

# Diversity–disturbance relationships: frequency and intensity interact



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**An influential ecological theory, the intermediate disturbance hypothesis (IDH), predicts that intermediate levels of disturbance will maximize species diversity. Empirical studies, however, have described a wide variety of diversity–disturbance relationships (DDRs). Using experimental populations of microbes, we show that the form of the DDR depends on an interaction between disturbance frequency and intensity. We find that diversity shows a monotonically increasing, unimodal or flat relationship with disturbance, depending on the values of the disturbance aspects considered. These results confirm recent theoretical predictions, and potentially reconcile the conflicting body of empirical evidence on DDRs.**

**Keywords:** disturbance; microcosm; *Pseudomonas fluorescens*; intermediate disturbance hypothesis

## 1. INTRODUCTION

Understanding how biological diversity responds to disturbance is a longstanding problem in ecology and evolution, with important ramifications for conservation and management of ecosystems [1–5]. A large body of theory predicts that ecological diversity is maximized at intermediate levels of disturbance [6,7], yet a wide range of diversity–disturbance relationships (DDRs) have been observed in empirical studies [8]. This inconsistency may arise because most studies consider a single aspect of disturbance, such as the frequency of disturbances over time, or their intensity, even though diversity probably depends on a combination of different aspects of disturbance [7]. Recent theory has formally addressed this prediction, finding, for example, that coexistence can peak at low, intermediate or high disturbance intensities depending on their frequency [9]. Here we show, using experimental populations of bacteria, that different DDRs can be observed within the same system,

depending on which aspect of disturbance is considered. These findings illustrate the need to consider multiple aspects of disturbance in order to fully understand the dynamics of disturbance-prone communities.

## 2. MATERIAL AND METHODS

We manipulated both the frequency and intensity of disturbances (mass mortality events [10]) in populations of *Pseudomonas fluorescens* SBW25 in microcosms. Each microcosm contained 6 ml liquid King's medium B in a 28 ml glass vial, incubated without shaking at 28°C [11]. In these conditions, *P. fluorescens* rapidly diversifies into genetically distinct morphotypes: the ancestral morph (smooth, SM) grows in the broth phase; the wrinkly spreader (WS) morph grows into a biofilm at the air–liquid interface and includes numerous sub-categories; the fuzzy spreader (FS) morph grows at the bottom of the tube [11]. Crucially, different morphotypes are readily distinguished on agar plates, allowing us to quantify ecological diversity at the end of the experiment [11]. We used a fully factorial experimental design, maintaining replicate populations over 16 days and imposing 0, 1, 4, 8 or 16 disturbances (frequency manipulation) with 10, 99.9 or 99.999 per cent mortality (intensity manipulation) at each disturbance.

We enacted disturbances by removing a fraction of cells (intensity) from each microcosm before transferring the surviving cells to a fresh microcosm [10,12]. Microcosms were homogenized by vortexing prior to every disturbance, to ensure that there was no bias towards particular morphotypes. There were three replicate microcosms at each combination of disturbance intensity (10, 99.9 or 99.999%) and frequency (0, 1, 4, 8, 16 disturbances). Disturbance frequencies were chosen to be consistent with previous work [10,12]. At the end of the experiment (16 days), diversity was estimated by vortexing, diluting and plating bacteria from every microcosm onto nutrient-rich KB agar plates, before counting the number of each morphotype after 48 h incubation at 28°C [10,11].

We tested whether the diversity–intensity relationship depended on the frequency of disturbances by the interaction term in a linear model, with diversity as the response variable and frequency and intensity as factors. Diversity scores were rank-transformed to account for non-normality. To analyse the correlation between diversity and frequency in each intensity treatment, we transformed disturbance frequencies to  $\log_2(\text{no. disturbances} + 1)$ . We tested for quadratic effects by comparing full and reduced models with *F*-tests. In some cases, it was unclear whether quadratic effects were due to unimodality or curvilinearity; to test this, we used Mitchell-Olds & Shaw tests [13,14]. WS frequencies were arcsine-transformed before analysis to stabilize the relationship between mean and variance.

## 3. RESULTS

The diversity–frequency relationship varied depending on the intensity of disturbances (interaction term:  $F_{8,30} = 6.07$ ,  $p = 0.0001$ ; figure 1a). At low intensity (10% mortality), there was no correlation between frequency and diversity ( $F_{1,13} = 0.04$ ,  $p = 0.84$ ). When disturbances were more intense (99.9% mortality), diversity was greatest at intermediate and high frequencies (quadratic term:  $F_{1,12} = 34.50$ ,  $p < 0.0001$ ), with a slight peak at an intermediate frequency (Mitchell-Olds & Shaw test:  $p = 0.01$ ). At the highest intensity (99.999% mortality), diversity was unimodally related to frequency (quadratic term:  $F_{1,12} = 25.92$ ,  $p = 0.0003$ ), showing a clear drop at the highest frequency, meaning that diversity at the highest frequency was considerably lower at high compared with intermediate intensity (Welch's *t*-test:  $t_2 = 8.28$ ,  $p = 0.01$ ). Another way to describe the interaction of intensity and frequency is by the shape of the diversity–intensity relationship at different frequencies: when disturbances were rare, diversity increased with intensity (lightest grey circles in figure 1a). When disturbances were frequent, diversity peaked at intermediate intensity (black circles, figure 1a). Thus, diversity was lowest at the extremes, where intensity and frequency were both very high or both very low.

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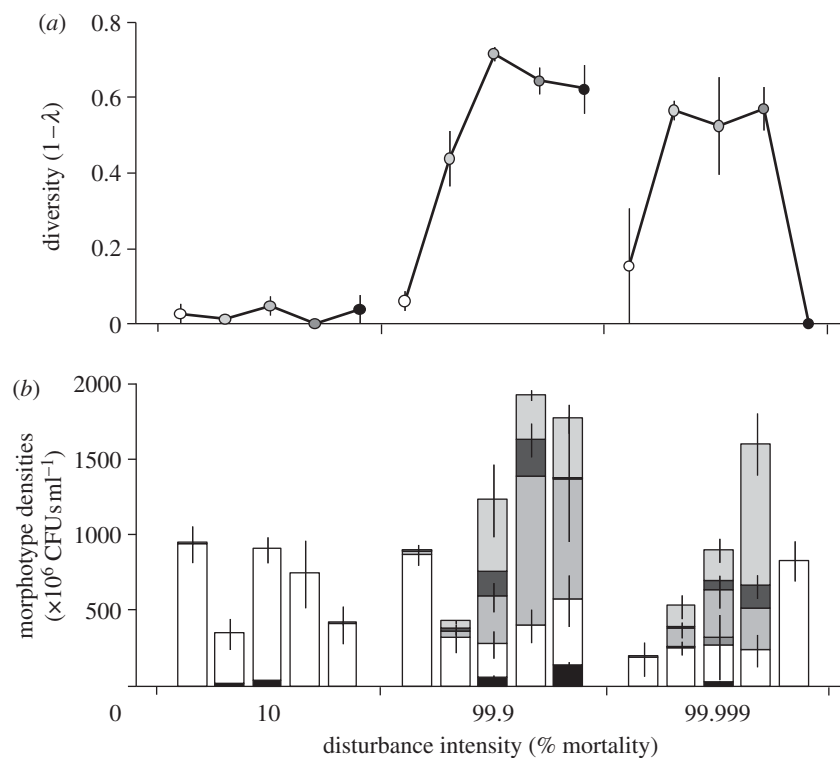


Figure 1. (a) Diversity, measured as the complement of Simpson's index ( $1 - \lambda$ , where  $\lambda = \sum p_i^2$  and  $p_i$  is the proportion of the  $i$ th morph), is shown for each of five disturbance frequencies (left to right in each series, 0, 1, 4, 8 or 16 disturbances over 16 days) at each of three disturbance intensities. Points show means  $\pm$  s.e. for three replicate microcosms. (b) Frequencies of different morphotypes (WS, four subcategories, each a different shade of grey; SM, white bars; FS, black bars) at each combination of disturbance intensity and frequency. Bars show mean  $\pm$  s.e. for three replicate microcosms at each disturbance frequency (from left to right in each group of five bars: 0, 1, 4, 8, 16 disturbances) and intensity.

Consequently, diversity can show a monotonically increasing, unimodal or flat relationship with disturbance depending on which aspect is considered.

Previous work at a single intensity (99.9%) showed that biofilm-forming morphotypes (WS) are most successful at intermediate disturbance frequencies [10,12]. Our results at 99.9 per cent intensity are entirely consistent with this pattern (figure 1b; quadratic term for WS frequency against disturbance frequency:  $F_{1,12} = 9.03$ ,  $p = 0.01$ ). We also find that increasing intensity to 99.999 per cent favours WS when disturbances are rare, but favours the broth-living morph (SM) at the highest frequency. SM was also relatively successful in low-intensity treatments. Thus, WS is most successful at intermediate frequencies and intensities of disturbance. The greater number of subcategories in WS compared with SM and FS causes total diversity to peak when the WS frequency is between 0.6 and 0.8, but we note that the same qualitative diversity patterns across disturbance treatments are obtained if we ignore WS sub-categories (frequency  $\times$  intensity interaction:  $F_{8,30} = 2.81$ ,  $p = 0.019$ ), showing unequivocally that the frequency–intensity interaction drives the coexistence of ecologically distinct sub-populations.

#### 4. DISCUSSION

Our results demonstrate that different DDRs can arise, even within a single empirical system, depending on an interaction between the frequency and intensity of

disturbances. This is because the relative abundances of the two main morphotypes in our experimental system, SM and WS, varied nonlinearly with both increasing intensity and frequency of disturbances. Consequently, we obtained monotonically increasing, unimodal or flat DDRs, depending on which aspects of disturbance were considered, and over what range of values.

Why does long-term growth rate (a measure of fitness), as reflected by morphotype density of SM and WS, vary nonlinearly with disturbance frequency and intensity? WS, which overproduces a cellulosic polymer [15], requires a threshold population density to form a mat at the air–broth interface [16]. WS and SM can only coexist when this mat forms [11]. At high frequencies of disturbance, WS, which has a lower intrinsic growth rate than SM, is unable to reach sufficient densities to form a strong mat [10,12,16]. Similarly, if disturbances are very intense, then WS may not reach threshold densities between disturbances. However, if left undisturbed, the WS mat eventually collapses, either through invasion by SM or because general environmental degradation reduces population size [17], or both, resulting in SM again dominating the system. This breakdown should be more rapid between disturbances if disturbance intensity is low, because a smaller proportion of the degraded growth media is renewed. Thus, both diversity and the success of WS are maximized at intermediate intensities and frequencies, and the peak in diversity across frequencies will be shifted in either direction depending on intensity, and vice versa.

Since WS has a lower intrinsic growth rate, and its mat may collapse, it can be seen as a weaker competitor than SM; thus it is natural to expect SM to dominate at low frequencies and intensities.

The recent model by Miller *et al.* [9] is qualitatively consistent with our results. It predicts that a unimodal relationship between diversity and disturbance frequency can result from a single type/species dominating at high and low disturbance frequencies, and that the unimodal relationship becomes relatively flat if disturbance intensity is reduced. Crucially, while this model is not a direct representation of our experimental system, the biological mechanisms underlying DDRs are similar. In the model, the growth rates of the two species respond differently and nonlinearly to changing disturbance intensity and frequency because of differences in growth rate, survival and competitive ability. In our experimental system, WS and SM also differ in all of these traits and show different nonlinear responses to changing disturbance intensity and frequency.

The model also predicts that a unimodal DDR at intermediate intensity can become bimodal at higher intensities. At intermediate intensity, one type/species dominates at high and low frequencies and both types coexist in the middle. At higher intensity, one type dominates at high and low frequency and the other dominates in the middle, so that coexistence occurs at middle–low and middle–high frequencies where neither type is dominant, resulting in a bimodal DDR. We did not observe such bimodal DDRs in our experiments. However, the fact that unimodal DDRs for *P. fluorescens* are based on a single type dominating at high- and low-disturbance frequencies, and that WS was dominant in some microcosms at intermediate disturbance frequencies, suggests that bimodal DDRs are possible in this system, but they may only be detected when a greater range of disturbance frequencies and intensities are included in the experimental design.

The diversity–frequency relationship in this experimental system was previously modelled [10] using a modified version of Levene's [18] model that explicitly considers distinct niches, where intra-niche competition is stronger than inter-niche competition. In this model, disturbances alter the relative contributions of the two niches to total population growth, with coexistence only possible when both niches support approximately equal numbers of individuals. This model can also display qualitatively different DDRs, including bimodal relationships, if the term describing population size after disturbance is varied (see electronic supplementary material). More complex models, such as metapopulation models incorporating disturbance and extinction of a proportion of demes [19], may also show similarly complicated DDRs when both intensity and frequency of disturbance are varied, but analysis of such models is beyond the scope of this paper.

These studies illustrate that several factors influence the shape of DDRs. For instance, Kondoh [19] showed that, in a metapopulation model, productivity influences the shape of observed DDRs. Empirical research has also demonstrated that competition–colonization trade-offs

in a metapopulation network of linked microcosms can generate distinct DDRs against disturbance frequency, depending on spatial scale [20]. In contrast, our data show that differing spatial scales and productivity gradients are not necessary to change the shape of DDRs. Rather, at a fixed local scale, differential responses to disturbance frequency and intensity form the biological basis for our findings. This suggests that closer attention to multiple disturbance aspects, and their interactions, may allow us to reconcile the large and conflicting body of empirical evidence on the relationship between disturbance and diversity [8].

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