyculture	4-ecotype polyculture
1	1	3	4	16	24	16
2	1	3	4	16	24	16
3	1	3	4	16	24	16
4	1	**1**	4	16	**8**	16
5	1	**2**	4	16	**16**	16
6	1	3	4	16	24	16
7	1	3	4	16	24	16

Table S4: Detailed design. The experiment included 92 tubs with each 4 black containers (flower pots) for a total of 92*4 = 368 experimental units.

Diversity	Composition	Total tubs
1	Ecotype 1	4
1	Ecotype 2	4
1	Ecotype 3	4
1	Ecotype 4	4
1	Ecotype 5	4
1	Ecotype 6	4
1	Ecotype 7	4
Total monocultures:	7	28
2	Ecotype 1_Ecotype 2	4
2	Ecotype 1_Ecotype 6	4
2	Ecotype 1_Ecotype 3	4
2	Ecotype 2_Ecotype 7	4
2	Ecotype 2_Ecotype 6	4
2	Ecotype 3_Ecotype 5	4
2	Ecotype 3_Ecotype 7	4
2	Ecotype 4_Ecotype 6	4
2	Ecotype 5_Ecotype 7	4
Total 2-ecotype polycultures:	9	36
4	Ecotype 1_Ecotype 2_Ecotype 3_Ecotype 7	4
4	Ecotype 2_Ecotype 4_Ecotype 5_Ecotype 7	4
4	Ecotype 2_Ecotype 3_Ecotype 5_Ecotype 6	4
4	Ecotype 1_Ecotype 3_Ecotype 4_Ecotype 6	4
4	Ecotype 1_Ecotype 5_Ecotype 6_Ecotype 7	4
4	Ecotype 3_Ecotype 4_Ecotype 6_Ecotype 7	4
4	Ecotype 1_Ecotype 2_Ecotype 4_Ecotype 5	4
Total 4-ecotype polycultures:	7	28
Unique combinations:	23	92

Table S5: Results of a Type III Analysis of Variance (ANOVA) with Satterthwaite's method for linear-mixed model with population growth rate as response variable in phase 1 and log-transformed mean abundance as response variable in phase 2. Fixed-effect terms were ecotype identity and diversity context in phase 1 and the same factors plus salinity in phase 2. Group and tub (nested within group) were included as random factors. *P*-values < 0.05 are shown in bold.

Phase 1	**Sum Sq**	**Mean Sq**	**NumDF**	**DenDF**	***F*-value**	***P*-value**
Ecotype identity	0.003239	0.0005398	6	133.418	44.405	**<0.001**
Diversity	0.000004	0.0000019	2	71.962	0.1594	0.853
Ecotype x Diversity	0.00006	0.000005	12	134.882	0.4074	0.9587
Phase 2	**Sum Sq**	**Mean Sq**	**NumDF**	**DenDF**	***F*-value**	***P*-value**
Ecotype identity	44.871	7.4785	6	121.233	175.2795	**<0.001**
Diversity06/02/2022 10:10:0006/02/2022 10:10:00	0.036	0.018	2	63.646	0.4226	0.65717
Salinity	0.004	0.0045	1	63.541	0.1043	0.74776
Ecotype x Diversity	1.165	0.0971	12	121.402	2.2757	**0.01214**
Ecotype x Salinity	0.036	0.006	6	121.233	0.1418	0.99032
Ecotype x Salinity	0.016	0.008	2	63.646	0.1882	0.82892
Ecotype x Diversity x Salinity	0.132	0.011	12	121.402	0.257	0.99423

Table S6: Results of a Type III Analysis of Variance (ANOVA) with Satterthwaite's method for linear-mixed model with biofilm score assessed on day 110 as response variable. Fixed-effect terms were ecotype identity, diversity context and salinity treatment (0 vs. 50 mM) and the interaction terms. Group and tub (nested within group) were included as random factors. *P*-values < 0.05 are shown in bold. Tukey postdoc testing showed strong evidence that ecotype 5 differed from ecotype 7 ($P = 0.0027$). In addition, there was weak evidence that ecotype 5 and 6 and ecotypes 2 and 7 were also different in terms of biofilm formation ($P = 0.0688$ and $P = 0.0550$, respectively).

Source of variation	**Sum Sq**	**Mean Sq**	**NumDF**	**DenDF**	***F*-value**	***P*-value**
Ecotype identity	4.6739	0.77898	6	236.239	2.1471	**0.049**
Diversity	0.2171	0.21709	1	68.695	0.5984	0.442
Salinity	2.7295	2.72954	1	65.763	7.5236	**0.008**
Ecotype identity x Diversity	2.2373	0.37289	6	293.291	1.0278	0.407
Ecotype identity x Salinity	2.3308	0.38847	6	241.125	1.0708	0.381
Diversity x Salinity	0.0005	0.00052	1	65.679	0.0014	0.970
Ecotype identity x Diversity x Salinity	1.9706	0.32844	6	296.708	0.9053	0.491

Table S7: Results of a Type III Analysis of Variance (ANOVA) with Satterthwaite's method for linear-mixed model with log-transformed number of fronds on day 110 as response variable and biofilm score plus all experimental treatment as explanatory variables. Group was included as random factor. Note that this represents only one day of the experiment. Two populations that had gone extinct by day 110 were excluded from these analysis, in order to be able to log-transform the response variable. *P*-values < 0.05 are shown in bold

Source of variation	Sum Sq	Mean Sq	NumDF	DenDF	*F*-value	*P*-value
Biofilm score	0.1438	0.14379	1	309.79	0.747	0.388
Salinity	0.0433	0.0433	1	309.99	0.225	0.636
Ecotype identity	10.0861	1.68101	6	309.3	8.730	**< 0.001**
Diversity	0.2055	0.20554	1	306.55	1.067	0.302
Biofilm score x Salinity	0.1486	0.1486	1	309.93	0.772	0.380
Biofilm score x Ecotype identity	2.8228	0.47047	6	308.71	2.443	**0.025**
Salinity x Ecotype identity	0.7412	0.12354	6	308.3	0.642	0.697
Biofilm score x Diversity	0.2222	0.22222	1	307.05	1.154	0.284
Salinity x Diversity	0.0235	0.02352	1	307.56	0.122	0.727
Ecotype identity x Diversity	1.1441	0.19069	6	308.42	0.990	0.432

Note: None of the three- or four-way interactions were significant and are thus not shown.

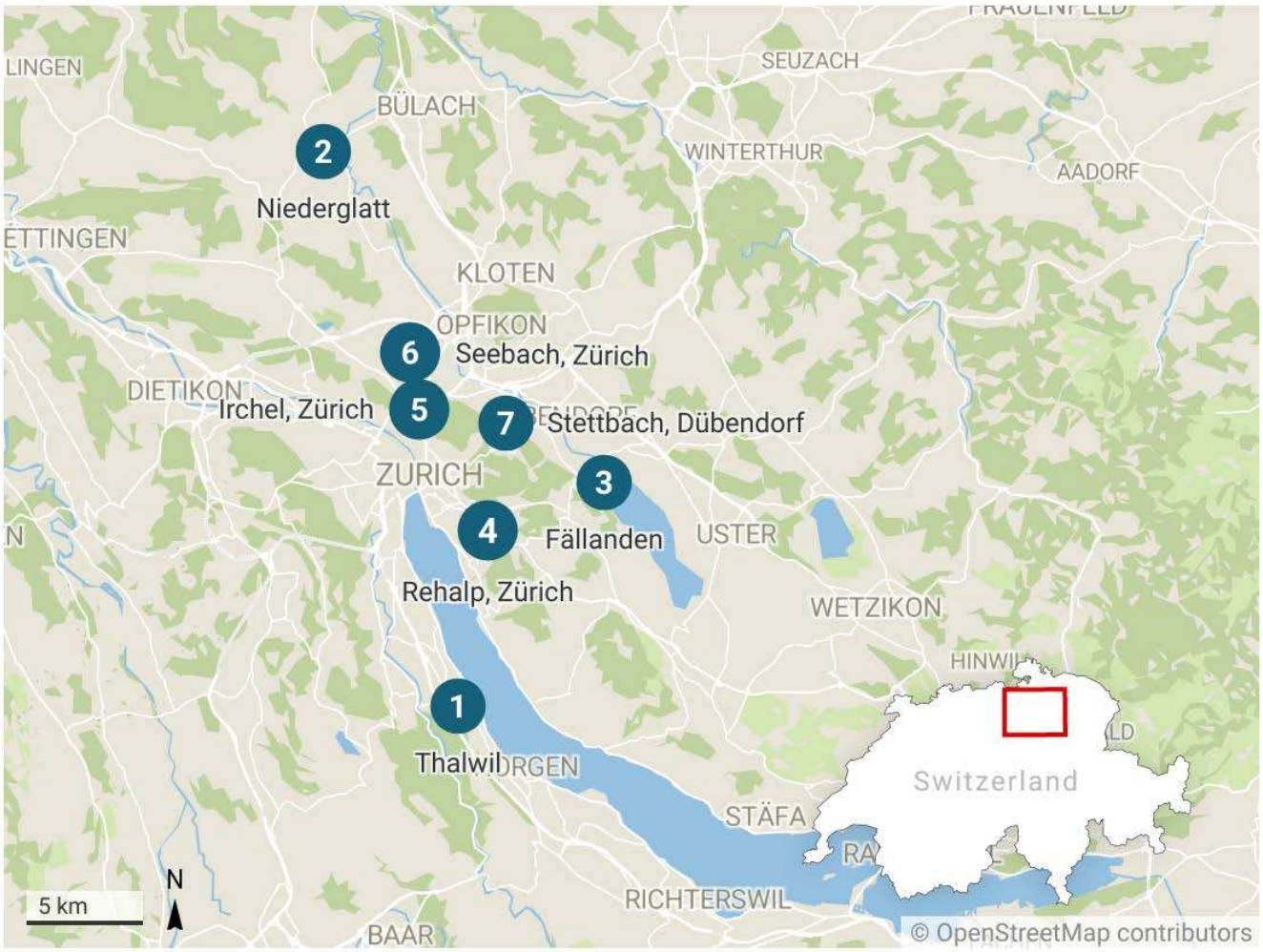

Figure S1: Sampling locations. Ecotypes are numbered according to ecotype number used throughout the manuscript. All ecotypes were collected in the Kanton Zurich, Switzerland in late summer/early fall 2020. The maximum distance between two locations was 24.16 km (distance between ecotypes 1 and 2), the minimum distance was 2.5 km (between ecotypes 5 and 6). For more details about the locations see Table S1. (Figure created with Datawrapper).

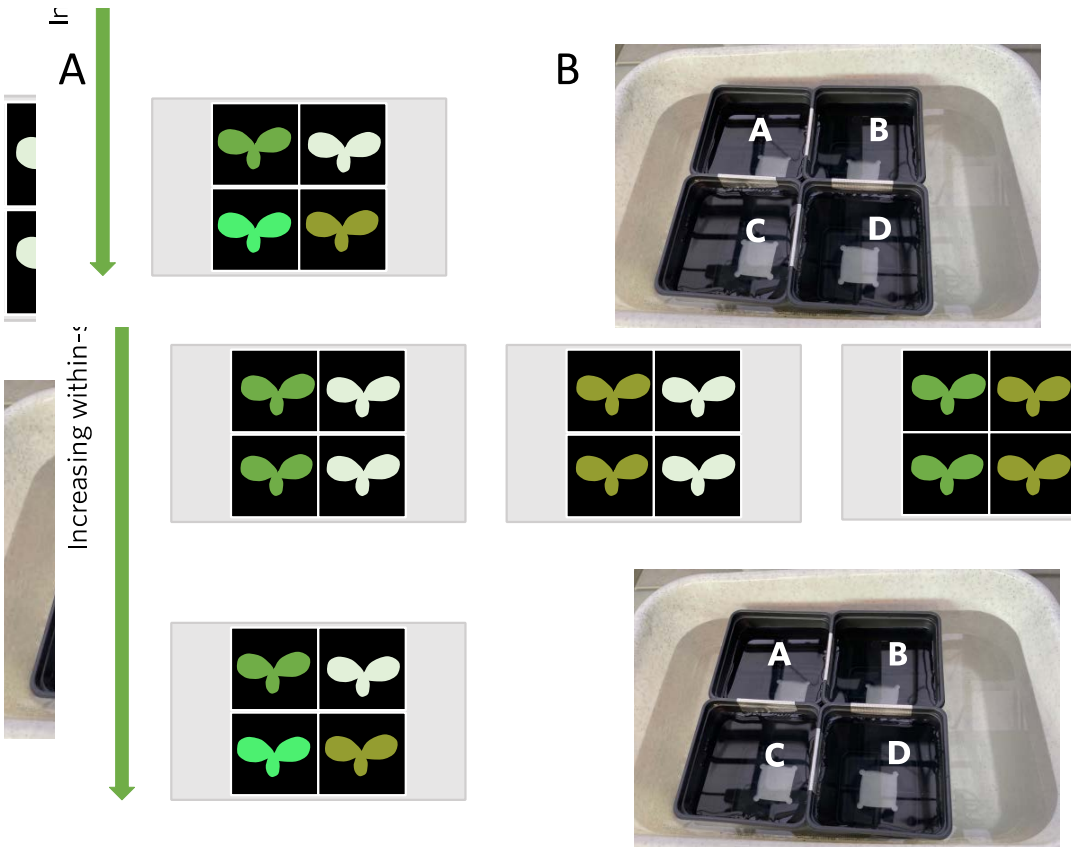

Figure S2: Schematic of the experimental set up. a shows how the diversity gradient was created and **b** the experimental units before and several weeks after addition of the duckweed individuals.

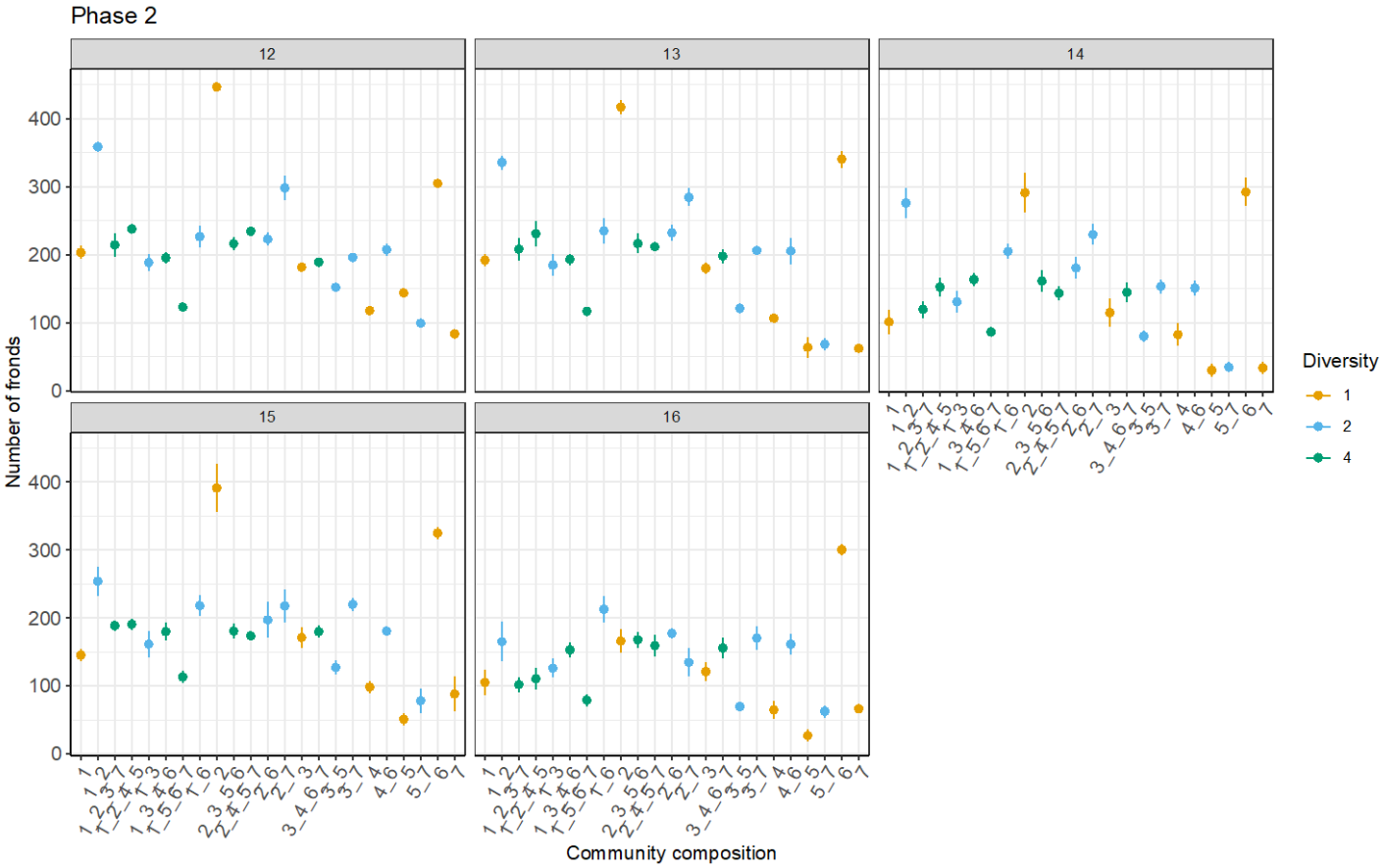

Figure S3: Differences among the community compositions in phase 2. The mean number of fronds for each unique community composition (on the x axis) at the for all time points during phase 2. Time point 12 represents the beginning of phase 2, time point 16 is the final measurement taken on day 126 of the experiment. Note that the monoculture of ecotype 6 was the best performing composition in phase 2, though its abundance declined over time.

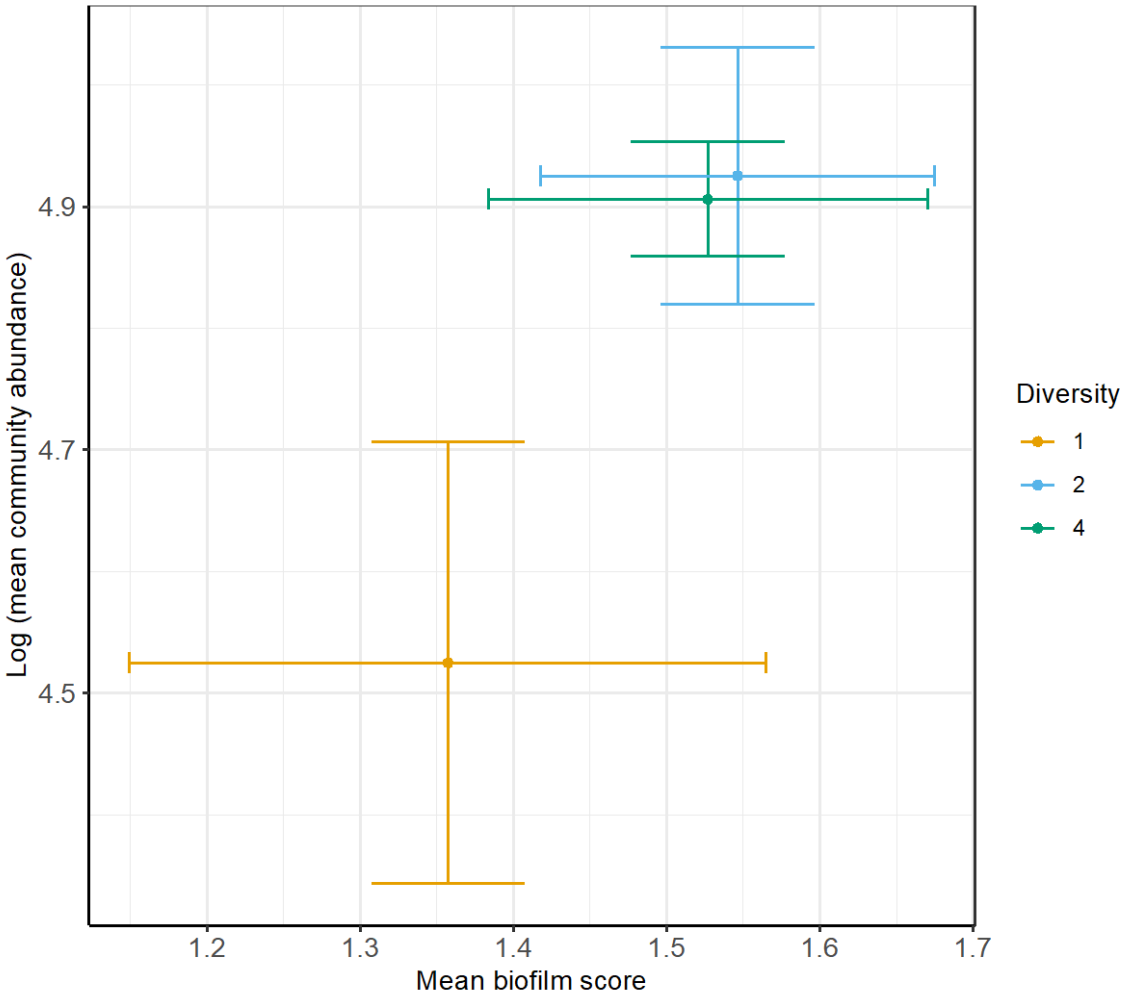

Figure S4: Log-transformed whole-population abundance in response to the mean biofilm score per population, as assessed on day 100 of the experiment. Shown are means and standard errors for both population abundances and the biofilm score. There was no significant effect of biofilm score on whole-population abundances ($F = 0.9980$, $P = 0.3207$).

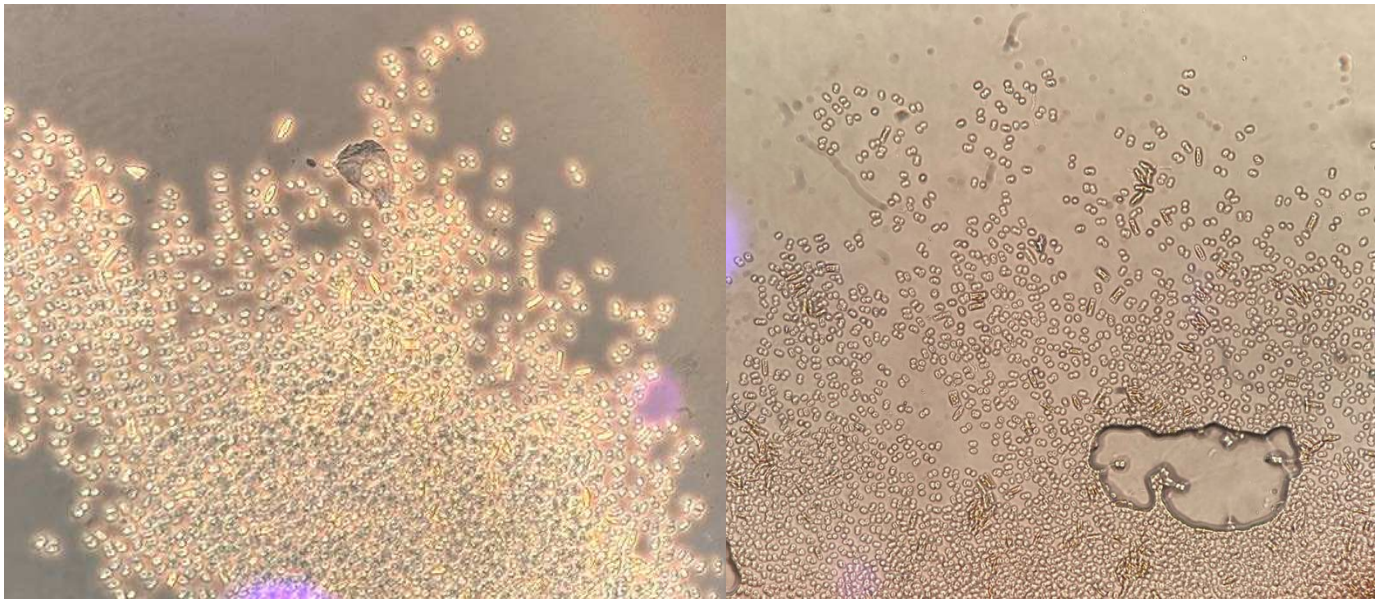

Figure S5: Microscopy image of two random sample of the algal biofilm that formed during the experiment. The algae were attributed to the groups of diatoms and green algae. The pictures were taken on 12 February 2021, shortly before the start of phase 2.

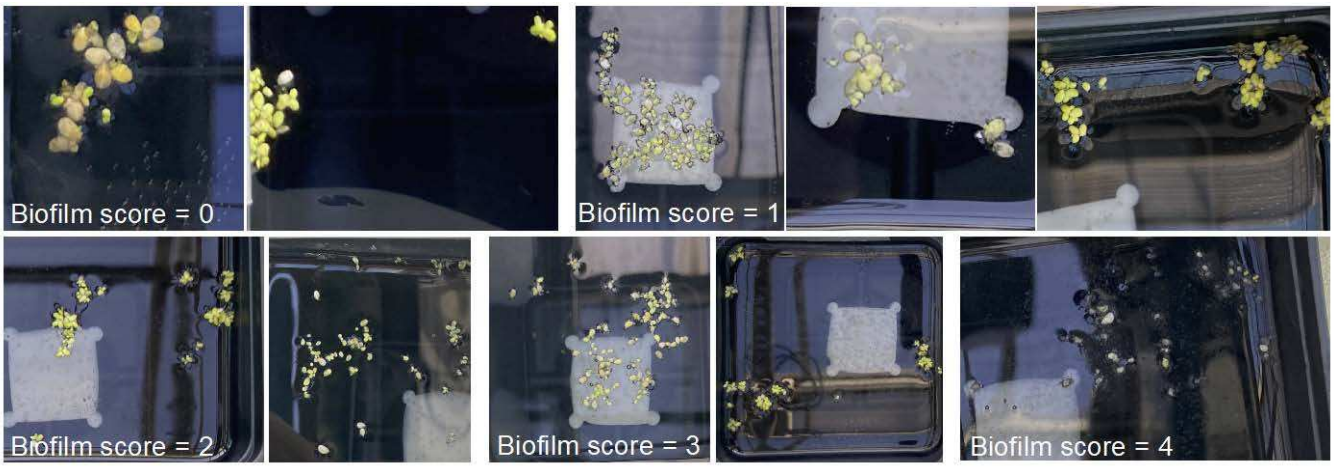

Figure S6: Examples of how the biofilm was scored. The biofilm on the water surface and the surface of the fronds was assessed and a score was given from 0 to 4. No visible biofilm scored a 0, even when fronds did not look healthy.