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

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Abstract

Aims

The pollution of freshwater ecosystems is threatening freshwater plant species diversity worldwide. Freshwater plants, such as duckweed (*Lemna minor*), are potentially sensitive to novel stressful environments. To test if ecotype diversity could increase resistance to stressful environments, I used seven *L. minor* populations and measured their growth rates with and without moderate salt stress across an ecotype diversity gradient.

Methods

The *L. minor* populations were grown over five months in 92 experimental mesocosms, either in ecotype monocultures or in polyculture with either one or three conspecific ecotypes (23 unique compositions). After growing the duckweed in unperturbed conditions (phase 1), the cultures were subjected to moderate salt stress (50mM NaCl) for several weeks (phase 2). The experiment was conducted in the presence of the natural epimicrobial community associated with the different ecotypes. In phase 2, a subset of these algae added an unintentional second stressor to the experiment.

Important findings

The ecotypes differed in their growth rates, the fastest growing at twice the rate of others. The diversity context further shaped the ecotype growth rates. Ecotype polycultures showed higher abundances towards the end of the experiment, thus over time, as the environment deteriorated, ecotype diversity gained in importance. These findings show that within-species variation in growth rates can translate to a positive effect of ecotype diversity on population abundance. Exposure of *L. minor* to moderate salt levels did not significantly impact growth rates, although the effect may have been masked by reduced algal stress in the saline environments.

Running head: Ecotype diversity effects in duckweed

Keywords: *aquatic plant, diversity experiment, glasshouse experiment, growth rate, intraspecific diversity, Lemna minor*

INTRODUCTION

Freshwater environments are increasingly under pressure from human-mediated climate change and activities. Freshwater biodiversity is consequently in decline as aquatic communities scramble to adapt rapidly to their deteriorating environments (Dudgeon *et al.* 2006; Tickner *et al.* 2020). Not only species diversity is in decline, but also within-species diversity is increasingly being lost (Leigh *et al.* 2019). However, intraspecific diversity is key for the persistence of communities in changing environments (Des Roches *et al.* 2018; Stange *et al.* 2021). One measure of intraspecific diversity is the concept of ecotypes (Gregor 1944; Turrill 1946). Ecotypes usually refer to populations within a species that are adapted to local environmental conditions (Hufford and Mazer 2003) and ecotype diversity is thus a measure of intraspecific diversity (Clemens and Schreck 2021). Ecotypes represent an important source for potential adaptive genetic variation (Blackmore *et al.* 2016). Ecotype diversity may increase a species' performance in varying environments because different ecotypes have different traits that allow them to perform well in different conditions (Gregor 1944; Wożakowska-Natkaniec 1977), thus creating response diversity within a single species.

Stressful environmental conditions lead to a reduction in fitness in populations, resulting in declining populations (Hoffmann and Hercus 2000; Agrawal and Whitlock 2010). Intraspecific diversity, including ecotype diversity, may increase resistance to biotic stressors and abiotic stressors (Jump *et al.* 2009). Genotypic diversity can maintain ecosystem functioning in the presence of stressors (Hughes and Stachowicz 2004) and prevent populations from extinction (Loria *et al.* 2022). However, there is still limited evidence as to how intraspecific diversity can increase the response diversity (Elmqvist *et al.* 2003) within a single species. Moreover, there is a lack of research concerning the effect of ecotype diversity on community functioning and stability. Here, I hypothesized that ecotype diversity will increase a single species' resistance to environmental stress.

To this aim, I used one of the world's smallest angiosperms, *Lemna minor* L. (common or lesser duckweed) as a model system. *L. minor* grows very fast, is easy to culture, and is a convenient and cheap model system to test ecological and evolutionary theory. This species has recently gained substantial interest to be used as a model organism in community ecology and eco-evolutionary dynamics (Laird and Barks 2018; Hart *et al.* 2019). Importantly, different ecotypes of *L. minor* have been shown to vary in their physiological properties (Ziegler *et al.* 2015). To make use of this intraspecific variation within *L. minor*, I collected seven ecotypes of *L. minor* from different waterbodies in an urbanized and agricultural areas that were isolated by several kilometres. It is known that in *L. minor* there is commonly high genetic variation among populations even within a close geographic range, but low genetic diversity within populations (Cole and Voskuil 1996; Xu *et al.* 2015). It is thus possible that each ecotype represents a different clonal population of a site-specific genotype. However, the collected ecotypes were not genotyped, and it is unknown whether the genotypic diversity within each ecotype was the same for all ecotypes. It is thus possible that the lowest diversity level in the experiment (ecotype monocultures) represented multiple genotypes for some or all ecotypes.

The aim was to test two hypotheses: First, whether the ecotype identity influences ecotype-level growth rates in the absence and presence of salt stress. This response diversity would be

a prerequisite to be able to test the second hypothesis if ecotype diversity could increase the resistance of *L. minor* to moderate salt stress. Finally, I tested whether the growth rates of ecotypes were influenced by the diversity context in which the ecotype was growing.

I grew the seven ecotypes alone or in the presence of either one or three other ecotypes, thus creating an ecotype diversity gradient ranging from monoculture to a 4-ecotype-polyculture. This allowed studying the effects of ecotype diversity on total population abundance. I carried out the experiment in two phases. Initially, I allowed *L. minor* to vegetatively grow with ample nutrients, light, and space for several weeks (phase 1). Subsequently, in phase 2, I subjected them to salt stress using sodium chloride (NaCl). Salt is as a stressor commonly found in *L. minor*'s freshwater habitats. In many northern areas where duckweeds are common, large amounts of salt are applied to roads and other surfaces in winter. The application of road salt can significantly increase the salinity of waterbodies in urban areas (Schuler *et al.* 2017), with negative consequences for aquatic ecosystems (Hébert *et al.* 2022; Hintz *et al.* 2022). In contrast to previous experiments that investigated the influence of NaCl on duckweed growth rate (Sree *et al.* 2015), I used wild populations (ecotypes) and not clonal strains that had been grown under laboratory conditions for a prolonged period of time. I conducted the experiment in a non-sterile environment with a community of algae and microbes in the water. This means that the ecotype diversity gradient in the experiment may have been paralleled by a microbial community diversity gradient.

Algal growth was held in check during phase 1 with frequent transfers into fresh water. However, during phase 2, an algal biofilm developed in all experimental cultures, which inhibited duckweed growth. As a result, I also investigated whether ecotypes were differentially affected by the algae and whether ecotype diversity would also increase resistance to this second, unintentional stress.

METHODS AND MATERIALS

Study species and collection

Lemna minor mostly reproduces clonally, producing new plants by budding, although in the wild it also occasionally flowers. It has a near-global distribution and occurs at high densities in slow-moving freshwater bodies in a wide range of environmental conditions (Landolt 1975).

The seven ecotypes were collected at different locations in and around Zurich, Switzerland. I sampled in Thalwil (ecotype 1), Neeracher Ried (ecotype 2), Fällanden (ecotype 3), Zurich, Rehalp (ecotype 4), Zurich, Irchel park (ecotype 5), Zurich, Seebach (ecotype 6), and Stettbach, Dübendorf (ecotype 7) in late summer/early fall 2020 (Fig. S1 for a map with the sampling locations, Table S1 for additional information including the coordinates). The conductivity was measured in a water sample using a handheld probe (Hanna instruments). Conductivity ranged from 183 µS/cm (Seebach, Zurich) to 552 µS/cm (Rehalp, Zurich) between collection areas but was < 1000 µs/cm in all locations, which corresponds to freshwater conditions (Table S2). Duckweed ecotypes were collected with approximately 5 L of water from the water body they were collected from. From each location, several hundred to several thousand individuals (fronds) were collected, capturing also the potential within-site intraspecific diversity. The duckweed populations were then moved to the glasshouse facility at University of Zurich and kept in plastic tubs in their own water supplemented with

tap water for ~6 weeks. After that they were transferred into tap water to reduce algal growth and kept in the glasshouse in an ecotype monoculture common garden until the start of the experiment (approximately two months).

Glasshouse experiment

The experiment was carried out at the glasshouse facilities of the University of Zurich, Switzerland in opaque plastic tubs (Universalwanne 9L, PP, Semadeni, Switzerland) containing 6 L of tap water and nutrients (see below). In total, there were 92 tubs which were divided into four compartments using black plastic containers (10 x 11 cm, GVZ rossat, Switzerland) to track growth of each ecotype individually (Fig. S2). A large hole was cut out from the bottom of the containers to maximize the underwater connection. The ecotypes were thus separated on the surface to prevent them from floating into each other's areas but shared the same water. At the beginning of the experiment, the fronds were placed inside the containers with tap water (Fig. S2) using an inoculation loop. *L. minor* individuals were thoroughly rinsed in tap water but not sterilized prior to the experiment to avoid sterilization-induced mortality and to maintain the natural epimicrobial community. Consequently, this experiment was not a strict common garden experiment because the environment may have been influenced by the epimicrobial communities associated with the collected ecotypes. Artificial light was programmed to be turned on from 10 am to 4 pm if the natural light was below 30 klux. The temperature was set at minimum 20° C during the day, 15°C during the night. The experimental design included seven monocultures (single-ecotype communities), nine 2-ecotype mixtures, and seven 4-ecotype mixtures for a total of 23 unique culture compositions. To measure diversity effects that are independent from composition effects, it was crucial to have several different specific community compositions for the highest diversity treatment (Bruelheide *et al.* 2014). The design aimed to be as balanced as possible with every ecotype occurring with the same frequency in all diversity levels (see Table S4). However, for ecotypes 4 and 5, there were not enough fronds (individuals) available, therefore, they appeared less frequently in the design. Apart from this limitation, combinations were drawn randomly (random extinction scenarios, Bruelheide *et al.* 2014). Each unique composition was replicated four times at the beginning of the experiment (23 * 4 = 92 containers). For the full experimental design, see Table S4.

The experiment was started on 11 Nov 2020 (day 0). On this day, each of the four containers per tub received on average 13 fronds (+/- 3 fronds) for a total of an average of 51 fronds (+/- 5 fronds) per tub as initial population size. In the monocultures, all four containers received the same ecotype. The tubs were placed in random fashion on the tables in the glasshouse and then covered with transparent plastic boards (4 mm Hobbyglas, Coop, Switzerland**)** to reduce evaporation. One board covered a group of four tubs that were located next to each other (the group was random due to the randomized positioning of the tubs in the glasshouse). 9 mL (0.7ml / L) of fertilizer (100% Hoagland's E Media, Cowgill and Milazzo 1989) was added on day nine after the start of the experiment for a final concentration of 0.125% fertilizer, which corresponded to approximately 0.124 mg/L of nitrogen (N) and 0.019 mg/L of phosphorus (P). To mitigate algal growth, the communities were transferred into fresh tap water with Hoagland's E medium approximately every two weeks. After the transfer, fresh

Hoagland solution was added to the tap water and thoroughly mixed. Initially, 9 mL / 6 L of nutrients (0.7 ml/ L) were added but due to the observed slow growth of the duckweed populations the concentration was increased to 18 mL / 6L (1.4 mL/ L) from day 57 on. The exponential growth of the fastest-growing ecotypes (especially ecotype 2) for the first months of the experiment suggests that nutrients were not limiting.

The experiment was then carried out in two phases. During phase 1 (day 0 to day 95 of the experiment), the populations were grown without any experimental treatment. During this time, I recorded population abundances for all ecotypes and cultures under unperturbed conditions. On days 95, 96 and 97 of the experiment, phase 2 was initiated by establishing moderate salt stress using sodium chloride (NaCl). To ensure that all four independent replicates started with relatively equal abundances, I standardized *L. minor* abundance among the four replicates of the same composition. To do so, I pooled all the duckweed individuals per ecotype and collected them in a global pool from which I redistributed the fronds to the replicates at roughly equal abundances. After this standardization process, I subjected half of the communities to salt stress. I added NaCl (Sigma-Aldrich, 99.5% purity) to half of the tubs (17.53 +/– 0.01 g / 6L) for a final concentration of 50 mM. This salt concentration has been shown to be harmful but not lethal to *L. minor* (Sree *et al.* 2015; O'Brien *et al.* 2020). The salt was added to pre-labelled replicates 3 and 4 for all experimental cultures. The NaCl was resuspended in the water by mixing. Phase 2 ran from day 97 to day to day 128 of the experiment (the end of the experiment). The duckweed populations continued to be transferred into fresh tap water with the same salt concentrations and nutrient concentrations every two weeks. However, despite these frequent transfers, the experiment had to be terminated after 31 days of phase 2 due to algal growth causing high duckweed mortality in both treatment and control tubs. The algae formed a biofilm on the water surface and grew on top of the duckweed fronds, and consequently the infested duckweed individuals generally died within days. Algal stress could not repeatedly be quantified during the experiment but was assessed once based on photographs taken on day 110. To do so, each population within a container was given a score from 0 (no visible biofilm) to 4 (very extensive biofilm formation). This was done by a single experimenter and scores were done blindly (see Figure S5 for some examples).
On day 112, the tubs had to be moved to an adjacent compartment due to construction work being carried out at the glasshouse facilities. The new compartment had adiabatic cooling but had otherwise similar conditions. Groups of four tubs that were in close proximity in the first compartment (termed a "group") stayed together after the move. Duckweed abundance was estimated using photographs (iPhone SE camera) and by counting all individual fronds with the counter function in Image J (Rasband, W.S. 1997). The tubs were photographed in total 16 times.

Data analysis

During phase 1, ecotype population growth rates were calculated as $\ln(N_2/N_1)/(t_1-t_2)$. For population growth rates in phase 1, I used the initial population abundance at the start of the experiment (t_1) and the final time point of phase 1 (t_2, day 89). For ecotype-level analyses, the average growth rate was calculated across all individual black plastic containers with a

specific ecotype growing in it (n = 56 for ecotypes 1, 2, 3, 5 and 6, n=40 for ecotype 4, and n=48 for ecotype 5). In phase 2, the duckweed populations stopped growing and even slightly declined. Therefore, calculating the growth rate would not be insightful. Instead, I used the mean abundance during phase 2 as response variable.

To test the outcome of the ecotype diversity manipulation for the full duration of the experiment, I used abundance as response variable. Total abundance per tub was summed for the four black containers to get an abundance estimate per tub (n=92).

To account for the effect of spatial position in the glasshouse, a factor "group" was created that corresponded to a group of 4 tubs that were covered by the same plastic board throughout the experiment and thus had similar light conditions. "Group" was used as random effect in statistical models when appropriate.

For the ecotype growth rates in phase 1, the effect of the treatment variables on growth rates (n=368) was analyzed with linear mixed models. Fixed-effect factors were ecotype diversity (1, 2 or 4 ecotypes), ecotype identity (ecotype 1, 2, 3, 4, 5, 6 or 7) and their interaction. Group was included as random-effect factor to account for spatial variation. In addition, I nested the container (unique composition) within group. Mixed models using restricted maximum likelihood (REML) were fitted using the function lmer in the R-package lme4 (Bates *et al.* 2015). To further investigate the significant effect of ecotype identity a post-hoc test (Tukey, lsmeans (Lenth 2016)) was used. Mean abundance per population in phase 2 (n=368) was log-transformed and consequently analyzed using the same mixed models as explained above.

For whole-population abundances (n=92) the time series was also split into the two phases. Whole-population abundance was log-transformed for all statistical analyses. Here, the fixed effects were ecotype diversity either as factor (1, 2 or 4 ecotypes), a linear term or a contrast between monocultures and polycultures, and time (linear, n = 16 time points). Group (to account for spatial variation) and community composition were included as random effects. Algal stress was analysed based on the biofilm score using the same models, with ecotype identity, community composition, diversity, and salinity as treatment variables. I also analyzed the effect of the severity of algal stress (biofilm score) on population abundance on day 110 of the experiment. Mixed models using restricted maximum likelihood (REML) were fitted using the function lmer in the R-package lme4 (Bates *et al.* 2015). Due to the imbalanced design (Table S3), test statistics were obtained with a type 3 ANOVA using the R package lmerTest (Kuznetsova *et al.* 2017). All analyses were conducted in R v 4.1.0 (R Development Core Team 2021).

RESULTS

Ecotype responses in the absence and presence of moderate salt stress

I found strong and significant effects of ecotype identity on growth rates in both mono- and polyculture in phase 1 (Fig. 1, 3A, Table S5). However, the diversity context in which the ecotypes were growing and the interaction between the diversity context and ecotype identity were not significant (Table S5). Thus, ecotype growth rates were not influenced by either diversity or the presence or absence of a specific different ecotype.

During phase 1, most ecotypes showed continued exponential growth (Fig. 1). An exception was ecotype 7, which stopped growing after only a few weeks and then maintained its

population size. A Tukey post-hoc test confirmed that ecotype number 2 significantly outperformed all the others. In contrast, ecotype number 7 showed a significantly lower growth rate than the others (but its growth rate was comparable to the one of ecotype 4). Ecotype 6 grew significantly better than ecotypes 3, 4, 5 and 7 but its growth rate was similar to the one of ecotype 1 and still significantly lower than the one of ecotype 6.
In phase 2 (Fig. 2, 3B), when salt exposure was combined with (unintentional) stress from algal contamination (Fig.5, Fig. S4), I also found strong and significant effects of ecotype identity on growth rates in both mono- and polyculture (Table S5). In addition, there was a significant effect of the diversity context for a subset of the ecotypes (significant interaction ecotype x diversity, table S5). When diversity is added as a contrast between monocultures and polycultures in the model, the contrast term was also significant (data not shown). In other words, ecotype growth varied significantly, and some ecotypes were influenced by the diversity context, especially the difference between growing alone vs growing in polyculture (Fig. 3B). However, salinity did not impact population growth rates and on average, diversity did also not influence growth rates.

Whole-population responses in the absence and presence of moderate salt stress

For whole-population analyses the population abundances for the four ecotypes growing in the black containers were summed to get a total population abundance per culture. For phase 1, I did not find significant effects of diversity on total abundance (Table 1). In phase 1, over time ecotype monocultures started to have on average lower total abundance and polycultures (both 2-ecotype and 4-ecotype polycultures) were on average more productive. (Fig. 4, Table 1). However, throughout phase 1 the best performing community composition was the monoculture of ecotype 2. The interaction between the comparison between mono- and polycultures and time was significant for all three diversity terms, but the effect was strongest for the comparison between monocultures and polycultures ($P = 0.009$, Time x Diversity interaction, Table 1). In phase 2, the diversity effect (only the contrast between mono- and polycultures) was significant, with 2- and 4-ecotype polycultures being significantly more productive than ecotype monocultures ($P = 0.021$, Table 2). This positive effect of diversity was consistent during phase 2 (Fig. 4). However, the best performing community in phase 2 was the monoculture of ecotype 6 (Fig. S3). There was no significant effect of salt addition on total abundance in phase 2 (Table 2, Fig. 4).
Algal stress (biofilm score on day 110 of the experiment) was slightly influenced by ecotype identity (Figure 5a, Table S6), for example the score was on average highest for ecotypes 2 and 5. Furthermore, salinity significantly reduced biofilm formation in all diversity levels (Figure 5b, Table S6). However, diversity had no effect (Figure 5b, Table S6). When the biofilm score was modelled as explanatory variable and the number of fronds on day 110 as response, there was no significant main effect of the biofilm score on the number of duckweed fronds on that particular day ($F = 0.747$, $P = 0.388$ for the main term, Table S7). However, the interaction term ecotype identity x biofilm score was significant ($F = 2.443$, $P = 0.025$ for the interaction term, Table S7). The significant interaction was driven by ecotypes 2 and 6, and to a lesser extent ecotype 7. There was thus an interactive effect of the severity of biofilm stress and ecotype identity on duckweed abundance on the day when the

biofilm score was assessed (Fig. 5c). There was no significant effect of the mean biofilm score per polyculture on whole-population abundance (Fig. S4).

DISCUSSION

Effects of ecotype diversity on whole-population abundance

Similar to species response diversity with consequences for whole-community resilience (Baskett *et al.* 2014), intraspecific diversity can have strong effects on community and ecosystem functioning (Des Roches *et al.* 2018). Consequently, I hypothesized that also ecotype diversity would lead to greater population abundances in a stressful environment because the different ecotypes may represent differentially adapted populations of *L. minor* (Wożakowska-Natkaniec 1977).

Indeed, I found that as the experiment progressed, the positive effect of ecotype diversity started to emerge (see significant interaction term Date x Diversity in Table 1 and significant diversity term in Table 2). At the end of the experiment, populations growing in polyculture were on average more abundant, though not more abundant than in the most productive monoculture of ecotype 6 (Fig. S3). The strengthening of the positive effect of ecotype diversity came as the growing conditions worsened, both due to the exposure to salt in phase 2 but also the appearance of increasingly more algae in the containers. Note that space was never limiting. The algae formed a dense biofilm covering the fronds and roots of the *L. minor* individuals. The algal biofilm (Fig. S5, S6) led to significant reductions in growth rates and mortality in all experimental cultures. However, there was no evidence that cultures with greater ecotype diversity were on average less overgrown by the biofilm. Therefore, I cannot conclude that the positive effect of ecotype diversity was driven by a greater resistance to the algal biofilm. I would like to emphasize though that the biofilm was only assessed once, two weeks after the addition of salt, which may not show the full picture.

It has previously been found that *L. minor* associates with a diverse and mostly beneficial microbial community (Ishizawa *et al.* 2017; O'Brien *et al.* 2020). This microbial community diversity may have increased in parallel with ecotype diversity, similarly to the positive relationship between terrestrial plants and their associated soil microbial diversity (Schmid *et al.* 2021), which in turn benefits the host plants (van der Heijden *et al.* 2008). Analogously, it is possible that the positive effect of ecotype diversity was in part due to a more diverse beneficial community of microbes.

In contrast to the stress imposed by the algae in the experiment, salt addition had no significant effects on population abundances. However, it could be that the effect of algal stress masked the effect of salt because salinity reduced the biofilm formation (Fig. 5b), which in turn had a positive effect on duckweed persistence. Previous studies conducted in laboratory conditions found that >50 mM of NaCl significantly reduced growth in *L. minor* (Sree *et al.* 2015). However, there is also evidence that *L. minor* can grow well under sustained salt stress in the laboratory (Ullah *et al.* 2021). Here, I found that *L. minor* can persist in near-brackish water, which adds to previous evidence that *L. minor* can be grown under a wide range of environmental conditions, including in saline environments.

Ecotype identity effects on growth rates

In line with previous studies investigating ecological differentiation in *Lemna minor* ecotypes (Wożakowska-Natkaniec 1977), I showed that ecotypes collected from different waterbodies of a maximum distance of 25 km showed differential growth rates in a new environment, i.e., a glasshouse compartment. In particular, ecotype 2 outperformed all the other ecotypes. In contrast, ecotype 7 grew significantly slower than all the other ecotypes.

Not only did the ecotypes vary in their growth rates, but ecotype growth rate also varied over time. For example, the best-performing ecotype in phase 1 (ecotype 2) could not maintain its growth rate in phase 2 and its higher population abundance was not buffering the impact of moderate salt stress, or the secondary stressor induced by the algal biofilm. Contrastingly, the best-performing ecotype at the end of phase 2 was only average during phase 1 (ecotype no. 6). The diversity context further influenced ecotype growth rates, i.e., some ecotypes grew better when they were in the presence of other ecotypes, whereas for some ecotypes it was beneficial to be growing in a monoculture. In phase 1, the two slowly growing ecotypes (4 and 7) tended to profit from growing in mixture, though the effect was not significant. In phase 2, the interaction term between ecotype identity and diversity was significant (P= 0.012, Table S5). The best performing ecotype (6) profited from growing alone. Contrastingly, the two low-performers in phase 2, ecotypes 5 and 7, grew better in ecotype mixtures, though for ecotype 5 it was only the case in the absence of salt (Fig. 3), indicating some facilitative mechanisms (Le Bagousse-Pinguet *et al.* 2014). Ecotype 7 grew very slowly from the beginning of the experiment, when there was no visible biofilm on the fronds and despite not being strongly affected by the biofilm in phase 2 (Figure 5a) continued to grow very slowly until the experiment was terminated.

The assessment of biofilm formation in phase 2 showed that the three most-affected ecotypes (1 and 6) were not consistently the worst performers. Instead, ecotype 6 persisted the best in phase 2 despite being strongly attacked by the algae. Furthermore, there was an interactive effect of the severity of algal stress and ecotype identity on abundance. Taken together, these results suggest that the ecotypes were differentially resistant to the biofilm. It is conceivable that ecotype 6 had adapted to stronger competition with harmful algae in the environment it was growing before the experiment (a small garden pond). Ecotype 5 sourced from a pond in an urban park showed a negative relationship between biofilm score and abundance, suggesting it is maladapted to the presence of harmful algae. However, it is important to note that the amount of biofilm is not independent of population abundance. The more fronds on the water surface, the better the biofilm could grow (seen also in the slightly positive relationship between whole-population abundance and mean biofilm score, Fig. S4).

The differential response of the ecotypes could be because different ecotypes are locally adapted to their environments, which resulted in varying degrees of maladaptation to the novel conditions in the greenhouse. Despite the dominance of asexual reproduction, *L. minor* maintains relatively high levels of genetic diversity (Vasseur *et al.* 1993) and ecotypes collected from different habitats often represent different genotypes, even when they occur in close distance to each other (Ho 2018; Hart *et al.* 2019; Tan *et al.* 2021). These genotypes are potentially adapted to different environmental conditions (Wożakowska-Natkaniec 1977).

For example, three genotypes of *L. minor* collected in France showed varying growth rates in a common garden experiment as well as differential responses to copper pollution (Roubeau Dumont *et al.* 2019). Thus, it is conceivable that the different populations collected in the field are either different single genotypes or represented by a specific set of genotypes that could also show differential growth rates in the experimental setting.
Alternatively, it could be that associated epimicrobial community that hitchhiked on the surface of the duckweed leaves and roots into the experiment had a very strong effect on duckweed growth. Recent studies have shown that there are strong interactions between the duckweed microbiome and the plants' fitness as well as response to stressors (O'Brien *et al.* 2020; Tan *et al.* 2021). A repeated assessment of biofilm formation or even the characterization of its composition and diversity would have been needed to further shed light on the underlying mechanisms. Regardless of the mechanism though, these results show that associated algae and other microbes can strongly influence population dynamics of *L. minor*. I acknowledge that I cannot fully disentangle the contribution of genetic variation between and within ecotypes from the effects of the microbial community. Nevertheless, my findings suggest that experiments conducted with *L. minor* in axenic conditions may overestimate growth rates and other fitness components but underestimate the strength of population dynamics over time. Further research needs to be conducted that carefully disentangles the effect of microbial community diversity from the effect of ecotype identity. Controlled common garden experiments should be combined with ones conducted in more natural conditions, in particular when studying stress tolerance of *L. minor* to evaluate their potential for phytoremediation.

Conclusions

L. minor is a promising candidate for many applications such e.g. as biofuel (Van Hoeck *et al.* 2015), bioremediators (Alvarado *et al.* 2008), crop (Chakrabarti *et al.* 2018), or protein source (Ullah *et al.* 2021). More studies need to be conducted with controlled intraspecific diversity gradients and a separation of the effects of genotype vs. the effects of epimicrobial flora on duckweed population fitness. Knowing more about the effects of its intraspecific diversity on abundance and growth rate will help to maximize yields for food production and to choose ecotypes (genotypes) best suited for local cost-effective growing conditions.

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SUPPLEMENTARY MATERIAL

Supplementary Tables S1–S7 and Figures S1–S4 are available online.

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Conflict of interest statement. The author declares that she has no conflict of interest.

DATA ACCESSIBILITY STATEMENT

Data are publicly available on Zenodo (DOI: 10.5281/zenodo.6371962).

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Tables

Table 1. Type III ANOVA results for linear-mixed model with log-transformed whole population abundance as response variable in phase 1. Fixed-effect terms were time point and diversity (factorial, linear and contrast between monocultures and polycultures). Group was included as random effect to account for spatial variation in the glasshouse and community composition was added as random effect to account for the variation induced by specific combinations of ecotypes. p-values < 0.05 are shown in bold.

Source of variation	**Sum Sq**	**Mean Sq**	**NumDF**	**DenDF**	**F value**	**Pr(>F)**
Diversity as factor						
Time	179.967	179.967	1	956.97	3078.0939	**<0.001**
Diversity	0.054	0.027	2	27.67	0.4623	0.635
Time x Diversity	0.406	0.203	2	956.97	3.469	**0.032**
Random terms	**Variance**	**St.Dev**				
Group (n=23)	0.01249	0.1118				
Composition (n=23)	0.02602	0.1613				
Residual (n = 1008 observations)	0.05847	0.2418				
Diversity as linear term						
Time	19.7685	19.7685	1	957.82	337.5893	**<0.001**
Linear diversity	0.0479	0.0479	1	29.56	0.818	0.37307
Time x linear diversity	0.2651	0.2651	1	957.82	4.5267	**0.034**
Random terms	**Variance**	**St.Dev**				
Group (n=23)	0.01245	0.1116				
Composition (n=23)	0.02476	0.1574				
Residual (n = 1008 observations)	0.05856	0.242				
Diversity contrast monocultures vs. polycultures						
Time	149.442	149.442	1	957.82	2558.3577	**<0.001**
Diversity contrast	0.055	0.055	1	29.33	0.9345	0.341597
Time x Diversity contrast	0.404	0.404	1	957.82	6.91	**0.009**
Random terms	**Variance**	**St.Dev**				
Group (n=23)	0.01245	0.1116				
Composition (n=23)	0.02476	0.1574				
Residual (n = 1008 observations)	0.05841	0.2417				

Table 2. Type III ANOVA results for linear-mixed model with log-transformed whole-population abundance as response variable in phase 2. The fixed effects was diversity (either factorial, linear or as a contrast between monocultures and polycultures) and salinity (control vs. 50mM). Time point and nested within each time point community composition were added as random effects. Group was excluded from the model because spatial variation did not influence the results in phase 2. p-values < 0.05 are shown in bold.

Source of variation	**Sum Sq**	**Mean Sq**	**NumDF**	**DenDF**	**F value**	**Pr(>F)**
Diversity as factor						
Diversity	0.232742	0.116371	2	108.030	2.743	0.069
Salinity	0.036214	0.036214	1	341.05	0.8536	0.356
Diversity x Salinity	0.04379	0.021895	2	341.05	0.5161	0.597
Random terms	**Variance**	**St.Dev**				
Community x time point	0.25873	0.5087				
Time point	0.04256	0.2063				
Residual (n= 459 observations)	0.04242	0.206				
Diversity as linear term						
Linear Diversity	0.152737	0.152737	1	109.04	3.6001	0.060
Salinity	0.002686	0.002686	1	342.04	0.0633	0.801
Linear diversity x Salinity	0.000834	0.000834	1	342.06	0.0197	0.889
Random terms	**Variance**	**St.Dev**				
Community x time point	0.26084	0.5107				
Time point	0.04246	0.2061				
Residual (n= 459 observations)	0.04243	0.206				
Diversity contrast monocultures vs. polycultures						
Diversity contrast	0.233605	0.233605	1	109.01	5.5127	**0.021**
Salinity	0.02165	0.02165	1	342.04	0.5109	0.475
Diversity contrast x Salinity	0.018084	0.018084	1	342.04	0.4268	0.514
Random terms	**Variance**	**St.Dev**				
Community x Time point	0.26084	0.5107				
Time point	0.04246	0.2061				
Residual (n= 459 observations)	0.04243	0.206				

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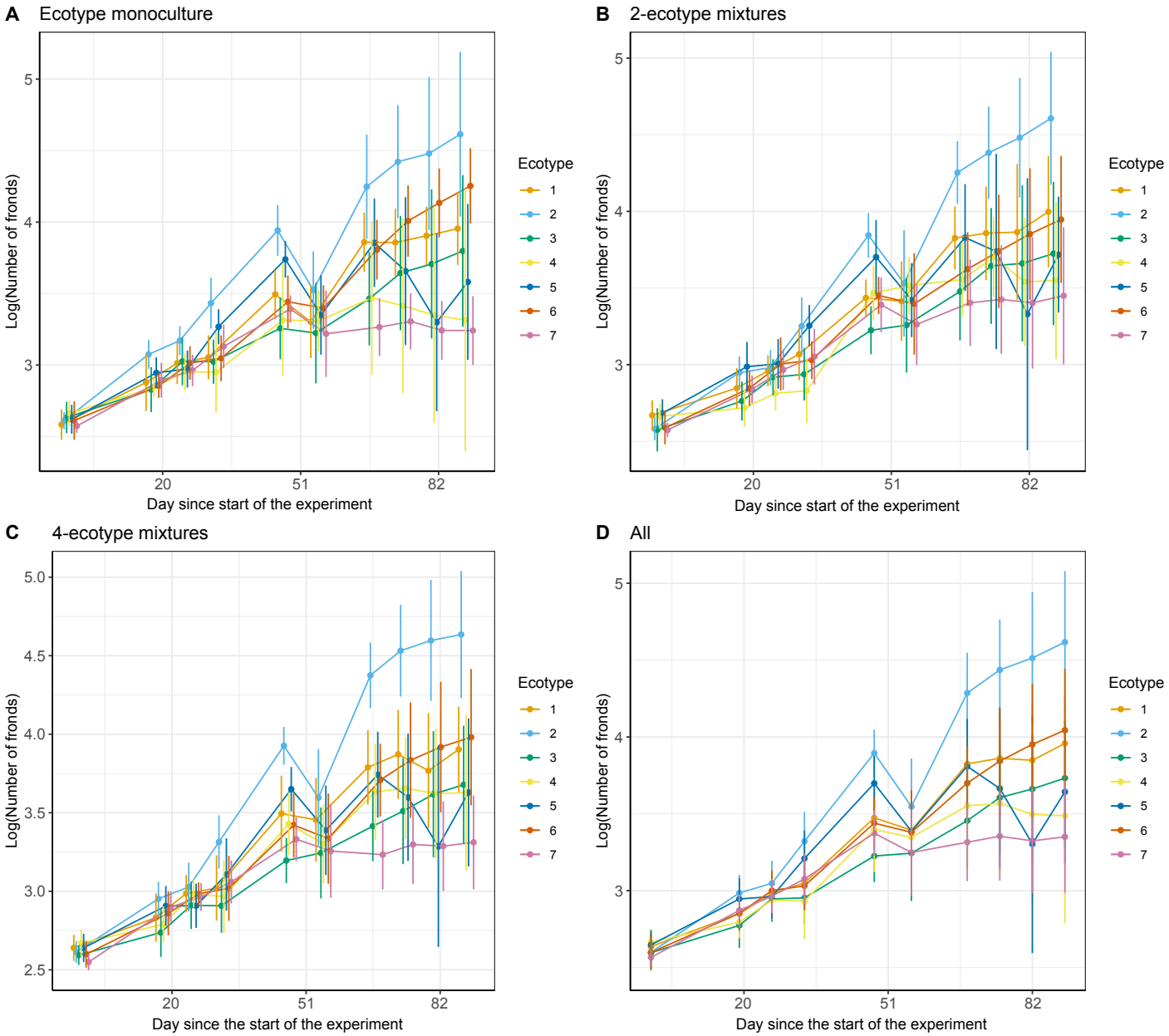

589 **Figure 1:** Population growth (log-transformed number of fronds) during phase 1. **a** Ecotype
590 monoculture, **b** 2-ecotype polyculture, **c** 4-ecotype polyculture and **d** across all three diversity
591 levels. Shown are means and standard errors. Fronds were counted based in image analysis
592 using the counter function in ImageJ.

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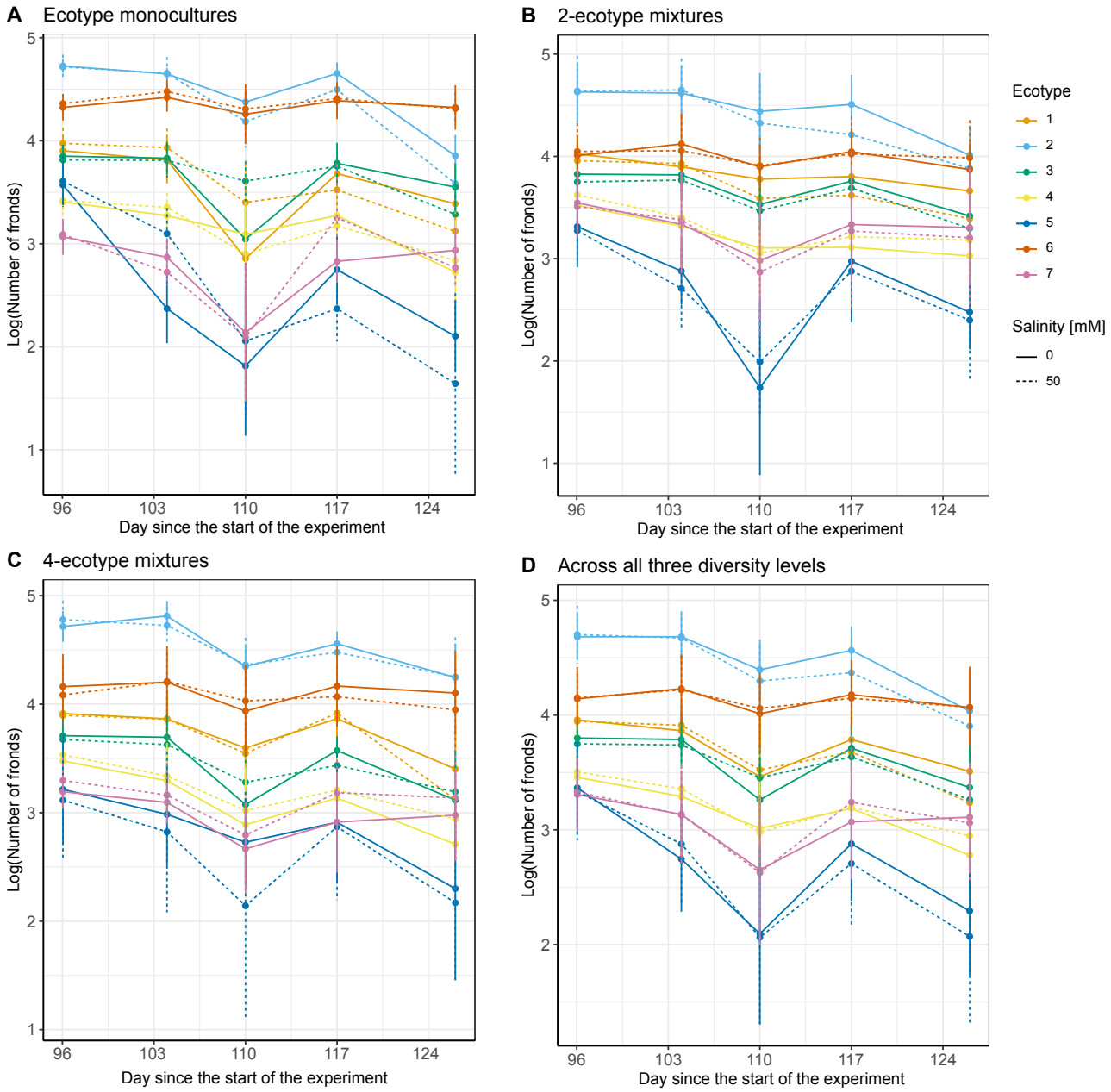


595 **Figure 2:** Population growth (log-transformed number of fronds) during phase 2. **a** Ecotype
596 monoculture, **b** 2-ecotype mixtures, **c** 4-ecotype mixtures and **d** across all three diversity
597 levels. Shown are means and standard errors. Note that abundances declined due to a
598 secondary stressor induced by algal biofilms in all experimental cultures. Salt addition did not
599 significantly decrease population growth (Table S5).
600
601

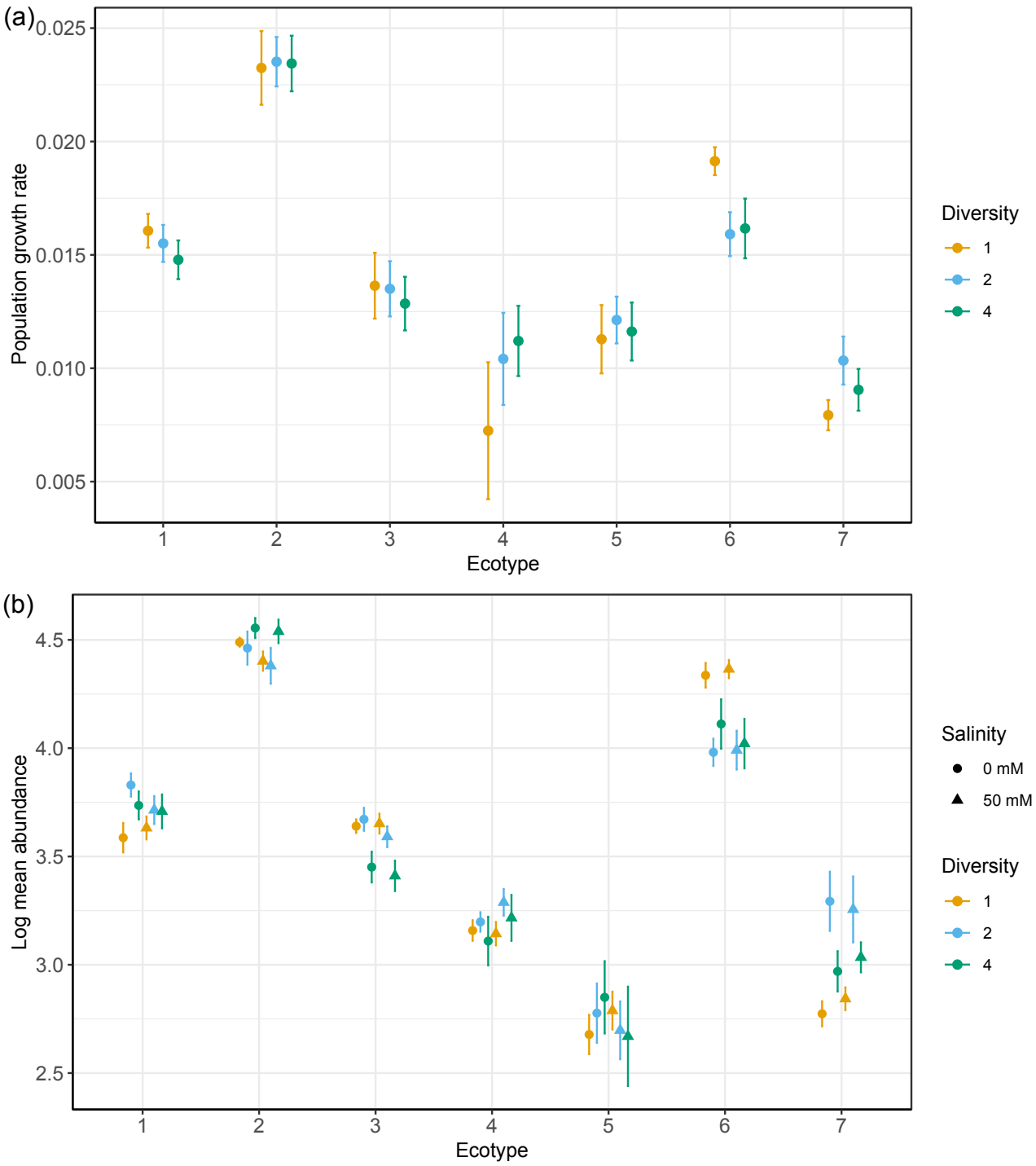
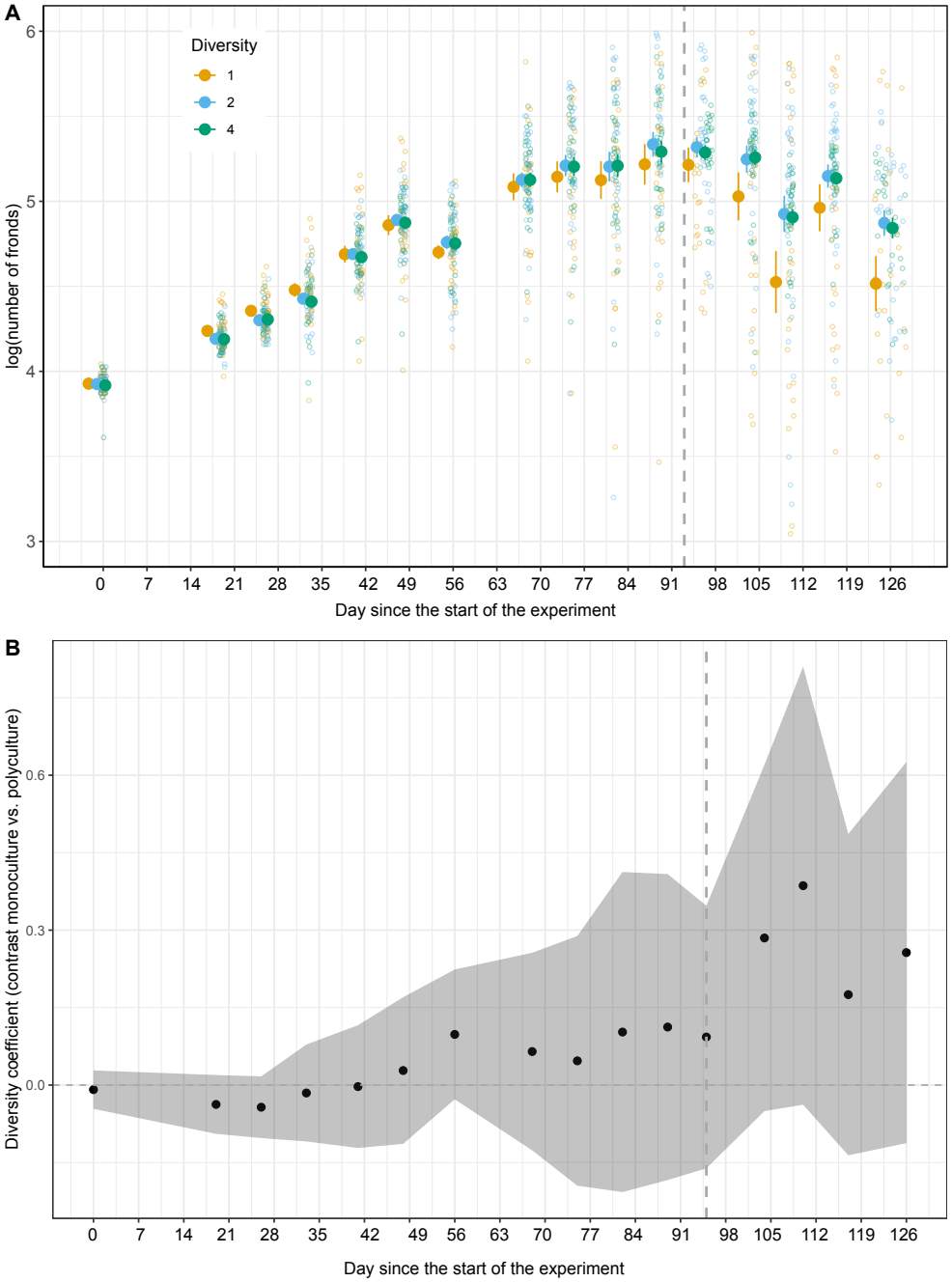


Figure 3: (**a)** Growth rates across phase 1. Shown are means and associated standard errors across cultures per diversity level. (**b)** Log-transformed mean abundance across phase 2 in the presence of salt (triangles) and in the absence of salt (round points). Shown are means and associated standard errors across cultures per diversity level and salinity treatment. For associated ANOVA test statistics see table S5.



608
609 **Figure 4: a** Community abundance (log-transformed number of individual duckweed fronds)
610 over time for each diversity level. The start of phase 2 (i.e., the addition of 50mM of NaCl to
611 half of the cultures) is indicated with a vertical dashed line. Shown are means and associated
612 standard errors per sampling time point (n=14) and diversity (n= 28 for monocultures, n = 36
613 for 2-ecotype polycultures, n= 28 for 4-ecotype monocultures, total n= 92) . Ecotype
614 monocultures: orange; 2-ecotype polycultures: blue; 4-ecotype polycultures: green. For
615 corresponding test statistics see Tables 1 and 2. **b** Model coefficients for the contrast between
616 monocultures and polycultures from a linear-mixed model including salinity as fixed-effect
617 factor and group as random-effect factor for each time point. The horizontal dashed line at 0
618 indicates that there is no diversity effect. Below 0, diversity had a negative impact on
619 community abundance (in the beginning of the experiment), above 0, diversity positively
620 influenced community abundance (from the middle to the end of the experiment). Shown are

621 the model estimates the 95% confidence intervals as shaded areas. The start of phase 2 (i.e.,
622 the addition of 50 mM of NaCl to half of the cultures) is indicated with a vertical dashed line.
623

Figure 5: Algal stress (biofilm score) on day 110, two weeks after the addition of salt. (**a**) Influence of each ecotype on the biofilm formation. (**b**) Influence of the salinity treatment on the biofilm. (c) Effect of the biofilm score on the mean abundance of each ecotype on day 110. Note that for ecotypes 3, 6 and 7, the biofilm score 4 was never given. Shown are means and associated standard errors. Associated test statistics are presented in the Supplementary Information (Table S6 and Table S7).