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Author for correspondence:

Claire Jacquet

e-mail: claire.jacquet@eawag.ch

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

Claire Jacquet^{1,2} and Florian Altermatt^{1,2}

¹Department of Aquatic Ecology, Swiss Federal Institute of Aquatic Science and Technology, Eawag, Dübendorf, Switzerland

²Department of Evolutionary Biology and Environmental Studies, University of Zurich, Zürich, Switzerland

CJ, 0000-0002-7913-0689; FA, 0000-0002-4831-6958

Current global change is associated with an increase in disturbance frequency and intensity, with the potential to trigger population collapses and to cause permanent transitions to new ecosystem states. However, our understanding of ecosystem responses to disturbances is still incomplete. Specifically, there is a mismatch between the diversity of disturbance regimes experienced by ecosystems and the one-dimensional description of disturbances used in most studies on ecological stability. To fill this gap, we conducted a full factorial experiment on microbial communities, where we varied the frequency and intensity of disturbances affecting species mortality, resulting in 20 different disturbance regimes. We explored the direct and long-term effects of these disturbance regimes on community biomass. While most communities were able to recover biomass and composition states similar to undisturbed controls after a halt of the disturbances, we identified some disturbance thresholds that had long-lasting legacies on communities. Using a model based on logistic growth, we identified qualitatively the sets of disturbance frequency and intensity that had equivalent long-term negative impacts on experimental communities. Our results show that an increase in disturbance intensity is a bigger threat for biodiversity and biomass recovery than the occurrence of more frequent but less intense disturbances.

1. Introduction

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

Indeed, most of the theoretical studies on ecological stability compared the stability of communities along a gradient of biological complexity (e.g. number of species, number and type of interactions), but focused on community responses to one type of disturbance only, which is in sharp contrast to the diversity of disturbance regimes that ecosystems are experiencing in nature [4,20,21]. Although the effect of varying disturbance intensity or frequency on communities has been intensively studied empirically (e.g. [22–25]), Donohue *et al.* [19] showed in a review that 83% of theoretical studies and 80% of experimental and observational studies on stability focused on one single disturbance component. Exploring the multidimensional nature of disturbances is even more critical in the context of global change, where disturbances

are increasing worldwide, in both frequency and intensity, and may trigger permanent transitions to new ecosystem states [26–28].

Many studies have demonstrated that disturbances such as fire and flooding decrease species richness and functional diversity [4,25,29]. However, this relationship may not be linear [24], and we still do not know how a community will resist and recover from disturbances of varying intensity or frequency. Disturbances can have a twofold effect: on the one hand, they can affect communities only temporarily, such that they will go back to their pre-disturbance state if the disturbances are stopped or mitigated [3,30,31]. On the other hand, they can trigger irreversible population collapses, leading in turn to long-term changes in community composition and biomass [32–34]. We hypothesize that such ghosts of disturbance past, by analogy with Connell's ghost of competition past [35], may be more likely to be induced by disturbances of very high intensity, but may also depend on specific combinations of disturbance frequency and intensity. For example, communities may recover from disturbances of intermediate intensity if they remain infrequent, but will not recover if their frequency increases.

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

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

2. Material and methods

(a) Microbial community

We conducted an experiment on an aquatic community composed of 12 protozoan species, one rotifer species and a set of common freshwater bacteria (*Serratia fonticola*, *Bacillus subtilis* and *Brevibacillus brevis*) as a food resource [39]. Bacteria in turn were supported on a plant-based nutrient medium (pre-autoclaved standard protist pellet suspension filtered through Whatman filters; 0.31 g protist pellets in 1 l of tap water). The 12 protozoan species were *Blepharisma* sp., *Chilomonas* sp., *Chlorogonium euchlorum*, *Colpidium* sp., *Cyclidium* sp., *Euglena gracilis*, *Euplotes aediculatus*, *Loxocephalus* sp., *Paramecium aurelia*, *Paramecium caudatum*, *Spirostomum* sp. and *Tetrahymena* sp., and the rotifer was *Cephalodella* sp. (subsequently all 13 are referred to as 'protists'). All of these species are bacterivores, whereas *C. euchlorum*, *E. gracilis* and *E. aediculatus* can also photosynthesize. Furthermore, *Blepharisma* sp., *Euplotes aediculatus* and *Spirostomum* sp. may not only feed on bacteria but can also predate on smaller protists (see electronic supplementary material, table S1 for more information on the species). Another angle of this experiment, namely the effect of pulsed disturbances on size–abundance pyramids during the first phase of the experiment, has already been analysed in a previous paper [42]. Here, in addition to their direct effect, we investigated the long-term legacy of the disturbance regimes, that is, after a halt of the disturbances, on community composition, species richness and total community biomass.

(b) Disturbance experiment

We performed a factorial experiment in which we varied the frequency and intensity of pulse disturbances affecting species density, resulting in a total of 20 different disturbance regimes. A pulse disturbance was achieved by boiling a subsampled fraction of the well-mixed community in a microwave at 800 W that killed all living protists (see also [41–43]). The disturbances were therefore density independent, as all species experienced the same level of density reduction. Afterwards, the medium was cooled down to room temperature and was given back to the microcosm within 45 min. By doing so, we kept the composition of the microcosm constant and avoided nutrient addition or loss. This procedure mimics disturbances such as fire and flooding, which initially reduce population density but may also enhance the regeneration of nutrients [25]. We disturbed microcosms at five intensities ($I = 10, 30, 50, 70$ and 90%) and at four frequencies ($f = 0.08, 0.11, 0.17$ and 0.33 , corresponding to a disturbance every 12, 9, 6 and 3 days, respectively). Each factorial treatment combination was replicated 6 times, giving in total 120 replicates. We additionally cultured 8 control microcosms in an undisturbed environment under the same conditions to define a reference community state. The disturbance experiment lasted for 21 days, or 10–50 generations depending on species (electronic supplementary material, table S1). One additional measurement was taken 39 days after the onset of the disturbance experiment in order to estimate the legacy effect of disturbance regimes on community biomass and composition, that is 20 and 26 days after the last disturbance event happened for frequencies $f = 0.11, 0.17, 0.33$, and frequency $f = 0.08$, respectively. The populations in the microcosms experiencing the lowest frequency ($f = 0.08$) had therefore 6 more days to recover compared with other microcosms, or 3–14 generations depending on species.

(c) Microcosm description

Each replicate consisted of a 250 ml Schott bottle filled with nutrient medium to 100 ml. The microcosms were assembled by first filling each Schott bottle with 30 ml of pre-autoclaved standard protist pellet suspension filtered through Whatman

filters (0.31 g protist pellets in 1 l of tap water, Carolina Biological Supply, Burlington NC, USA), and 5 ml of a bacteria solution composed of three species (*Serratia fonticola*, *Bacillus subtilis* and *Brevibacillus brevis*). After 24 h, to allow time for bacterial growth, we added 65 ml of protist solution with each protist species at carrying capacity (5 ml per species). All communities were allowed to grow for one week before disturbance treatments started to be implemented. General laboratory procedures follow the protocols described previously [39], and build upon previous work on the effect of pulse disturbances on diversity [41,44], size–abundance pyramids [42] and invasion dynamics [45].

(d) Sampling

We sampled 0.2 ml of the well-mixed microcosms daily to quantify total community biomass (i.e. total bioarea in $\mu\text{m}^2 \mu\text{l}^{-1}$) using a standardized video procedure [39,46]. In brief, a constant volume (14.9 μl) of each sample was placed under a dissecting microscope connected to a camera and a computer for the recording of videos (4 s per video, that is 100 video frames). Then, using image processing software (ImageJ, National Institute of Health, USA) and the R package *bemovi* [47], we extracted the number of moving organisms per video frame and the size of each individual (mean cell area in μm^2). We estimated community biomass as the sum area of all individuals averaged by video frames, assuming proportionality between area and mass. Other traits, such as organisms' speed and shape, were used to filter out background movement noise (e.g. particles from the medium). Finally, we assessed manually the presence or the absence of each protist species at $t = 39$ (i.e. visual analysis of the videos) in order to determine the composition and species richness of each microcosm at the end of the experiment. This visual assessment of species identity is well-established and known to be precise [46].

(e) Statistical analyses

We tested for the direct effect of the disturbance regimes on average community biomass (i.e. total bioarea in $\mu\text{m}^2 \mu\text{l}^{-1}$), which was calculated over 21 time points for each microcosm. We performed Welch two-sample *t*-tests (null hypothesis: average community biomass in a given treatment (six replicates, temporal mean) is not different from average community biomass in the controls (eight replicates, temporal mean)). Similarly, we tested for the legacy effect of the disturbance regimes on community biomass ($\mu\text{m}^2 \mu\text{l}^{-1}$), species richness and species occurrences (i.e. proportion of replicates in which the species was present). For each variable and disturbance regime, we performed a Welch two-sample *t*-test (null hypothesis: the average value in the treatment (six replicates at $t = 39$) is not different from the average value in the controls (eight replicates at $t = 39$)). All *p*-values were adjusted for multiple testing using the Benjamini–Hochberg correction [48], which controls for false discovery rate (i.e. the expected proportion of false discoveries among the rejected hypotheses). To illustrate the direct and legacy effects of varying disturbance regimes on community biomass, we computed the difference between log average biomass in the treatments and log average biomass in the controls.

(f) Theoretical expectations

We used an analytical model inspired from harvesting theory and derived in Harvey *et al.* [41] to make theoretical expectations regarding the sets of disturbance frequency and intensity that should have equivalent long-term effects on community properties. This model has been initially used in a meta-ecosystem context to predict the amount of detritus produced in a community experiencing different scenarios of disturbance frequency and intensity [41]. It has been combined recently to the metabolic

theory of ecology to predict the effect of varying disturbance regimes on size–abundance scaling relationships [42]. The model states that a population following a logistic growth can persist in a disturbed environment only if its growth rate balances the long-term effect of the disturbance regime, that is:

$$r > -\frac{\ln(1-I)}{T}, \quad (2.1)$$

where r is population growth rate (mass/time), I is disturbance intensity (fraction of mass) and $T = 1/f$ is the time between two disturbances (time). From inequality (2.1), one can predict the set of disturbance regimes a population can sustain according to its growth rate.

Another important prediction of inequality (2.1) is that different combinations of disturbance frequency and intensity will have equivalent effects on effective population growth rate. Therefore, the right-hand side of inequality (2.1) can be used as a proxy for disturbance regime strength, which allows to classify varying combinations of disturbance frequency and intensity according to their expected effect on effective population growth rate. Here, we extended inequality (2.1) to assemblages of co-occurring species and assumed that all species' populations follow a logistic growth and are constrained by intraspecific competition only. We formulated the following theoretical expectation: the disturbance regimes that have equivalent effects on effective population growth rate should have equivalent legacy effects on community composition and species richness as well. We then compared the ranking of the disturbance regimes regarding their expected effect on effective population growth rate to the legacy effect of the disturbance regimes on species richness, composition and biomass of experimental communities.

3. Results

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

The experiment resulted in a set of time series showing the dynamics of community biomass in response to varying combinations of disturbance frequency and intensity, creating a two-dimensional landscape of disturbance regimes (figure 1; electronic supplementary material, figure S1). The temporal variability of community biomass in the controls is illustrated in figure 1 (in grey), which may be related to species extinctions or changes in nutrient concentration. High disturbance intensity ($I = 90\%$) and frequency ($f = 0.33$), as well as low disturbance frequency ($f = 0.08$) had a significant direct effect on communities by decreasing community biomass during the disturbance experiment (figure 2a; electronic supplementary material, table S2). Conversely, community biomass did not differ significantly from undisturbed communities in intermediate disturbance regimes (e.g. $I = 30\%$). The disturbance regimes with the lowest frequency ($f = 0.08$) did not have a significant legacy effect, that is, twenty-six days after the last disturbance event (figure 2b; electronic supplementary material, table S2). However, we observed a 'ghost of past disturbances' after reaching a certain disturbance threshold: community biomass was significantly lower than controls for intensity $I = 90\%$ with frequency $f = 0.11, 0.17, 0.33$ and $I = 70\%$ with $f = 0.33$. Note that these disturbance regimes were also the ones having the strongest direct effect on community biomass (figure 2a). Finally, one disturbance regime of intermediate strength, that is $I = 30\%$ with $f = 0.33$, had a significant positive legacy effect on community biomass. Other disturbance regimes of intermediate strength had a

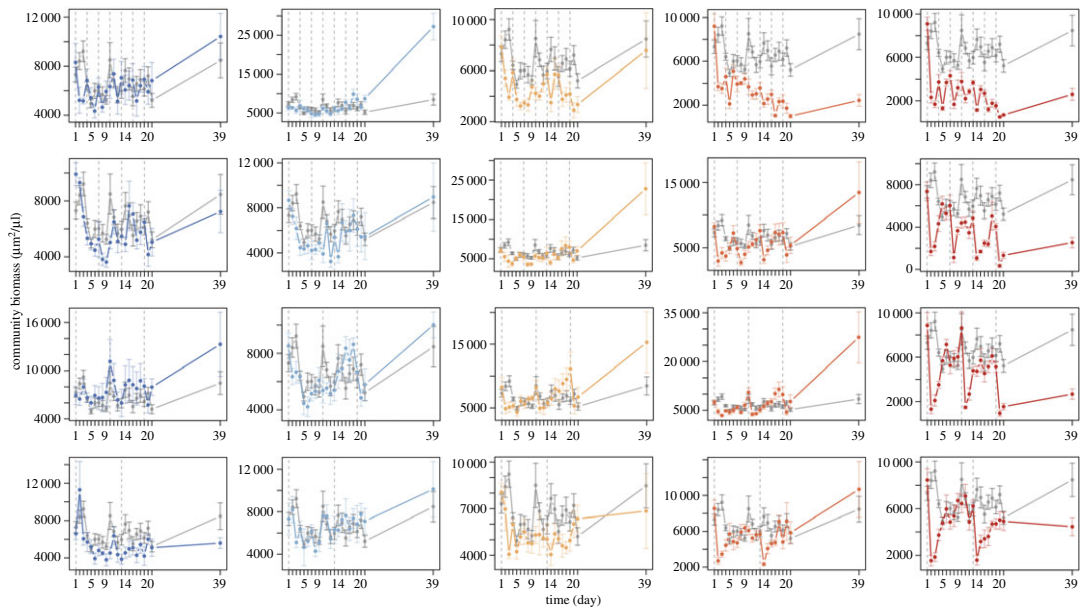


Figure 1. Temporal dynamics of community biomass (approximated by total bioarea of organisms, in $\mu\text{m}^2 \mu\text{l}^{-1}$) for different combinations of disturbance frequency and intensity (coloured lines) compared with undisturbed controls (grey lines). Rows depict a gradient of disturbance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

positive effect on community biomass, that is $I=50\%$ with $f=0.17$ and $I=70\%$ with $f=0.11$ (figure 2b, blue squares). However, the legacy effect of these treatments was extremely variable among replicates (see error bars in figure 1) and community biomass was therefore not significantly different from the controls according to the Welch two-sample t -tests (electronic supplementary material, table S2).

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

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

We used these reference communities to assess the legacy effect of disturbances on species richness and community composition (see electronic supplementary material, table S4 for detailed species presence/absence in the 128 microcosms). A significant legacy effect of disturbances on species richness was only found in the four strongest disturbance regimes (i.e. $I=90\%$ with $f=0.11, 0.17$ and 0.33 and $I=70\%$ with $f=0.33$),

which matched the disturbance thresholds found for community biomass (figure 3; electronic supplementary material, table S2). These disturbance regimes not only led to a long-term change in species richness but also to a clear switch in the functional composition of the species present (figure 4). While most of the disturbed communities were composed of one predatory species (*Blepharisma* sp.) and two autotrophic species (*Euglena gracilis* and *Euplotes aediculatus*), communities that experienced the strongest disturbance regimes (i.e. $I=90\%$ with $f \geq 0.11$ and $f=0.33$ with $I \geq 70\%$) were composed of bacterivorous species only. Interestingly, the occurrence of *Chilomonas* sp. was significantly higher in communities that experienced these disturbance regimes, which was strongly correlated to the absence of *Blepharisma* sp. and *Euglena gracilis*, suggesting a disruption of competitive exclusion (figure 4; electronic supplementary material, table S5).

(c) Theoretical equivalences between disturbance regimes

The proxy for disturbance regime strength (right-hand side of inequality 1) outlined two important points that were consistent with the experimental results. First, it illustrated how community response to increasing disturbance intensity is expected to be nonlinear, with relatively small effects of low intensities compared with stronger ones (figure 5). The disturbance regimes that had the strongest effect on effective population growth rate according to the model were also the ones having a significant long-term impact on the species richness, biomass and composition of the experimental communities. Specifically, the disturbance regimes with a strength above 0.25 d^{-1} (figure 5), that is $I=90\%$ with

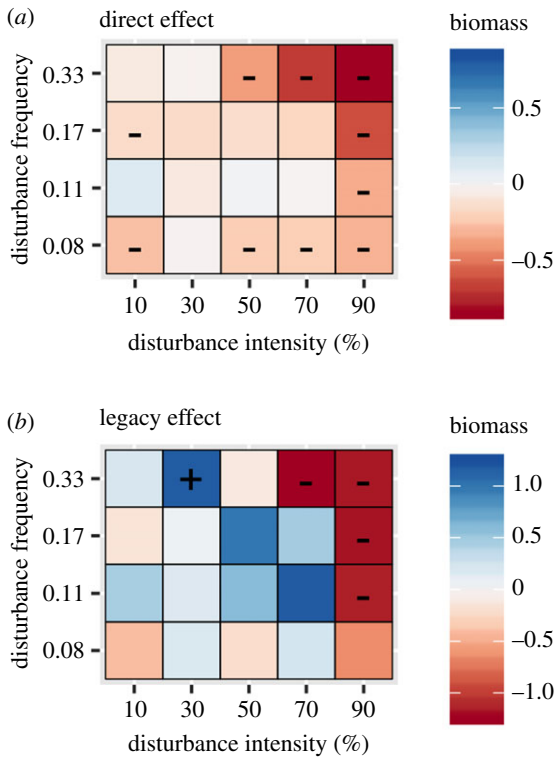


Figure 2. Direct and legacy effects of varying disturbance regimes on community biomass compared with undisturbed communities. Each square corresponds to the difference between log average biomass in the treatments and log average biomass in the controls. Red and blue colours illustrate a negative or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

$f = 0.33$, 0.17 or 0.11 and $I = 70\%$ with $f = 0.33$, led to novel communities, characterized by a lower biomass and species richness, as well as a different functional composition dominated by bacterivorous species (figures 2–4).

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

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

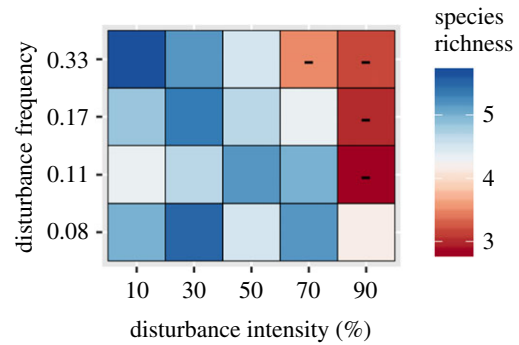


Figure 3. Legacy effect of disturbance regimes on species richness. Each square corresponds to the average over six replicates at $t = 39$. A negative sign given in a square illustrates a significant effect of the treatment relative to the control (p -value < 0.1 , individual statistics of Welch two-sample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

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

4. Discussion

We experimentally investigated the effect of repeated pulse disturbances affecting the abundances of all species in a similar way, on the biomass and composition of a protist community. We specifically studied two dimensions of environmental disturbances and their interactions by varying disturbance frequency and intensity, resulting in 20 different disturbance regimes. While high disturbance intensity ($I = 90\%$) and frequency ($f = 0.33$), as well as low disturbance frequency ($f = 0.08$), had a significant direct effect on community biomass, a long-lasting legacy of disturbances was observed only in the communities exposed to disturbance intensity $I = 90\%$ at all frequencies but the lowest and $I = 70\%$ with $f = 0.33$ (highest frequency). This ghost of disturbance past was characterized by communities critically different from the undisturbed ones, with a significantly lower species richness and total biomass. The functional composition of these communities changed as well, such that predatory and autotrophic species were replaced by bacterivorous species.

From a theoretical perspective, the model predicted that communities can recover from a wide range of disturbance regimes if the disturbances are stopped or mitigated, but will not recover their initial state once a threshold in disturbance regime strength is reached. This is in accordance with our experimental findings, where most of the communities approached a state similar to the reference state when the disturbance stopped, even if they were significantly impacted during the disturbance events. Above a threshold, however, disturbances had an irreversible effect on community biomass and composition. Hence, we could experimentally (i) demonstrate that the reference state of the protist community is an attractor (or stable state), and (ii) identify the combinations of

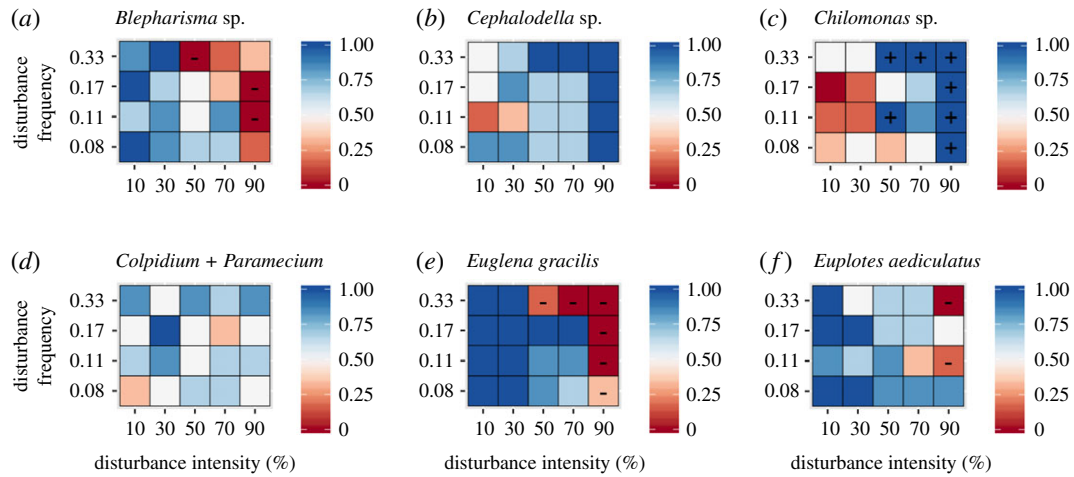


Figure 4. Legacy effects of disturbance regimes on species average presence. Each square corresponds to the average presence over six replicates at $t = 39$. A positive or negative sign in a square illustrates respectively a significant positive or negative effect of the treatment relative to the control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

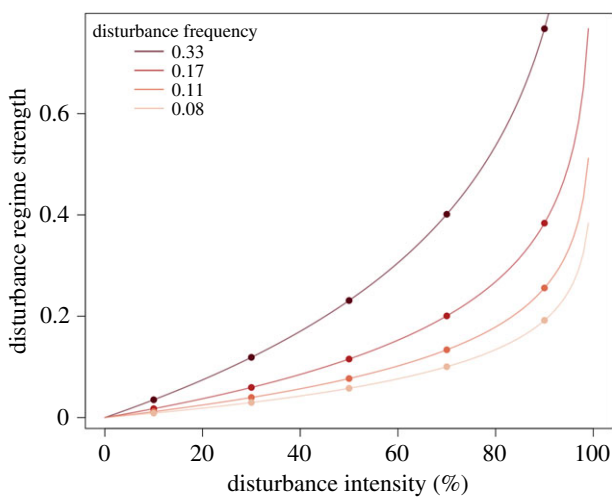


Figure 5. Disturbance regime strength (right-hand side of inequality 1), corresponding to the effect of disturbances on effective population growth rate (day^{-1}), for varying combinations of disturbance frequency (colour gradient) and intensity (x -axis). The points represent the 20 disturbance regimes performed during the experiment (e.g. $I = 50\%$ with $f = 0.33$ on the line in brown). From the model, different combinations of disturbance frequency and intensity are expected to have equivalent effects on species mortality (e.g. $\{I = 30\%; f = 0.33\}$ and $\{I = 70\%; f = 0.11\}$). (Online version in colour.)

disturbance frequency and intensity that pushed the community to another state, corresponding to a disturbance strength of 0.25 d^{-1} for this community (right-hand side of inequality 1). We could not assess if the new community state resulting from the disturbance regimes is stable or unstable, as the return of the original species in the communities was prevented in our experiment. However, this could also be determined experimentally, by testing species' abilities to recolonize the communities showing a legacy effect of past disturbances [49].

The model we used provides a qualitative baseline to determine which disturbance regimes should have equivalent effects on communities. However, this model is based on population growth rate only and cannot be used to determine *a priori* the threshold above which disturbances will have an irreversible impact on community properties. Indeed, the disturbance threshold we observed for the experimental protist

community (i.e. disturbance strength of 0.25 d^{-1} , inequality 1) emerges from the dynamics of interacting species that compete or predate on each other. Hence, a promising way forward would be the integration of the effect of species interactions to the model in order to determine communities' disturbance thresholds. In a recent study, Arnoldi *et al.* [50] showed that the effective growth rates in a community of interacting species correspond to $r^* = rN^*/K$, where r is species intrinsic growth rate, N^* is the equilibrium abundance within the community and K is carrying capacity (i.e. abundance in monocultures). This result has direct implications for the response of competitive communities to disturbances. Indeed, by replacing r by r^* in equation (2.1), we can demonstrate that, for similar growth rates, species with low competitive abilities (i.e. low N^*/K), will be more sensitive to a given disturbance regime than strong competitors (i.e. high N^*/K).

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

The competitive abilities of most of the protist species we used in our experiment have been extensively studied in previous works [55,56] and explain well the composition of the microcosms at the end of the experiment. Indeed, the five species that went systematically extinct in the control communities (i.e. *Cyclidium* sp., *Chlorogonium euchlorum*, *Loxocephalus* sp., *Spirostomum* sp. and *Tetrahymena* sp.) have low competitive abilities relative to other protist species [55]. Second, the

two species that were systematically present in the control communities, that is *Euglena gracilis* and *Euplotes aediculatus*, are strong competitors and significantly decreased the N^*/K of *Chilomonas* sp. in a relative yield experiment [55]. Interestingly, we observed the combined negative effects of competitive interactions and disturbances on the occurrence of *Chilomonas* sp. at low disturbance intensity (i.e. $I=10\%$ and 30% , figure 4) as predicted by the theory [50]. At high disturbance intensity, however, *Chilomonas* sp. could persist due to its high growth rate and significantly benefited from the extinction of its competitors or predators (figure 5 and electronic supplementary material, table S5).

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

In order to explore the effect of disturbance frequency and intensity on community recovery, we made several choices regarding other aspects of disturbances that may limit the transferability of our results to other disturbance types. First, the experiment explored the effects of repeated pulse disturbances that punctually decreased population density, in contrast to press perturbations that correspond to a sustained alteration of species density [31]. Nonetheless, previous studies have shown that equation (2.1) can be easily adapted to the study of press disturbances that affect effective population growth rate in a continuous way [42,52]. Then, similar effects on communities would be expected for this type of press disturbances. Second, the disturbances affected all species in a similar way, with the same fraction of population biomass being removed from the system. However, some species might be more resistant than others to environmental

disturbances, which can influence the recovery dynamic of the whole community [50]. Similarly, some disturbances can only affect a subset of the community, such as size-selective harvesting [57]. Third, as protist microcosms are closed systems, we did not address here the recovery mechanisms related to species recolonization from neighbour communities nor the spatial extent of disturbances, which is another important dimension to consider to predict the legacy effects of disturbances on communities [19,22,44,58,59].

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

Data accessibility. The data supporting the experimental results are archived in the Dryad Digital Repository: <http://dx.doi.org/10.5061/dryad.zkh189378> [60].

Authors' contributions. C.J. conducted the experimental research, analysed the data and wrote the first draft of the manuscript. C.J. and F.A. designed research and contributed to the edition of the paper.

Competing interests. We declare we have no competing interests.

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