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The ghost of disturbance past: long-term effects of pulse disturbances on community biomass and composition

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1 **Abstract**

2 Current global change is associated with an increase in disturbance frequency and intensity,
3 with the potential to trigger population collapses and to cause permanent transitions to new
4 ecosystem states. However, our understanding of ecosystem responses to disturbances is still
5 incomplete. Specifically, there is a mismatch between the diversity of disturbance regimes
6 experienced by ecosystems and the one-dimensional description of disturbances used in most
7 studies on ecological stability. To fill this gap, we conducted a full factorial experiment on
8 microbial communities, where we varied the frequency and intensity of disturbances affecting
9 species mortality, resulting in twenty different disturbance regimes. We explored the direct and
10 long-term effects of these disturbance regimes on community biomass. While most
11 communities were able to recover biomass and composition states similar to undisturbed
12 controls after a halt of the disturbances, we identified some disturbance thresholds that had
13 long-lasting legacies on communities. Using a model based on logistic growth, we identified
14 qualitatively the sets of disturbance frequency and intensity that had equivalent long-term
15 negative impacts on experimental communities. Our results show that an increase in disturbance
16 intensity is a bigger threat for biodiversity and biomass recovery than the occurrence of more
17 frequent but less intense disturbances.

18

19 **Keywords:** perturbations, extreme events, resistance, recovery, protist communities,
20 ecosystem functioning.

21 INTRODUCTION

22 Understanding the response of ecological systems to disturbances is a long-standing
23 goal in ecological research [1–5]. Important progress has been made in identifying the structural
24 properties of communities, such as species richness [6], interaction types [7,8] or network
25 structure [9–12], that influence their capacity to resist and recover from a disturbance. The
26 multidimensional nature of community stability is now well recognized [13–15], and several
27 complementary measures of stability have been proposed to capture its different aspects, such
28 as resistance, recovery or temporal variability [16–18]. Surprisingly, however, the equally
29 diverse and multidimensional nature of environmental disturbances, which vary in their
30 intensity, frequency, duration and spatial extent, has received much less attention [19].

31 Indeed, most of the theoretical studies on ecological stability compared the stability of
32 communities along a gradient of biological complexity (e.g. number of species, number and
33 type of interactions), but focused on community responses to one type of disturbance only,
34 which is in sharp contrast with the diversity of disturbance regimes that ecosystems are
35 experiencing in nature [4,20,21]. Although the effect of varying disturbance intensity or
36 frequency on communities has been intensively studied empirically (e.g. [22–25]), Donohue et
37 al. [19] showed in a review that 83% of theoretical studies and 80% of experimental and
38 observational studies on stability focused on one single disturbance component. Exploring the
39 multidimensional nature of disturbances is even more critical in the context of global change,
40 where disturbances are increasing worldwide, both in frequency and intensity, and may trigger
41 permanent transitions to new ecosystem states [26–28].

42 Many studies have demonstrated that disturbances, such as fire and flooding, decrease
43 species richness and functional diversity [4,25,29]. However, this relationship may not be linear
44 [24], and we still do not know how a community will resist and recover from disturbances of
45 varying intensity or frequency. Disturbances can have a twofold effect: on the one hand, they

46 can affect communities only temporarily, such that they will go back to their pre-disturbance
47 state if the disturbances are stopped or mitigated [3,30,31]. On the other hand, they can trigger
48 irreversible population collapses, leading in turn to long-term changes in community
49 composition and biomass [32–34]. We hypothesize that such ghost of disturbance past, by
50 analogy with Connell’s ghost of competition past [35], may be more likely induced by
51 disturbances of very high intensity, but may also depend on specific combinations of
52 disturbance frequency and intensity. For example, communities may recover from disturbances
53 of intermediate intensity if they remain infrequent but will not recover if their frequency
54 increases.

55 While we have identified the need for studying different disturbance dimensions (and
56 their interactions) on community stability, this is notoriously difficult in natural ecosystems for
57 both logistic and ethical reasons. Thus, many studies in disturbance ecology only investigate a
58 small subset of a disturbance dimension (e.g. [36–38]). Experimental work is therefore
59 particularly relevant to get highly replicated and factorially studied data on disturbances.
60 Microbial communities provide a unique opportunity to study the effect of a large diversity of
61 disturbance regimes on community stability. The same community can be replicated easily over
62 a wide number of microcosms that will experience different disturbance regimes [39], and allow
63 the study of disturbances over many generations within days to weeks. This approach allows to
64 have replicates for each treatment as well as a reference state, that is, undisturbed communities,
65 which is essential to calculate most stability dimensions, such as resistance and recovery. This
66 reference state is extremely difficult to assess in the field because of inherent environmental
67 fluctuations and the slower generation time of some organisms that require long field surveys
68 (often years to decades) to observe full recovery.

69 Here, we experimentally investigated the effect of repeated pulse disturbances of
70 varying frequency and intensity, affecting the abundances of all species in a similar way, on the

71 biomass and composition of a community of freshwater protozoans. We focused on disturbance
72 frequency and intensity for two reasons: (i) these two disturbance dimensions are both expected
73 to increase with global change and (ii) they are experimentally tractable and easier to
74 manipulate than other dimensions (e.g. disturbance spatial extent). We specifically focused on
75 two major components of community stability [40]: (i) the capacity of the community to resist
76 to a disturbance regime (i.e. resistance, or direct effect of disturbances) and (ii) the capacity of
77 the community to recover from a disturbance regime when the disturbances stopped (i.e.
78 recovery, or legacy effect of disturbances). Using an analytical model based on logistic growth
79 [41,42], we then investigated which disturbance regimes are expected to have equivalent long-
80 lasting legacies on communities and compared these expectations to the experimental results.

81

82 MATERIALS AND METHODS

83 (a) Microbial community

84 We conducted an experiment on an aquatic community composed of 12 protozoan species,
85 one rotifer species and a set of common freshwater bacteria (*Serratia fonticola*, *Bacillus subtilis*
86 and *Brevibacillus brevis*) as a food resource [39]. Bacteria, in turn were supported on a plant-
87 based nutrient medium (pre-autoclaved standard protist pellet suspension filtered through
88 Whatman filters (0.31 g protist pellets in 1 l of tap water). The 12 protozoan species were
89 *Blepharisma* sp., *Chilomonas* sp., *Chlorogonium euchlorum*, *Colpidium* sp., *Cyclidium* sp.,
90 *Euglena gracilis*, *Euplotes aediculatus*, *Loxocephalus* sp., *Paramecium aurelia*, *Paramecium*
91 *caudatum*, *Spirostomum* sp., and *Tetrahymena* sp., and the rotifer was *Cephalodella* sp.
92 (subsequently all 13 are referred to as “protists”). All of these species are bacterivores, whereas
93 *C. euchlorum*, *E. gracilis* and *E. aediculatus* can also photosynthesize. Furthermore,
94 *Blepharisma* sp., *Euplotes aediculatus*, and *Spirostomum* sp. may not only feed on bacteria but
95 can also predate on smaller protists (see table S1 for more information on the species). Another

96 angle of this experiment, namely the effect of pulsed disturbances on size-abundance pyramids
97 during the first phase of the experiment, has already been analysed in Jacquet et al. [42]. Here,
98 in addition to their direct effect, we investigated the long-term legacy of the disturbance
99 regimes, that is, after a halt of the disturbances, on community composition, species richness
100 and total community biomass.

101

102 **(b) Disturbance experiment**

103 We performed a factorial experiment in which we varied the frequency and intensity of
104 pulse disturbances affecting species density, resulting in a total of twenty different disturbance
105 regimes. A pulse disturbance was achieved by boiling a subsampled fraction of the well-mixed
106 community in a microwave at 800 W that killed all living protists (see also [41–43]). The
107 disturbances were therefore density independent, as all species experienced the same level of
108 density reduction. Afterwards, the medium was cooled down to room temperature and was
109 given back to the microcosm within 45 minutes. By doing so, we kept the composition of the
110 microcosm constant and avoided nutrient addition or loss. This procedure mimics disturbances
111 such as fire and flooding, which initially reduce population density but may also enhance the
112 regeneration of nutrients [25]. We disturbed microcosms at five intensities: $I = 10, 30, 50, 70$
113 and 90 % and at four frequencies: $f = 0.08, 0.11, 0.17$ and 0.33, corresponding to a disturbance
114 every 12, 9, 6 and 3 days, respectively. Each factorial treatment combination was replicated 6
115 times, giving in total 120 replicates. We additionally cultured 8 control microcosms in an
116 undisturbed environment under the same conditions to define a reference community state. The
117 disturbance experiment lasted for 21 days, or 10–50 generations depending on species (table
118 S1). One additional measurement was taken 39 days after the onset of the disturbance
119 experiment in order to estimate the legacy effect of disturbance regimes on community biomass
120 and composition, that is 20 and 26 days after the last disturbance event happened for frequencies

121 $f = 0.11, 0.17, 0.33$, and frequency $f = 0.08$ respectively. The populations in the microcosms
122 experiencing the lowest frequency ($f = 0.08$) had therefore 6 more days to recover compared to
123 other microcosms, or 3–14 generations depending on species.

124

125 (c) Microcosm description

126 Each replicate consisted of a 250 ml Schott bottle filled with nutrient medium to 100 ml.
127 The microcosms were assembled by first filling each Schott bottle with 30 ml of pre-autoclaved
128 standard protist pellet suspension filtered through Whatman filters (0.31 g protist pellets in 1 l
129 of tap water, Carolina Biological Supply, Burlington NC, USA), and 5 ml of a bacteria solution
130 composed of three species (*Serratia fonticola*, *Bacillus subtilis* and *Brevibacillus brevis*). After
131 24 hours, to allow time for bacterial growth, we added 65 ml of protist solution with each protist
132 species at carrying capacity (5 ml per species). All communities were allowed to grow for 1
133 week before disturbance treatments started to be implemented. General lab procedures follow
134 the protocols described in Altermatt et al. [39], and build upon previous work on the effect of
135 pulse disturbances on diversity [41,44], size-abundance pyramids [42], and invasion dynamics
136 [45].

137

138 (d) Sampling

139 We sampled 0.2 ml of the well-mixed microcosms daily to quantify total community
140 biomass (i.e. total bioarea in $\mu\text{m}^2/\mu\text{l}$) using a standardized video procedure [39,46]. In brief, a
141 constant volume (14.9 μl) of each sample was placed under a dissecting microscope connected
142 to a camera and a computer for the recording of videos (4 s per video, that is 100 video frames).
143 Then, using image processing software (IMAGEJ, National Institute of Health, USA) and the
144 R-package *bemovi* [47], we extracted the number of moving organisms per video frame and the
145 size of each individual (mean cell area in μm^2). We estimated community biomass as the sum

146 area of all individuals averaged by video frames, assuming proportionality between area and
147 mass. Other traits, such as organisms' speed and shape, were used to filter out background
148 movement noise (e.g. particles from the medium). Finally, we assessed manually the presence
149 or absence of each protist species at $t = 39$ (i.e. visual analysis of the videos) in order to
150 determine the composition and species richness of each microcosm at the end of the experiment.
151 This visual assessment of species identity is well-established and known to be precise [46].

152

153 **(e) Statistical analyses**

154 We tested for the direct effect of the disturbance regimes on average community biomass
155 (i.e. total bioarea in $\mu\text{m}^2/\mu\text{l}$), which was calculated over 21 time points for each microcosm. We
156 performed Welch two-sample t-tests (null hypothesis: average community biomass in a given
157 treatment (6 replicates, temporal mean) is not different from average community biomass in the
158 controls (8 replicates, temporal mean)). Similarly, we tested for the legacy effect of the
159 disturbance regimes on community biomass ($\mu\text{m}^2/\mu\text{l}$), species richness and species occurrences
160 (i.e. proportion of replicates in which the species was present). For each variable and
161 disturbance regime, we performed a Welch two-sample t-test (null hypothesis: the average
162 value in the treatment (6 replicates at $t = 39$) is not different from the average value in the
163 controls (8 replicates at $t = 39$). All p-values were adjusted for multiple testing using the
164 Benjamini-Hochberg correction [48], which controls for false discovery rate (i.e. the expected
165 proportion of false discoveries amongst the rejected hypotheses). To illustrate the direct and
166 legacy effects of varying disturbance regimes on community biomass, we computed the
167 difference between log average biomass in the treatments and log average biomass in the
168 controls.

169

170 **(f) Theoretical expectations**

171 We used an analytical model inspired from harvesting theory and derived in Harvey et al. [41]
172 to make theoretical expectations regarding the sets of disturbance frequency and intensity that
173 should have equivalent long-term effects on community properties. This model has been
174 initially used in a meta-ecosystem context to predict the amount of detritus produced in a
175 community experiencing different scenarios of disturbance frequency and intensity [41]. It has
176 been combined recently to the metabolic theory of ecology to predict the effect of varying
177 disturbance regimes on size-abundance scaling relationships [42]. The model states that a
178 population following a logistic growth can persist in a disturbed environment only if its growth
179 rate balances the long-term effect of the disturbance regime, that is:

$$180 \quad r > - \frac{\ln(1-I)}{T} \quad (1)$$

181 where r is population growth rate (mass/time), I is disturbance intensity (fraction of mass) and
182 $T=1/f$ is the time between two disturbances (time). From inequality (1), one can predict the set
183 of disturbance regimes a population can sustain according to its growth rate.

184 Another important prediction of inequality (1) is that different combinations of
185 disturbance frequency and intensity will have equivalent effects on effective population growth
186 rate. Therefore, the right-hand side of inequality (1) can be used as a proxy for disturbance
187 regime strength, which allows to classify varying combinations of disturbance frequency and
188 intensity according to their expected effect on effective population growth rate. Here, we
189 extended inequality (1) to assemblages of co-occurring species and assumed that all species'
190 populations follow a logistic growth and are constrained by intraspecific competition only. We
191 formulated the following theoretical expectation: the disturbance regimes that have equivalent
192 effects on effective population growth rate should have equivalent legacy effects on community
193 composition and species richness as well. We then compared the ranking of the disturbance
194 regimes regarding their expected effect on effective population growth rate to the legacy effect

195 of the disturbance regimes on species richness, composition and biomass of experimental
196 communities.

197

198 **RESULTS**

199 **(a) Direct versus legacy effects of disturbances on community biomass**

200 The experiment resulted in a set of time series showing the dynamics of community biomass
201 in response to varying combinations of disturbance frequency and intensity, creating a two-
202 dimensional landscape of disturbance regimes (figure 1 and figure S1). The temporal variability
203 of community biomass in the controls is illustrated in figure 1 (in grey), which may be related
204 to species extinctions or changes in nutrient concentration. High disturbance intensity ($I = 90\%$)
205 and frequency ($f = 0.33$), as well as low disturbance frequency ($f = 0.08$) had a significant direct
206 effect on communities by decreasing community biomass during the disturbance experiment
207 (figure 2a and table S2). Conversely, community biomass did not differ significantly from
208 undisturbed communities in intermediate disturbance regimes (e.g. $I = 30\%$). The disturbance
209 regimes with the lowest frequency ($f = 0.08$) did not have a significant legacy effect, that is,
210 twenty-six days after the last disturbance event (figure 2b and table S2). However, we observed
211 a “ghost of past disturbances” after reaching a certain disturbance threshold: community
212 biomass was significantly lower than controls for intensity $I = 90\%$ with frequency $f = 0.11$,
213 0.17 , 0.33 and $I = 70\%$ with $f = 0.33$. Note that these disturbances regimes were also the ones
214 having the strongest direct effect on community biomass (figure 2a). Finally, one disturbance
215 regime of intermediate strength, that is $I = 30\%$ with $f = 0.33$, had a significant positive legacy
216 effect on community biomass. Other disturbance regimes of intermediate strength had a positive
217 effect of community biomass, that is $I = 50\%$ with $f = 0.17$ and $I = 70\%$ with $f = 0.11$ (figure
218 2b, blue squares). However, the legacy effect of these treatments was extremely variable among

219 replicates (see error bars in figure 1) and community biomass was therefore not significantly
220 different from the controls according to the Welch two-sample t-tests (table S2).

221

222 **(b) Legacy effect of disturbances on species richness and community composition**

223 Of the initially 13 protist species, not all persisted until the end of the experiment in the
224 control communities. Competitive exclusion or ecological drift resulted in communities
225 composed of a maximum of eight species. Indeed, five species could not be detected in any
226 control communities at the end of the experiment, namely *Cyclidium* sp., *Chlorogonium*
227 *euchlorum*, *Loxocephalus* sp., *Spirostomum* sp., and *Tetrahymena* sp. Additionally, *Colpidium*
228 sp., *Paramecium caudatum* and *Paramecium aurelia* could not be reliably differentiated. We
229 therefore merged and treated them as one single species. Consequently, average species
230 richness in the control communities was $S = 4.88 \pm 1.13$, with *Euglena gracilis* and *Euplotes*
231 *aediculatus* being systematically present (see table S3 for detailed average species presences
232 over the eight control communities).

233 We used these reference communities to assess the legacy effect of disturbances on species
234 richness and community composition (see table S4 for detailed species presence/absence in the
235 128 microcosms). A significant legacy effect of disturbances on species richness was only
236 found in the four strongest disturbance regimes (i.e. $I = 90\%$ with $f = 0.11, 0.17$ and 0.33 and I
237 $= 70\%$ with $f = 0.33$), which matched the disturbance thresholds found for community biomass
238 (figure 3 and table S2). These disturbance regimes not only led to a long-term change in species
239 richness but also to a clear switch in the functional composition of the species present (figure
240 4). While most of the disturbed communities were composed of one predatory species
241 (*Blepharisma* sp.) and two autotrophic species (*Euglena gracilis* and *Euplotes aediculatus*),
242 communities that experienced the strongest disturbance regimes (i.e. $I = 90\%$ with $f \geq 0.11$ and
243 $f = 0.33$ with $I \geq 70\%$) were composed of bacterivorous species only. Interestingly, the

244 occurrence of *Chilomonas* sp. was significantly higher in communities that experienced these
245 disturbance regimes, which was strongly correlated to the absence of *Blepharisma* sp. and
246 *Euglena gracilis*, suggesting a disruption of competitive exclusion (figure 4 and table S5).

247

248 (c) Theoretical equivalences between disturbance regimes

249 The proxy for disturbance regime strength (right-hand side of inequality 1) outlined two
250 important points that were consistent with the experimental results. First, it illustrated how
251 community response to increasing disturbance intensity is expected to be nonlinear, with
252 relatively small effects of low intensities compared to stronger ones (figure 5). The disturbance
253 regimes that had the strongest effect on effective population growth rate according to the model
254 were also the ones having a significant long-term impact on the species richness, biomass and
255 composition of the experimental communities. Specifically, the disturbance regimes with a
256 strength above 0.25 day^{-1} (figure 5), that is $I = 90\%$ with $f = 0.33, 0.17$ or 0.11 and $I = 70\%$ with
257 $f = 0.33$, led to novel communities, characterized by a lower biomass and species richness, as
258 well as a different functional composition dominated by bacterivorous species (figures 2–4).

259 Second, the proxy for disturbance regime strength (right-hand side of inequality 1)
260 highlighted the existence of equivalent combinations of disturbance frequency and intensity
261 regarding their impact on population dynamics such as $I = 90\%$ with $f = 0.17$ and $I = 70\%$ with
262 $f = 0.33$, which had equivalent legacy effects on the experimental communities as well (fig. 2b).
263 Similarly, the disturbance regimes of intermediate strength that had a positive legacy effect on
264 community biomass in the experiment, that is $I = 30\%$ with $f = 0.33$, $I = 50\%$ with $f = 0.17$, I
265 $= 70\%$ with $f = 0.11$, were also expected to have equivalent effects on communities according
266 to the model.

267 We also observed some discrepancies between the theoretical expectations and the
268 experimental results. Indeed, the positive effect of the disturbance regimes with intermediate

269 strength on community biomass compared to very mild regimes could not be predicted by a
270 model based on population growth rate only. Moreover, the model provided the expectation
271 that the disturbance regime $I = 90\%$ with $f = 0.33$ would have the strongest effect on
272 communities. However, the legacy effect of the latter regime was not significantly stronger than
273 $I = 90\%$ with $f = 0.17$ or 0.11 . Nonetheless, the experimental results were qualitatively
274 consistent with the theoretical identification of the four strongest disturbance regimes.

275

276 **DISCUSSION**

277 We experimentally investigated the effect of repeated pulse disturbances affecting the
278 abundances of all species in a similar way, on the biomass and composition of a protist
279 community. We specifically studied two dimensions of environmental disturbances and their
280 interactions by varying disturbance frequency and intensity, resulting in twenty different
281 disturbance regimes. While high disturbance intensity ($I = 90\%$) and frequency ($f = 0.33$), as
282 well as low disturbance frequency ($f = 0.08$), had a significant direct effect on community
283 biomass, a long-lasting legacy of disturbances was observed only in the communities exposed
284 to disturbance intensity $I = 90\%$ at all frequencies but the lowest and $I = 70\%$ with $f = 0.33$
285 (highest frequency). This ghost of disturbance past was characterized by communities critically
286 different from the undisturbed ones, with a significantly lower species richness and total
287 biomass. The functional composition of these communities changed as well, such that predatory
288 and autotrophic species were replaced by bacterivorous species.

289 From a theoretical perspective, the model predicted that communities can recover from
290 a wide range of disturbance regimes if the disturbances are stopped or mitigated, but will not
291 recover their initial state once a threshold in disturbance regime strength is reached. This is in
292 accordance with our experimental findings, where most of the communities approached a state
293 similar to the reference state when the disturbance stopped, even if they were significantly

294 impacted during the disturbance events. Above a threshold, however, disturbances had an
295 irreversible effect on community biomass and composition. Hence, we could experimentally (i)
296 demonstrate that the reference state of the protist community is an attractor (or stable state),
297 and (ii) identify the combinations of disturbance frequency and intensity that pushed the
298 community to another state, corresponding to a disturbance strength of 0.25 day^{-1} for this
299 community (right-hand side of inequality 1). We could not assess if the new community state
300 resulting from the disturbance regimes is stable or unstable, as the return of the original species
301 in the communities was prevented in our experiment. However, this could also be determined
302 experimentally, by testing species abilities to recolonize the communities showing a legacy
303 effect of past disturbances [49].

304 The model we used provides a qualitative baseline to determine which disturbance
305 regimes should have equivalent effects on communities. However, this model is based on
306 population growth rate only and cannot be used to determine *a priori* the threshold above which
307 disturbances will have an irreversible impact on community properties. Indeed, the disturbance
308 threshold we observed for the experimental protist community (i.e., disturbance strength of 0.25
309 day^{-1} , inequality 1) emerges from the dynamics of interacting species that compete or predate
310 on each other. Hence, a promising way forward would be the integration of the effect of species
311 interactions to the model in order to determine communities' disturbance thresholds. In a recent
312 study, Arnoldi et al. [50] showed that the effective growth rates in a community of interacting
313 species correspond to $r^* = rN^*/K$ where r is species intrinsic growth rate, N^* is the equilibrium
314 abundance within the community and K is carrying capacity (i.e. abundance in monocultures).
315 This result has direct implications for the response of competitive communities to disturbances.
316 Indeed, by replacing r by r^* in equation (1), we can demonstrate that, for similar growth rates,
317 species with low competitive abilities (i.e. low N^*/K), will be more sensitive to a given
318 disturbance regime than strong competitors (i.e. high N^*/K).

319 Extending the model to communities of interacting species would also help understand
320 under which conditions disturbance regimes of intermediate strength can have a positive effect
321 on community biomass. Indeed, the experimental observation of higher community biomass
322 for intermediate disturbance regimes compared to milder disturbances could not be predicted
323 by the model in its current form. This result echoes the extensive literature on the intermediate
324 disturbance hypothesis, which proposes that species richness will be highest at intermediate
325 levels of disturbance [51–54]. However, the effect we observed in our experiment is distinct
326 from a diversity-disturbance relationship. Indeed, the positive effect of intermediate levels of
327 disturbance was observed on community biomass only, not on species richness. Furthermore,
328 the positive effect of intermediate levels of disturbance on community biomass was only
329 observed on the long-term, that is, after the halt of the disturbances, not during the disturbance
330 experiment.

331 The competitive abilities of most of the protist species we used in our experiment have
332 been extensively studied in previous works [55,56] and explain well the composition of the
333 microcosms at the end of the experiment. Indeed, the five species that went systematically
334 extinct in the control communities (i.e. *Cyclidium* sp., *Chlorogonium euchlorum*, *Loxocephalus*
335 sp., *Spirostomum* sp., and *Tetrahymena* sp.) have low competitive abilities relative to other
336 protist species [55]. Second, the two species that were systematically present in the control
337 communities, that is *Euglena gracilis* and *Euplotes aediculatus*, are strong competitors and
338 significantly decreased the N^*/K of *Chilomonas* sp. in a relative yield experiment [55].
339 Interestingly, we observed the combined negative effects of competitive interactions and
340 disturbances on the occurrence of *Chilomonas* sp. at low disturbance intensity (i.e. $I = 10\%$ and
341 30% , figure 4) as predicted by the theory [50]. At high disturbance intensity, however,
342 *Chilomonas* sp. could persist due to its high growth rate and significantly benefited from the
343 extinction of its competitors or predators (figure 5 and table S5).

344 The change in community functional composition in response to strong disturbances we
345 reported, where predatory and autotrophic species were replaced by bacterivorous species,
346 would deserve further investigations. The explanation we proposed is based on observed
347 growth rate differences between species. However, species vulnerability to disturbances may
348 also be linked to their functional role, with a higher resistance of bacterivorous species, such as
349 *Chilomonas* sp., compared to predatory or autotrophic species, such as *Blepharisma* sp. and
350 *Euglena gracilis*. Given that we cannot causally separate effects of growth rate differences and
351 functional groups, we suggest that further experiments on communities showing no correlations
352 between species growth rate and trophic groups would be helpful to generalize the response of
353 community functional composition to disturbances.

354 In order to explore the effect of disturbance frequency and intensity on community
355 recovery, we made several choices regarding other aspects of disturbances that may limit the
356 transferability of our results to other disturbance types. First, the experiment explored the
357 effects of repeated pulse disturbances that punctually decreased population density, in contrast
358 to press perturbations that correspond to a sustained alteration of species density [31].
359 Nonetheless, previous studies have shown that equation (1) can be easily adapted to the study
360 of press disturbances that affect effective population growth rate in a continuous way [42,52].
361 Then, similar effects on communities would be expected for this type of press disturbances.
362 Second, the disturbances affected all species in a similar way, with the same fraction of
363 population biomass being removed from the system. However, some species might be more
364 resistant than others to environmental disturbances, which can influence the recovery dynamic
365 of the whole community [50]. Similarly, some disturbances can only affect a subset of the
366 community, such as size-selective harvesting [57]. Third, as protist microcosms are closed
367 systems, we did not address here the recovery mechanisms related to species recolonization
368 from neighbour communities nor the spatial extent of disturbances, which is another important

369 dimension to consider to predict the legacy effects of disturbances on communities
370 [19,22,44,58,59].

371 We showed both theoretically and experimentally how different combinations of
372 disturbance frequency and intensity can have equivalent legacy effects on community total
373 biomass and species richness. Indeed, the combinations of disturbance frequency and intensity
374 that had negative legacy effects on the experimental communities corresponded to the
375 disturbance regimes expected to have the strongest impacts. This finding can be used to
376 generalize empirical studies that only tracked data for one disturbance regime to equivalent
377 combinations of disturbance frequency and intensity. Finally, our results outline the
378 disproportional consequences of high disturbance intensity due to the nonlinear relationship
379 between disturbance regime strength and disturbance intensity. We therefore expect that legacy
380 effects of disturbances on communities, triggered by repeated pulse disturbances affecting all
381 species in a similar way, are more likely to be correlated to an increase in the intensity of
382 extreme events rather than on the increase in the frequency of small to intermediate
383 disturbances.

384 **Data accessibility.** The data supporting the experimental results are archived in the Dryad
385 Digital Repository: <https://doi.org/10.5061/dryad.zkh189378>.

386 **Author contributions.** CJ and FA designed research, CJ conducted the experimental research,
387 analysed the data and wrote the first draft of the manuscript. CJ and FA both contributed to the
388 edition of the paper.

389 **Competing interests.** We declare we have no competing interests.

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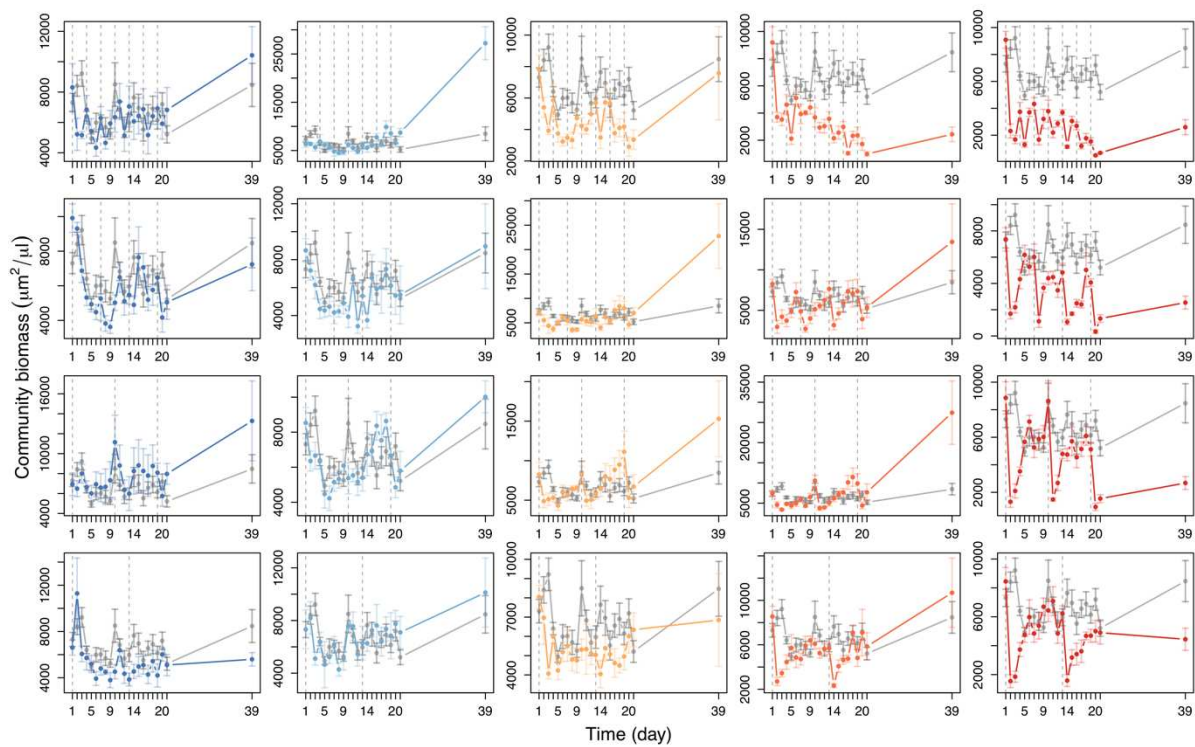
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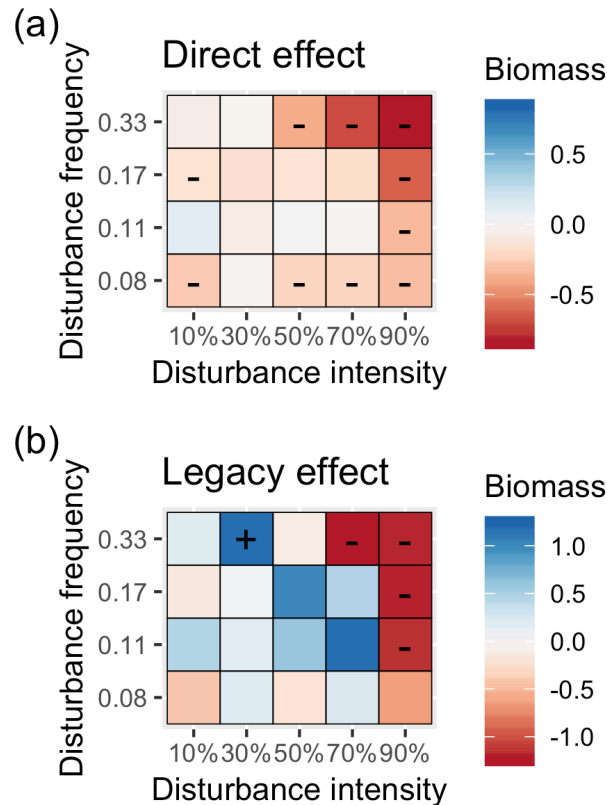
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547 **FIGURE LEGEND**

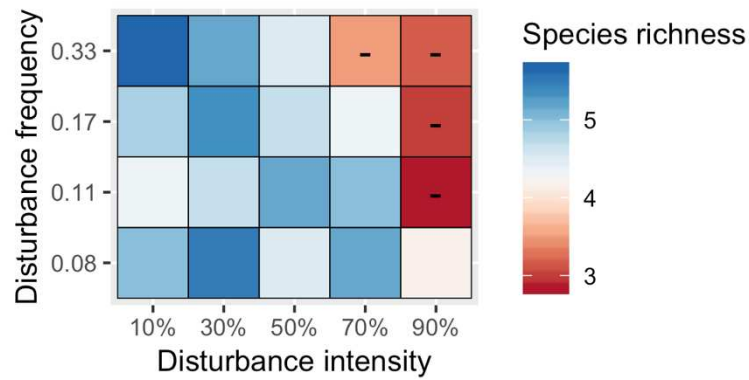
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549 **Figure 1.** Temporal dynamics of community biomass (approximated by total bioarea of
 550 organisms, in $\mu\text{m}^2/\mu\text{l}$) for different combinations of disturbance frequency and intensity
 551 (coloured lines) compared to undisturbed controls (grey lines). Rows depict a gradient of
 552 disturbance frequency (panels from bottom to top: $f = 0.08, 0.11, 0.17$ and 0.33 respectively),
 553 while columns and the corresponding colour gradient correspond to a gradient of disturbance
 554 intensity (panels from left to right: $I = 10, 30, 50, 70,$ and 90% respectively). Points and error
 555 bars correspond to the mean value and \pm s.e. of community biomass for each sampling day over
 556 6 replicates for the treatments (in colour) and 8 replicates for the controls (in grey), respectively.
 557 Dotted vertical lines represent the timing of disturbance events. The last disturbance events
 558 were on day 18, and legacy effects after the halt of disturbances were assessed on day 39. Y-
 559 axes scale differently across panels (see figure S1 for an illustration of biomass temporal
 560 dynamics between $t = 1$ and $t = 21$ with equal ranges of Y-axes across panels).



561

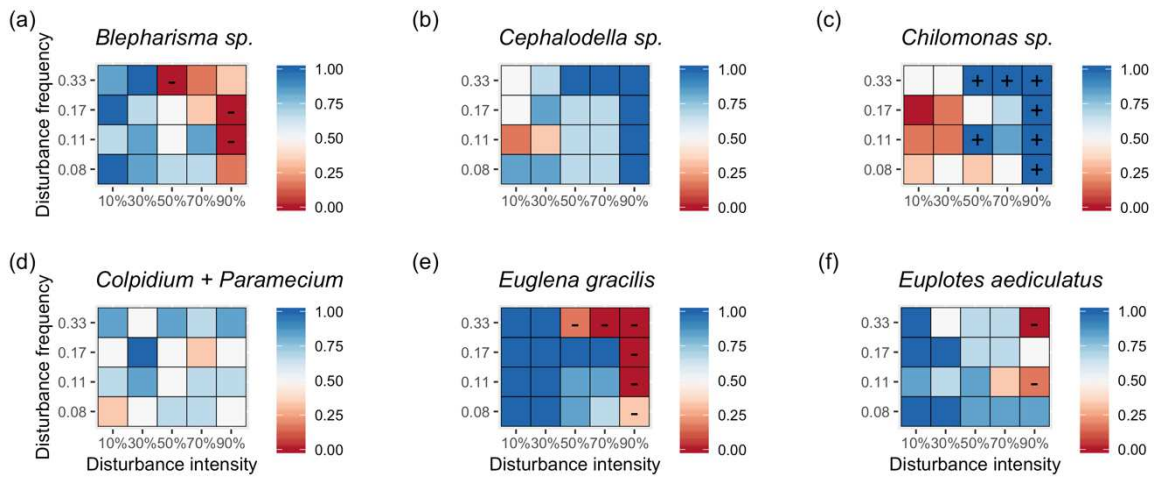
562 **Figure 2.** Direct and legacy effects of varying disturbance regimes on community biomass
 563 compared to undisturbed communities. Each square corresponds to the difference between log
 564 average biomass in the treatments and log average biomass in the controls. Red and blue colours
 565 illustrate a negative or positive difference between treatments and controls respectively. The
 566 colour gradient is centred on zero (i.e. no difference, in white) and its range is defined by the
 567 maximum absolute difference observed between treatments and controls, corresponding to [-
 568 0.85 – 0.85] for direct effects (panel a) and [-1.24 – 1.24] for legacy effects (panel b). Direct
 569 effects correspond to average values observed during the disturbance experiment (21 time
 570 points, 6 replicates per treatments). Legacy effects correspond to average values observed at t
 571 = 39, that is, 20 days after the last disturbance event. A positive or negative sign given in a
 572 square illustrates a significant positive or negative effect of the treatment relative to the control
 573 (p -value < 0.1, individual statistics of Welch two-sample t-tests with p -values corrected for
 574 false discovery rate, are given in table S2).



575

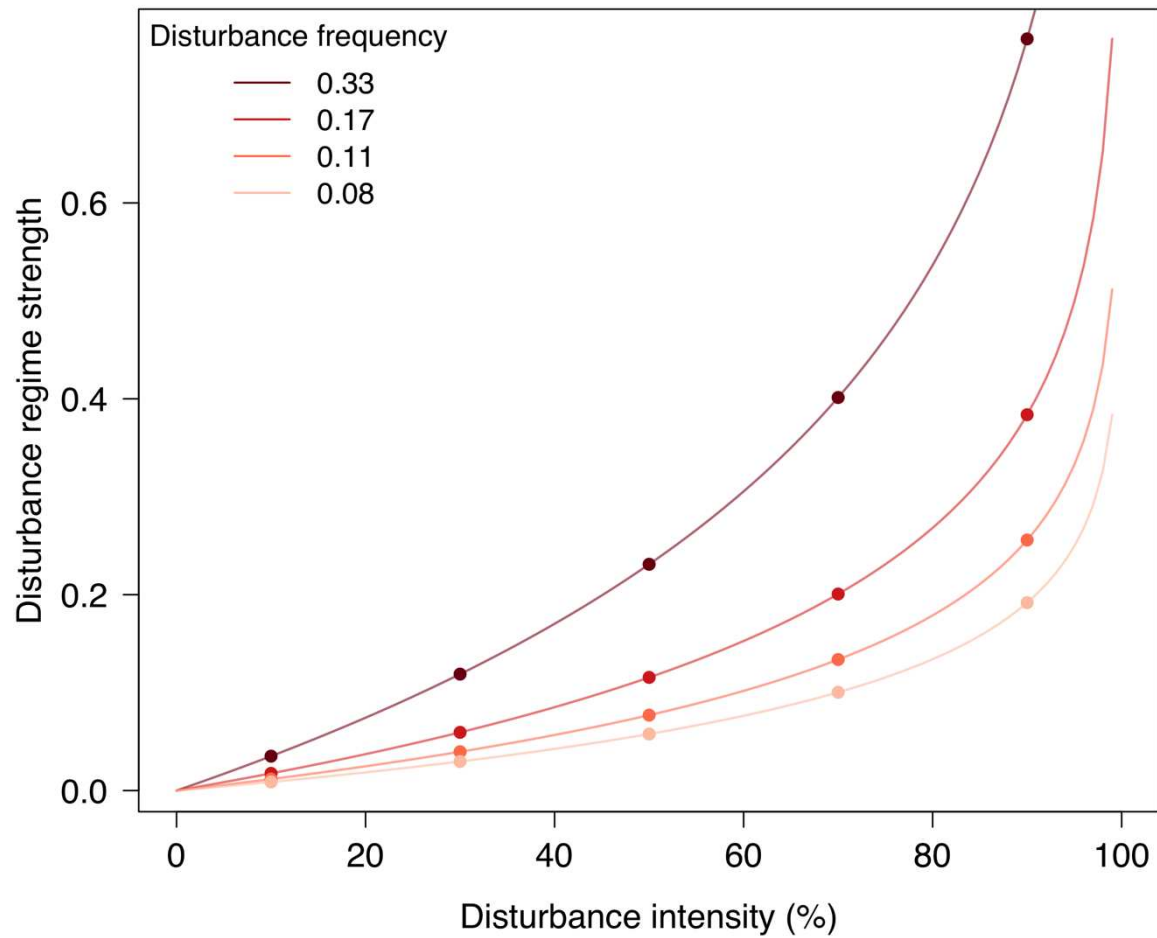
576 **Figure 3.** Legacy effect of disturbance regimes on species richness. Each square corresponds
577 to the average over 6 replicates at $t = 39$. A negative sign given in a square illustrates a
578 significant effect of the treatment relative to the control (p -value < 0.1 , individual statistics of
579 Welch two-sample t-tests with p -values corrected for false discovery rate, are given in table
580 S2).

581



582

583 **Figure 4.** Legacy effects of disturbance regimes on species average presence. Each square
 584 corresponds to the average presence over 6 replicates at $t = 39$. A positive or negative sign in a
 585 square illustrates respectively a significant positive or negative effect of the treatment relative
 586 to the control (p -value < 0.1 , Welch two-sample t -tests with p -values corrected for false
 587 discovery rate).



588

589 **Figure 5.** Disturbance regime strength (right-hand side of inequality 1), corresponding to the
 590 effect of disturbances on effective population growth rate (day^{-1}), for varying combinations of
 591 disturbance frequency (colour gradient) and intensity (x-axis). The points represent the twenty
 592 disturbance regimes performed during the experiment (e.g. $I = 50\%$ with $f = 0.33$ on the line in
 593 brown). From the model, different combinations of disturbance frequency and intensity are
 594 expected to have equivalent effects on species mortality (e.g. $\{I = 30\%; f = 0.33\}$ and $\{I = 70\%$
 595 with $f = 0.11\}$).