

## LETTER

# Effects of multiple stressors on the dimensionality of ecological stability

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**Abstract**

Ecological stability is a multidimensional construct. Investigating multiple stability dimensions is key to understand how ecosystems respond to disturbance. Here, we evaluated the single and combined effects of common agricultural stressors (insecticide, herbicide and nutrients) on four dimensions of stability (resistance, resilience, recovery and invariability) and on the overall dimensionality of stability (DS) using the results of a freshwater mesocosm experiment. Functional recovery and resilience to pesticides were enhanced in nutrient-enriched systems, whereas compositional recovery was generally not achieved. Pesticides did not affect compositional DS, whereas functional DS was significantly increased by the insecticide only in non-enriched systems. Stressor interactions acted non-additively on single stability dimensions as well as on functional DS. Moreover we demonstrate that pesticides can modify the correlation between functional and compositional aspects of stability. Our study shows that different disturbance types, and their interactions, require specific management actions to promote ecosystem stability.

**KEYWORDS**

multiple stressors, disturbance, ecological stability, mesocosm experiment, community ecology, populations, resilience, community composition, functional ecology, recovery

**INTRODUCTION**

Ecosystems are exposed to several co-occurring disturbances (Millennium Ecosystem Assessment, 2005). Human-mediated disturbances differ greatly in nature (climate change, species invasion, chemical pollution), temporal extension (press, pulse) and intensity (Jacquet & Altermatt, 2020; Turner, 2010). Understanding the impact of anthropogenic disturbances across levels of biological organisation is critical to improve our capacity to manage and restore natural systems (De Laender et al., 2016). Recently, the overall understanding of ecosystems stability in relation to disturbance has improved greatly, starting with the work by Donohue et al., (2013), which unravelled the multidimensional nature of stability.

According to their work, the dimensionality of stability (DS) requires the assessment of several ecological properties, including resistance, resilience, recovery and invariability (Donohue et al., 2013, 2016).

Different stability properties can be strongly or poorly correlated among each other (Donohue et al., 2013; Hillebrand et al., 2018). When such properties are strongly correlated, the overall DS is reduced, resembling a one-dimensional concept, and all the correlating metrics give similar information (Domínguez-García et al., 2019). Conversely, when stability properties are not correlated, the dimensionality increases, meaning that every single metric provides a unique information on the system's stability. Consequently, DS has important policy and ecosystem management implications. When DS

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is high, managers could decide to maximise one stability property (e.g. resistance) without influencing other stability's aspects. When DS is low, no action on a single stability property can be taken without affecting the others and, therefore, management actions cannot specifically address one stability property in isolation. Moreover when DS is low, the sign of these correlations becomes pivotal. If two stability properties correlate negatively, a trade-off exists, which prevents maximising both stability aspects. In this way, one stability property can be promoted only at the expenses of the other. When the correlation is positive, such trade-off does not exist, allowing to tackle both stability properties simultaneously.

The measurement of several components of stability is not yet a common practice in ecology. Despite the large number of metrics existing to quantify stability, most of the available studies (>90%) used a one-dimensional approach (Donohue et al., 2016), therefore failing to describe its multidimensional nature. Furthermore, Kéfi et al., (2019) pointed out that there is a disbalance towards the investigation of stability in species-poor systems. Among empirical studies, almost 30% involved systems with <10 species, and a negligible number of experiments used a species assemblage large enough to resemble a natural ecosystem (Kéfi et al., 2019).

The majority of studies addressing ecological stability have investigated the response of ecosystems to one single stressor (Kéfi et al., 2019). However, ecosystems are exposed to multiple disturbances at a time (Birk et al., 2020; Lemm et al., 2020). Multiple stressors research has accordingly grown in recent years (Orr et al., 2020), providing invaluable information on stressors interactions and unravelling mechanisms behind the observed responses. Meta-analyses have tried to summarise common stressors interactions across ecosystems (Birk et al., 2020; Crain et al., 2008; Jackson et al., 2016) and new methods have been proposed to understand and classify non-additive interactions (Piggott et al., 2015c; Tekin et al., 2020; Thompson et al., 2018). However, the multidimensional conception of stability has never been applied in multiple stressors' research. Disentangling the multidimensionality of stability is key to unlock our understanding of—and our ability to predict—how biological systems respond to multiple disturbances. The urge of understanding how multiple stressors affect the different stability components has become pressing since interactions between stressors have been shown to be dependent on temporal scales (Garnier et al., 2017). That is, non-additive interactions between stressors can modify several stability dimensions, from early response to pulse disturbance (e.g. resistance) and recovery (e.g. resilience and recovery), to long-term responses to press disturbances (e.g. temporal invariability).

Here, we investigate the effects of multiple stressors on the dimensionality of stability in freshwater communities, using data from a species-rich mesocosm experiment. We quantified four stability properties: resistance,

recovery, resilience and invariability, and analysed how stability is influenced by single and multiple disturbances in both, functional and compositional aspects, using a three-trophic level aquatic community. Additionally, we assessed the correlation between stability properties related to different disturbance types and between compositional and functional stability properties. As stressors, we selected two pesticides with different toxicological properties. We used the herbicide (diuron) to selectively reduce primary producers' diversity and abundance, and an insecticide (chlorpyrifos) to reduce consumers' diversity and abundance. Both pesticides were applied at a dose that is expected to affect 50% of the primary producers and consumers, respectively. Also, we assessed whether nutrients enrichment (as a press stressor) can influence the DS to the pesticides. For this, we treated half of the mesocosms with a press (continuous) addition of nutrients (N and P) starting one month before the application of the pesticides, while the other half were not treated with nutrients. Nutrients enrichment is known to reduce diversity and to homogenise species assemblages at different scales (Donohue et al., 2009; Isbell et al., 2013; Woodward, 2012). Ecosystem stability has been shown to change non-monotonically with diversity (Pennekamp et al., 2018). That is, diversity can increase as well as decrease stability. However, the underpinning mechanism driving the occasionally reported negative correlation between increasing diversity and stability remains poorly understood (Loreau & de Mazancourt, 2013; Pennekamp et al., 2018).

Through this study we addressed the following hypotheses:

H1: Single and multiple disturbances influence individual stability properties as well as the DS.

The metrics we used to quantify DS can be affected non-additively by multiple stressors (Garnier et al., 2017; Halstead et al 2014). Consequently, we may expect to find significant stressors interactions on single stability dimensions as well as on the overall DS. Since DS depends on the correlations between stability properties, we expect those correlations to be modified by disturbance.

The recent advancement in theoretical studies may help to formulate predictions on how DS behave under multiple stress situations. The work by Radchuk et al., (2019) does not only show that disturbance type determines DS, but also indicates that disturbances resembling pesticide applications generally increase functional DS, whereas compositional DS was not affected by disturbance.

Pesticides cause non-random effects according to their toxicological mode of action (De Laender et al., 2016). Using an equivalent dose that affects the same percentage of species of the most sensitive taxonomic group, we expect the herbicide to produce a smaller impact on the individual stability dimensions since primary producers (phytoplankton) usually show a higher adaptation capacity as compared to consumers, in part, due

to their fast population growth (Hillebrand et al., 2018). Similarly, we expect the herbicide to result in a lower effect on DS as compared to the insecticide.

H2: Compositional and functional stability properties of nutrient-enriched systems are less affected by pesticides than those of non-enriched ones.

Species identity and richness determines communities' responses to disturbance as well as the speed at which communities recover from perturbations (Loreau & de Mazancourt, 2013). That is, species identities influence different stability properties (Pennekamp et al., 2018; White et al., 2020). Accordingly, we expect nutrient enrichment to modify the species identity and richness (composition) of the community, promoting a less diverse assemblage dominated by few tolerant species and an overall increase of biomass (Donohue et al., 2009). A more homogeneous community dominated by tolerant species is expected to be less affected by disturbance, both functionally and compositionally, and to display differences in stability properties (Belando et al., 2017).

H3: Disturbance type influences the correlation between compositional and functional stability properties.

Diverse species assemblages are expected to have higher functional stability over time, as they consent faster changes in composition via asynchronous fluctuations in response to disturbance (Hautier et al., 2014; Yachi & Loreau, 1999). In such cases, perturbations may compromise compositional stability, but functional stability can be maintained (Allan et al., 2011), thus resulting in a negative correlation between the two stability realms. However, studies have found generally positive correlations between functional and compositional stability properties (Baert et al., 2016b; Hillebrand et al., 2018), and a recent meta-analysis has shown that correlations between functional and compositional stability are generally positive across ecosystems exposed to pulse disturbances (Hillebrand & Kunze, 2020). Thus, we expect to find mainly positive correlations between compositional and functional stability properties in our study. Yet, whether different disturbance types (i.e. press vs. pulse, or pesticides affecting different taxonomic groups) influence this general relationship has not been explicitly tested.

## MATERIALS AND METHODS

### Mesocosm experiment

We performed an outdoor mesocosm experiment at the IMDEA Water Institute (Alcalá de Henares, Madrid, Spain) between April and July of 2019. Each mesocosm was filled with approximately 40 cm of sediments and 850 L of water from an artificial lagoon. The community, composed of macrophytes (*Myriophyllum sp.* and *Elodea sp.*) and invertebrates collected from unpolluted water bodies in the vicinity of Alcalá de Henares, was

allowed to establish and homogenise among experimental units for two months prior to the start of the experiment.

A full factorial design ( $n = 3$ ), including chlorpyrifos (two levels: 1  $\mu\text{g/L}$  and absent), diuron (two levels: 18  $\mu\text{g/L}$  and absent) and nutrients (added, not added) was used in a randomised fashion. Nutrients (P and N) were applied twice per week as a solution containing 1.820 g of  $\text{NH}_4\text{NO}_3$  and 0.208 g of  $\text{KH}_2\text{PO}_4$ , which resulted in a nutrient addition of 750  $\mu\text{g/L}$  of N and 75  $\mu\text{g/L}$  of P, respectively. These nutrients levels correspond to a eutrophic system, whereas mesocosms not treated with nutrients resembled oligo-mesotrophic systems. Nutrient additions started 4 weeks before the pesticide application. Details on chemical and nutrient applications, sampling and analysis are provided in Polazzo et al., (2020).

Phytoplankton, zooplankton and macroinvertebrates communities were sampled one time before pesticides application and several times afterwards. For a complete list of the sampling days see Table S1 in the Supplementary Information (SI). In total we identified 128 taxa (68 phytoplankton taxa, 22 zooplankton taxa and 38 macroinvertebrates taxa). Details on sampling techniques are provided in Polazzo et al., (2020).

### Criteria for organism groups selection

We calculated four stability properties at the community level for three different organism groups: phytoplankton, zooplankton and macroinvertebrates. Each group was composed of several species which are differently impaired by the treatments, reflecting the range of sensitivity typical of natural species assemblages. Phytoplankton was heavily impaired by the herbicide, which reduced algae diversity. Zooplankton and macroinvertebrates were mostly impaired by the insecticide, which reduced abundance and diversity in both groups. However, indirect effects, resulting from stress-driven changes in species density, propagated throughout the food-web, changing the structure of non-target groups composition. Stability was calculated also for some populations. Methods and results of the populations' analysis are reported in the SI.

### Stability properties

We quantified four stability properties: resistance, resilience and invariability. We calculated the four stability properties for both composition and function. Compositional stability was calculated using the Bray–Curtis similarity as a state variable (Donohue et al., 2013; Radchuk et al., 2019), whereas functional stability was based on total abundance (Hillebrand & Kunze, 2020). Details on the measurement, calculation and interpretation of the stability properties are provided in Table 1.

**TABLE 1** Stability properties studied. The table shows definitions, how they were measured and interpretation of the different metrics. Measurement of stability properties were based on Hillebrand et al., (2018)

| Property: definition  | Type of endpoint | How was it measured?  | Formula   | Limits and interpretation   |
|---|------------------|---|---|---|
| <u>Resistance</u> : ability of a system to withstand stress (Hillebrand et al 2018)                                   | Functional       | log response ratio of the abundance in a treatment compared to a control at the first sampling after treatment (day 15)                     | $Res_{func} = \ln \left( \frac{Ab_t}{Ab_c} \right)$   | $Res_{func}$ of 0 reflects maximum resistance (abundance in treatment and control are the same) Resistance <of 0 means low resistance. Resistance >of 0 overperformance (increase in abundance)               |
|   | Compositional    | Bray-Curtis similarity of the community composition between treatment and control at the first sampling after treatment (day 15)            | $Res_{com} = BC \left( \frac{Comp_t}{Comp_c} \right)$   | $Res_{com}$ ranges between 0 and 1: 0 = low resistance, 1 = maximum resistance.   |
| <u>Recovery</u> : capacity of a system to return to undisturbed state following a disturbance (Ingrisch & Bahn, 2018) | Functional       | log response ratio of the abundance in a treatment compared to a control at the final sampling (day 50)                                     | $Res_{func} = \ln \left( \frac{Ab_t}{Ab_c} \right)$   | $Res_{func}$ of 0 reflects maximum (complete) recovery (abundance in treatment and control are the same); $Res_{func} < 0$ means low resistance. Recovery >of 0 means overperformance (increase in abundance) |
|   | Compositional    | Bray-Curtis similarity of the community composition between treatment and control at the final sampling (day 50)                            | $Rec_{com} = BC \left( \frac{Comp_t}{Comp_c} \right)$   | $0 \leq Rec_{com} \leq 1$ ; $Rec_{com}$ of 1 reflects maximum (complete) recovery (composition in treatment and control are the same); the closer $Rec_{com}$ to 0 the more incomplete is the recovery        |
| <u>Resilience</u> : engineering resilience; the speed of recovery after perturbation (Pimm, 1984)                     | Functional       | Slope of log response ratio of the abundance in a treatment compared to a control over time   | $i + (Rsln_{func}) * t = \ln \left( \frac{Ab_t}{Ab_c} \right)$<br>;<br>where<br>$i = \text{intercept}, t = \text{time}$ | $Rsln_{func} = 0$ means no recovery. $Rsln_{func} > 0$ means recovery. $Rsln_{func} < 0$ , means further deviation from the control   |
|   | Compositional    | Slope of the regression of similarity (Bray-Curtis) over time between treatment and control   | $i + Rsln_{com} * t = BC \left( \frac{Comp_t}{Comp_c} \right)$<br>where<br>$i = \text{intercept}, t = \text{time}$      | $Rsln_{com} = 0$ means no recovery. $Rsln_{com} > 0$ means recovery. $Rsln_{com} < 0$ , means further deviation from the control.   |
| <u>Invariability</u> (temporal): inverse of temporal variability of a state variable (Wang et al., 2017)              | Functional       | Inverse standard deviation of residuals from the linear model regressing log response ratio of the abundance (treatment vs control) on time | $Inv_{func} = \frac{1}{sd(resid(Rsln_{func}))}$   | The larger $Inv_{func}$ the higher is invariability, i.e. the less fluctuating is log response ratio around the linear temporal trend.  |
|   | Compositional    | Inverse standard deviation of residuals from the linear model regressing Bray-Curtis similarity on time                                     | $Inv_{com} = \frac{1}{sd(resid(Rsln_{com}))}$   | The larger $Inv_{com}$ the higher is invariability, i.e. the less fluctuating is Bray-Curtis similarity around the linear temporal trend.   |

ANOVA was used to test for effects of treatments on invariability, resistance, resilience and recovery, separately for the functional and compositional stability components (White et al., 2020).

## Dimensionality of stability

We measured DS as multidimensional ellipsoids constructed from the matrix of pairwise covariances between all stability properties (Donohue et al., 2013).

Disturbance can affect both volume and shape of the ellipsoid (Donohue et al., 2013), thus we considered both, using semi-axis length to characterise ellipsoids' shape. The semi-axis length was measured as  $\lambda_i^{0.5}$ , where  $\lambda_i$  is the  $i^{\text{th}}$  eigenvalue of the covariance matrix for a given treatment for each organism group. Ellipsoid volume ( $V$ ) was calculated as follows:

$$V = \frac{\pi^{n/2}}{\Gamma\left(\frac{n}{2} + 1\right)} \prod_{i=1}^n (\lambda_i^{0.5}), \quad (1)$$

where  $n$  is the dimensionality of the covariance matrix. All measurements of stability were standardised prior to the volume calculation by subtracting the mean and then dividing by the standard deviation (Donohue et al., 2013). Then, for each set of semi-axis, we divided them by the maximum length within a set, thus the maximum standardised length was equal to 1. By doing so, the largest theoretically possible volume equalled 1 (i.e. all the semi-axis lengths are 1), which represents a spheroidal shape and, consequently, high DS (Figure 1, Radchuk et al., 2019). Dividing the calculated volume by the theoretical volume, we obtained a proportional volume which varies between 1 (high DS, spheroidal shape) and 0 (low DS, “cigar”-like shape of the ellipsoid).

To assess the effects of the treatments on DS (H1), we fitted generalised linear mixed-effects models (GLMMs, Gamma distribution) with either ellipsoid volume or semi-axis length as a response. Stressors and their interactions were used as fixed effect predictors, whereas organism group was used as random effect (including both slope and intercept). To test for significance, we used likelihood – ratio tests (LRT).

### Pairwise correlations between stability properties

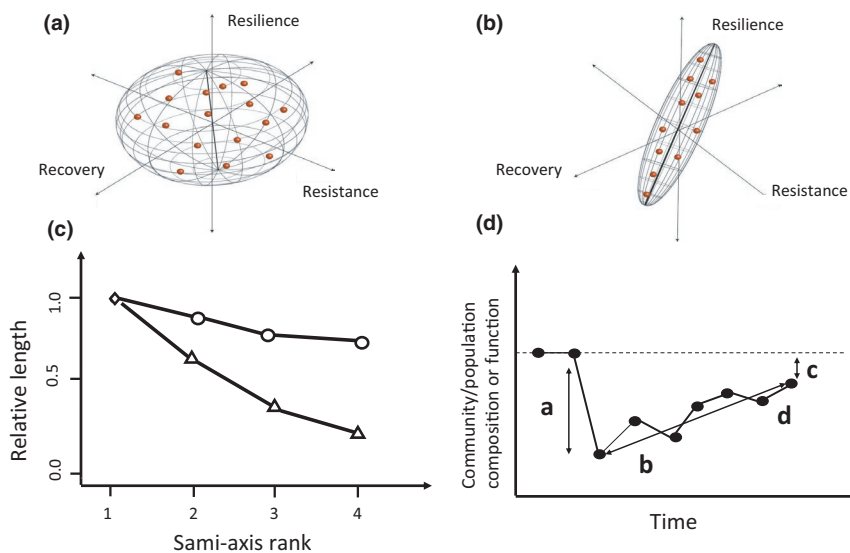
To test whether the treatments affect the correlations between different stability properties, we first calculated the Pearson correlation coefficient for each pair of stability properties within each treatment. We did that for

each group (phytoplankton, zooplankton and macroinvertebrates), both for function and composition. Then, we fitted GLMMs (Gaussian distribution) using the correlation coefficient as the response variable. As fixed effects we included stressors and their interactions, while organism group was used as random effect. Again, LRT was used to test for significance.

### Correlations between compositional and functional stability properties

To test whether there are some predisposed correlations between functional and compositional aspects of stability, and whether disturbance can modify these correlations (H3), first we calculated the pair-wise correlation coefficient (Pearson rho) between the same stability properties for function and composition. We first calculated the correlations across all treatments to look for the general correlation between functional and compositional stability. Then, we calculated the correlation coefficients for the single treatments. Eventually, we fitted GLMMs (Gaussian distribution) using the correlation coefficient as the response variable, the stressors and their interactions as fixed effects, and the organism group as random effect. LRT was used to test for significance.

All statistical analyses were conducted using R (version 3.5.1; R Core Team, 2018) in Rstudio (2020). GLMMs were run using the package lme4 (Bates et al., 2015). For all fitted models, the model diagnostics included tests of the normality of residuals. When testing for significance, we always considered  $p$ -values  $< 0.05$  as significant.



**FIGURE 1** Graphical representation of the dimensionality of stability (a, b and c) and schematic representation of how the four stability aspects were measured (d) after Hillebrand et al 2018. The multidimensional ellipsoids represent a system with high dimensionality—spheroid shape (a) and a systems with low dimensionality – “cigar” shape (b) in a 3-dimensional space (thus, representing only 3 stability properties). Their relative axis lengths are shown in (c), where dots represent the semi-axis length of the system with high dimensionality (spheroid), and triangles represent the semi-axis length of the system with low dimensionality (“cigar”). In (d), a = resistance, b = resilience, c = recovery and (d) = residuals used to calculate temporal invariability

## RESULTS

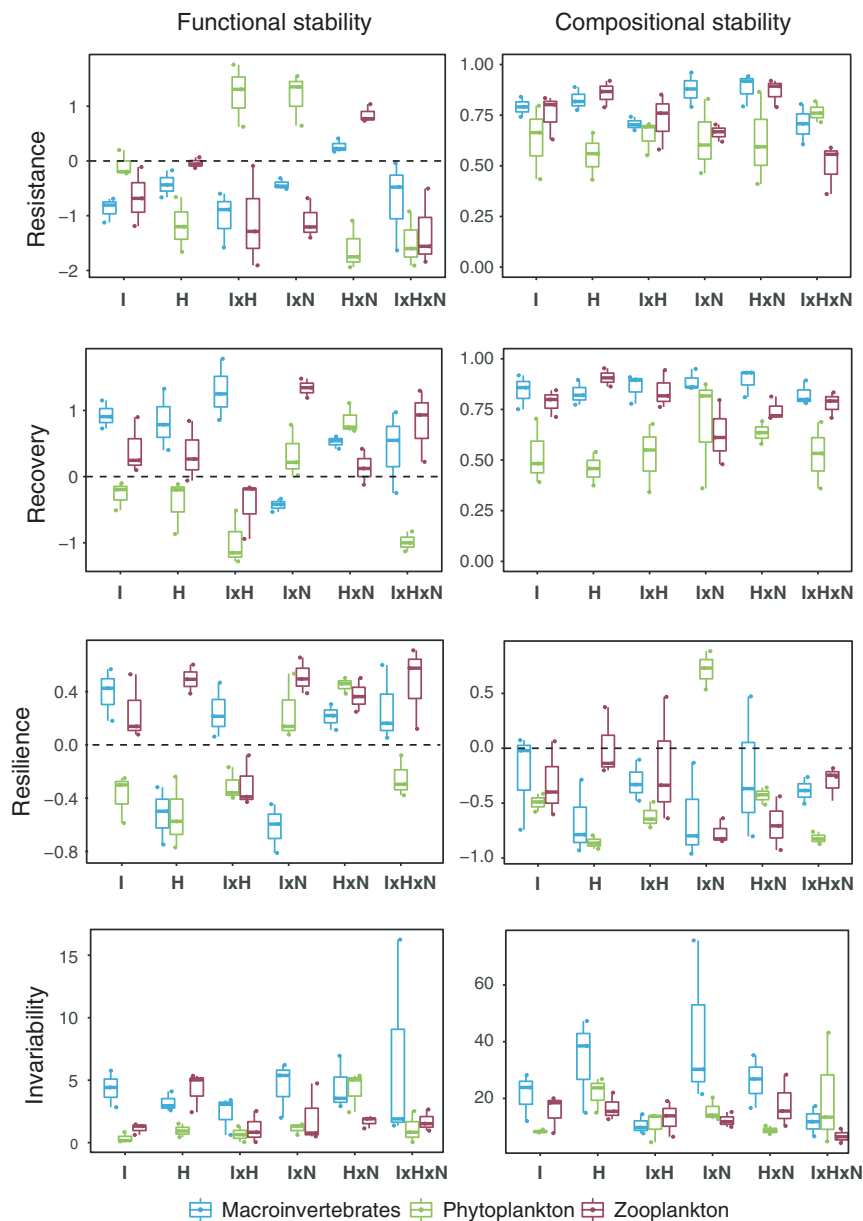
### Effects of disturbance on individual stability properties and on the dimensionality of stability

#### *Non-enriched systems*

Phytoplankton functional resistance and invariability were both impaired by the herbicide, whereas the mixture of the insecticide and the herbicide improved phytoplankton resistance (Figure 2, Table S2). Zooplankton functional recovery and resistance were impaired by the pesticide mixture (Figure 2, Table S2). Macroinvertebrates functional

resistance was impaired by the insecticide, whereas resilience was significantly dampened by the herbicide treatment. Phytoplankton compositional resistance and recovery were reduced by the herbicide treatment, as well as by the pesticide mixture. Zooplankton compositional resistance and recovery were significantly impaired by the insecticide treatment. Macroinvertebrates compositional resistance was reduced by the insecticide treatment, whereas the herbicide impaired compositional recovery and resilience. Overall, compositional resilience of macroinvertebrates was higher in systems treated with the pesticide mixture.

Disturbance type did not significantly affect the ellipsoid volume for what concerns composition in



**FIGURE 2** Box and whisker plots showing how the four dimensions of stability (resistance, recovery, resilience and invariability) responded to disturbance. Functional responses were based on abundance data, whereas compositional responses were based on community composition (Bray—Curtis similarity). Please note that functional resistance and recovery use a different scale than compositional resistance and recovery (see Table 1). I: chlorpyrifos; H: herbicide; IxH: mixture insecticide—herbicide; N: nutrients enrichment

non-enriched systems (Figure 3, Table S4,  $p$ -values always  $>0.05$ ). The dimensionality was influenced by pesticides for functional stability in non-enriched systems. The insecticide significantly increased the ellipsoid volume ( $\chi^2 = 13.0$ ,  $p < 0.001$ ), whereas the herbicide decreased it ( $\chi^2 = 6.63$ ,  $p = 0.01$ ). The pesticide mixture behaved non-additively ( $\chi^2 = 11.07$ ,  $p < 0.001$ ).

Two out of the six pair-wise correlations were affected by disturbance in non-enriched systems for compositional stability (Table S8). For functional stability, only the correlation between resistance and invariability was modified by the pesticide mixture.

### Nutrient-enriched systems

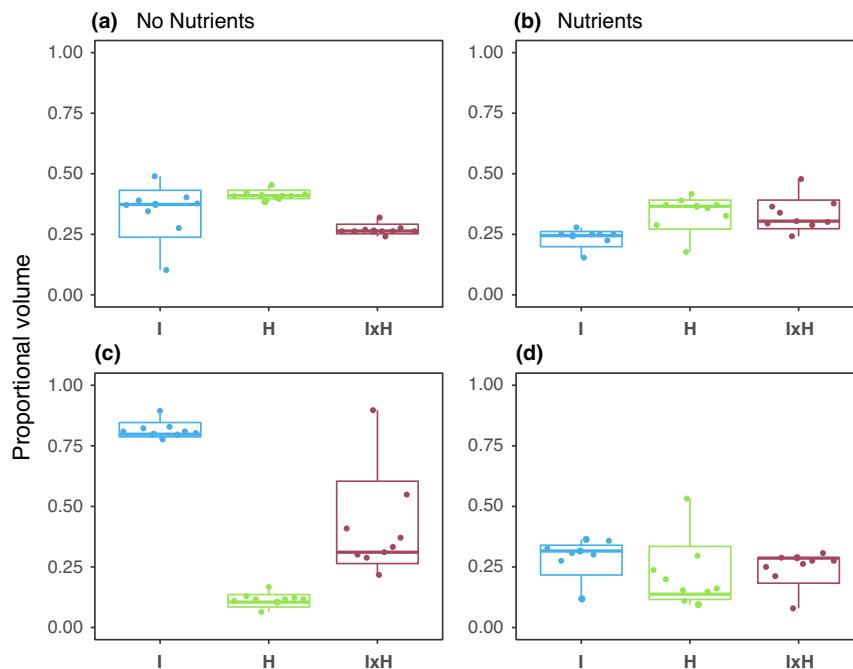
In enriched systems, phytoplankton functional resistance was significantly decreased by the herbicide treatment, while recovery and resilience were decreased only by the mixture of pesticides. Functional invariability was increased under the herbicide treatment, whereas the insecticide significantly decreased it. Zooplankton functional resistance was impaired by the insecticide, whereas functional recovery and resilience were significantly higher in systems treated with the insecticide or the pesticide mixture. Macroinvertebrates functional resistance and recovery were decreased by the insecticide treatment, whereas systems treated with the herbicide showed higher resilience. Phytoplankton compositional recovery was impaired by the herbicide.

Zooplankton compositional resistance was significantly lower under the insecticide treatment, whereas it was significantly increased by the herbicide. The insecticide also significantly impaired the compositional recovery of zooplankton, whereas the mixture of the pesticides boosted both compositional recovery and resilience. Zooplankton compositional invariability was reduced by the insecticide, but increased by the herbicide. Macroinvertebrates compositional resistance was increased by the herbicide treatment, but reduced by the pesticide mixture. Macroinvertebrates compositional recovery was increased significantly by the herbicide treatment.

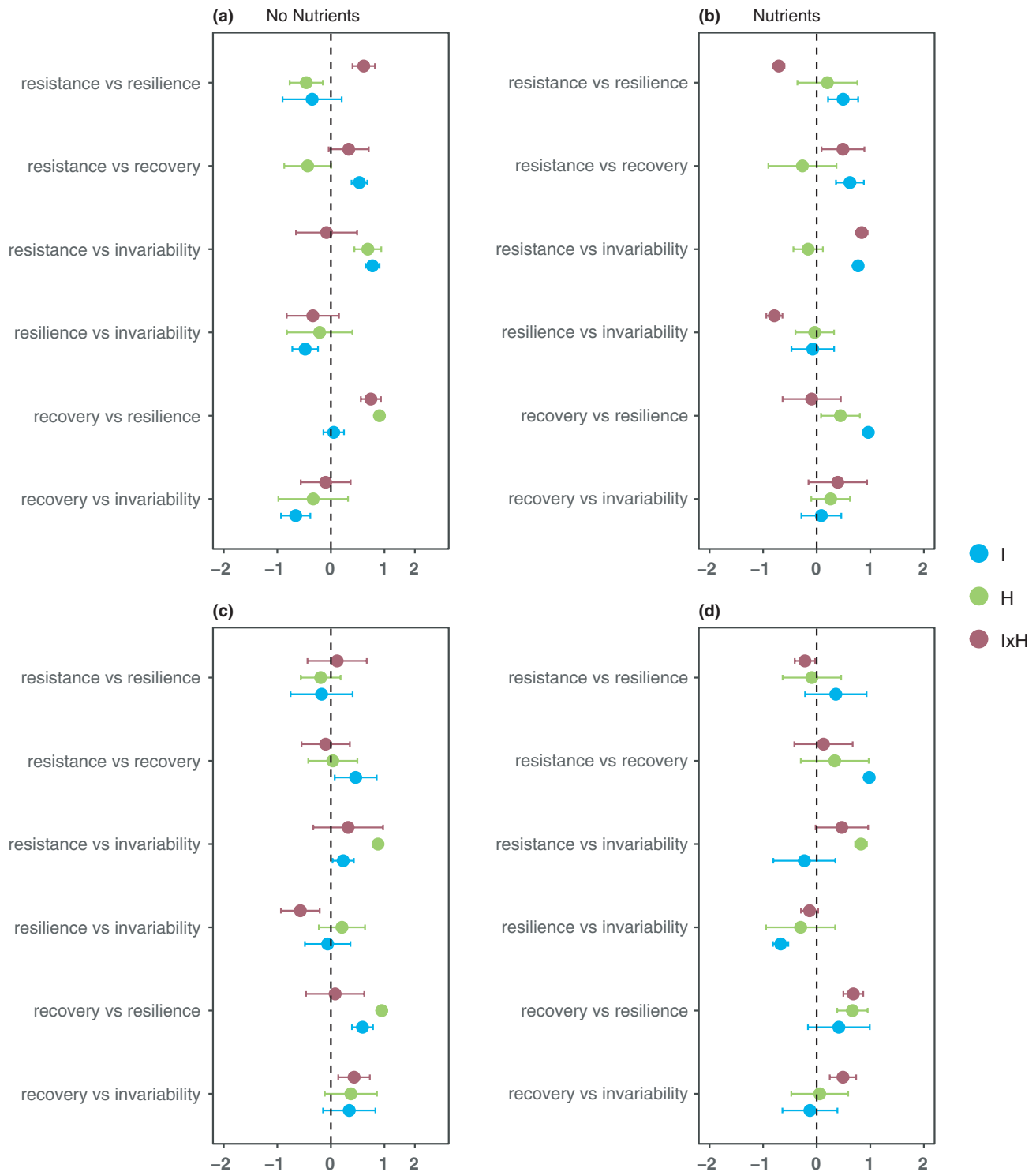
Conversely to non-enriched systems, we did not find any difference in the ellipsoid volumes of functional stability in nutrient-enriched systems. No differences were found for compositional stability either.

For composition stability, four out of the six stability parameter correlations were influenced by disturbance in the nutrient-enriched systems (Table S8). As for functional stability, the correlation between resistance and invariability was modified by the insecticide, which shifted the correlation from generally positive to negative.

Overall, we found consistent trends in the correlation between different aspects of stability across enriched and non-enriched systems (Figure 4). Resilience and resistance were generally positively correlated, and negative values were often related to significant effects of the treatments. Recovery and resilience showed a positive consistent correlation across treatments,



**FIGURE 3** Proportional ellipsoid volumes under different disturbances at the community level. Plots (a) and (b) show the ellipsoid volumes calculated for compositional stability, (c) and (d) show the ellipsoid volumes calculated for functional stability. Plots on the left side show the results of the analysis for non-enriched systems, whereas those on the right side show the results of the enriched ones. I: chlorpyrifos; H: herbicide; IxH: mixture insecticide - herbicide



**FIGURE 4** Correlation between aspects of stability at the community level for composition (a, b) and function (c, d). Plots on the left (a, c) show correlations for non-enriched systems. Plots on the right (b, d) show correlations for nutrient-enriched systems. I: insecticide; H: herbicide; IxH: mixture insecticide—herbicide

deviating only with a significant disturbance effect. Accordingly, resistance and recovery were positively correlated in most of the cases. Resistance and invariability were also generally positively correlated, while resilience and invariability were mostly negatively

correlated. Recovery and invariability were generally positively correlated.

To sum up, differences in the effects of the disturbance between enriched and non-enriched systems on single stability properties emerged for functional recovery and



resilience, which were improved in enriched systems. No clear differences between enriched and non-enriched systems appeared for single compositional stability properties, nor for functional resistance and invariability. When looking at the effects of disturbance on functional DS, differences were clear. Functional DS of non-enriched systems was modified by single and combined disturbances, whereas functional DS of enriched systems was never affected by disturbance. No effect of disturbance was detected on compositional DS regardless of the nutrients status.

### Effects of disturbance on the correlation between compositional and functional stability

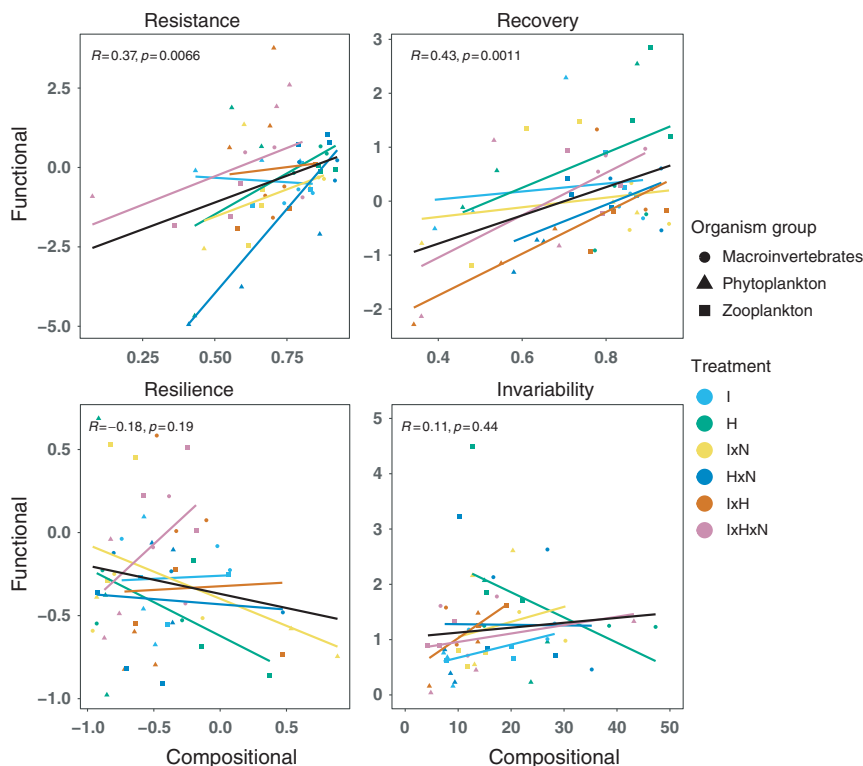
Overall, we found all correlation between composition and functional stability to be positive, with the exception of resilience, which was non-significantly negatively correlated ( $R = -0.18, p = 0.19$ ). Deviations from this general trend were associated to a significant effect of disturbance. In non-enriched systems, only one correlation between the compositional and functional invariability was affected by the herbicide ( $\chi^2 = 5.89, p = 0.01$ ) and the mixture of the pesticides ( $\chi^2 = 5.24, p = 0.02$ , Figure 5, Table S12). In enriched systems, the correlation between functional and compositional resistance was modified

by the insecticide ( $\chi^2 = 27.69, p < 0.001$ ), and invariability was affected by the herbicide ( $\chi^2 = 5.32, p = 0.02$ ) and the mixture of the pesticides ( $\chi^2 = 5.04, p = 0.03$ , Figure 5, Table S12).

## DISCUSSION

### Multiple stressors effects on the dimensionality of stability

We investigated whether and how pesticides affecting different trophic levels modify individual stability properties and the overall DS. We found that the pesticides affected the single stability properties of the different organism groups (H1) according to their expected sensitivity. That is, phytoplankton's stability properties were mainly affected by the herbicide application, whereas macroinvertebrates and zooplankton were significantly affected by the insecticide. Non-additive effects of the pesticides' mixture were found for all stability properties for functional and compositional stability. Indirect effects of the herbicide were also found to be significant on several stability properties of zooplankton and macroinvertebrates. Likewise, the insecticide significantly impaired some stability properties of phytoplankton. These significant indirect effects suggest that bottom-up



**FIGURE 5** Correlations between the same aspects of compositional and functional stability. Each dot represents one sample. Coloured lines represent the correlation within a single treatment, whereas the black line represents the correlation between compositional and functional aspects of stability across all treatments. Each plot shows the calculated Spearman correlation coefficient ( $R$ ) across the treatments and the corresponding  $p$ -value. I: insecticide; H: herbicide; IxH: mixture insecticide—herbicide; N: nutrients enrichment

and top-down effects play a large role in shaping both functional and compositional stability at the community level (White et al., 2020). Moreover we found consistent differences between enriched and non-enriched systems for functional stability properties across organism groups. Generally, functional recovery and resilience were higher in nutrient-enriched systems compared to the non-enriched ones, while no clear differences were noted for compositional stability properties between the two nutrient availability scenarios.

Our study shows that neither the pesticides applied individually nor as a mixture influenced the dimensionality of compositional stability, in line with theoretical predictions (Radchuk et al., 2019). Inversely, pesticides did influence DS of functional stability (confirming H1). The insecticide significantly increased functional DS, and the joint application of the insecticide and the herbicide resulted in a higher functional DS compared to the herbicide alone. The herbicide treatment showed the smallest functional DS across all the measurements.

DS has been suggested to be driven by two mechanisms: changes in species interactions (Radchuk et al., 2019) and stochasticity (Yang et al., 2019). DS is commonly lower in systems with strong competitive interactions, as competitive interactions are usually more deterministic (Radchuk et al., 2019). Conversely, more stochastic species interactions likely result in weaker pair-wise correlations among stability properties, thus leading to higher DS (Radchuk et al., 2019; Yang et al., 2019). In our experiment, the herbicide treatment reduced the abundance of phytoplankton (Figure 2), which may have increased the strength of competition among consumers, leading to lower DS. Simultaneously, the herbicide reduced phytoplankton richness and increased dominance (Table S15). Communities sharing a limited number of traits (high dominance) have low resource use efficiency (Hodapp et al., 2019). Hence, the increase of dominance led to an increase in competitive interactions, contributing to the overall lower DS. Conversely, the insecticide and the mixture of pesticides may have reduced the strength of the interactions by reducing the abundance of sensitive consumer species, leading to an increase of DS. Sustaining this hypothesis, the phytoplankton abundance was not increased by the insecticide treatment. However, phytoplankton richness increased in the insecticide treatment, leading to an increased resources use efficiency and, thus, to a lower competition and DS.

We found consistent trends in the correlations between stability properties (see Results section), which are in line with those reported in other experimental and theoretical studies (Donohue et al., 2013; Radchuk et al., 2019). Most deviations from those trends were associated to a significant effect of the pesticides on the correlation between different stability properties. All stressors (single or multiple) were able to modify those correlations differently. This is, single pesticide or pesticide mixtures can modify the nature of the correlations between stability properties, which has

important ecological implications (Donohue et al., 2013). For instance, resistance and recovery were always positively correlated across the treatments, both for compositional and functional stability, as by definition, the smaller the initial change, the higher is the likelihood of recovery (Hillebrand & Kunze, 2020; Hillebrand et al., 2018). Yet, herbicide disturbance decoupled such positive correlation for compositional stability, regardless of nutrient status (Figure 4). This suggests that the features promoting the resistance of community composition to disturbance may, sometimes, be negatively related to the characteristics promoting recovery from perturbation and vice versa. This is in line with previous studies, in which features promoting both compositional and functional resistance under disturbance were found to be negatively correlated with recovery (Baert et al., 2016b; Isbell et al., 2015).

### **Nutrients enrichment and multidimensional stability**

Interestingly, functional DS was modified by the treatments only in non-enriched systems, while in nutrient-enriched systems, no significant differences in DS among treatments were detected (H2). The lack of pesticide effects in the DS in nutrient-enriched systems might be explained by the fact that their effect on abundance was lower compared to the non-enriched ones (Table S15-S17). Nutrients are known to reduce the negative effects of a wide range of disturbances on biomass and abundance, from fine sediment addition (Matthaei et al., 2010; Piggott et al., 2015b, a) to chemicals (Halstead et al., 2014), as a result of enhanced primary productivity and increased energy fluxes. Nutrients enrichment acted as an environmental filter, reducing diversity and promoting the dominance of tolerant species, less sensitive to additional disturbances, such as pesticides (Roessink et al., 2005). Based on our results we conclude that, in eutrophic systems, pesticide disturbances may not cause a significant change in functional stability, allowing the use of a restricted number of metrics (e.g. one or two) to quantify systems' stability. Conversely, the conservation of mesotrophic or oligotrophic ecosystems impacted by pesticides may need to consider specific actions targeting different stability properties (i.e., resistance, recovery, resilience, invariability) independently from each other.

Since our measurement of functional stability is based on abundance as state variable, the lower effect of the pesticides on abundance in nutrient-enriched systems may have kept DS lower and similar between treatments, despite a general reduction in species richness. Although these results seem to suggest a negative correlation between diversity and stability, we claim that, in this specific case, the higher functional stability observed in less diverse systems was not related with the total number of species. Actually, the change in species identity and the consequent dominance effect (see Fox, 2005) caused by

the nutrients promoted a community which is functionally more stable (Baert et al., 2016a). Therefore, our study suggests that the turnover rate in species identities and the dominance effect, rather than differences in species diversity, may drive the observed negative correlation between stability and diversity. Moreover stochasticity may have contributed to determine the difference in DS found between nutrient-enriched and non-enriched systems (Yang et al., 2019). Indeed, more diverse communities are expected to have more unpredictable dynamics, and thus be characterised by higher stochasticity compared to species poor communities, whose dynamics are largely regulated by the dynamics of the dominant species (Reuman et al., 2006; Yang et al., 2019). A previous study has shown that communities with lower stochasticity have lower DS under disturbance (Radchuk et al., 2019). Consistently, our results show that nutrient-enriched systems (lower diversity and, thus, lower stochasticity) had generally lower functional DS.

### **Effects of disturbance type on the correlation between compositional and functional stability**

In line with other studies, we found mostly positive correlation between compositional and functional aspects of stability (Hillebrand & Kunze, 2020; Hillebrand et al., 2018). Resistance and recovery showed a significant positive correlation across the treatments, suggesting that if compositional stability stays constant after disturbance application, high levels of functional stability will be maintained. Similarly, our results show that communities recovering their composition, will likely recover their function as well. Conversely, resilience and invariability did not show significant correlations between functional and compositional aspects of stability. Compositional resilience had negative values in most cases, indicating that community's composition further departed from the control after disturbance application. In contrast, functional resilience was often positive, suggesting that abundance can be recovered rapidly by communities that progressively diverged compositionally. Studies on ecological restoration as well as experiments on pulse disturbances have provided similar examples showing that functional recovery can be achieved even in absence of compositional recovery (Borja et al., 2010; Hillebrand et al., 2018). This generally faster functional recovery compared to compositional recovery forces us to consider the time-dependency of stability responses to disturbance, across which our predictive capacity still requires further research (Garnier et al., 2017; White et al., 2020; Yang et al., 2019).

Our study shows that the correlation between functional and compositional resistance can be modified by disturbance type. The generally positive correlation between functional and compositional resistance was significantly shifted by the insecticide only in

nutrient-enriched systems. This is because our systems were rich in species and the press disturbance exerted by the addition of nutrients selected, from the large initial pool, tolerant species which could maintain high functional level, even if community composition was severely compromised by disturbance (Allison & Martiny, 2008). Theoretical and empirical studies have shown that despite large changes in community composition, ecosystem functions can be maintained (Allan et al., 2011; Hautier et al., 2014; Yachi & Loreau, 1999). In our systems, the compensatory dynamics of tolerant species, which benefited by the nutrients enrichment and the decreased competition or predation promoted by the joint effect of the insecticide and the nutrients, maintained high functional levels despite a strong compositional deviation to control systems. A single mesocosm experiment is obviously insufficient to determine universal rules about the nature of the correlation between functional and compositional stability. However, our study suggests that nutrient enrichment plays a fundamental role in this relationship for species rich communities.

### **Recommendations for further research**

To date, the majority of available studies have focused on DS at the community level and on species poor systems (Kéfi et al., 2019). Our study is one of the first using species-rich systems containing several trophic levels to quantify DS in its multidimensional conception. More experimental work is needed to unravel the mechanisms driving disturbance effects on DS under different ecological scenarios. In this regard, meaningful diversity manipulations across different trophic levels would greatly benefit the long-standing debate on how and to what extent biodiversity can influence ecosystem stability. Furthermore, which state variable(s) is the most appropriate to quantify multidimensional stability, especially for functional aspects, is still an open question. Indeed, functional stability's properties are mostly measured using biomass or abundance as state variable, which relate mainly with the functioning of the food-web, rather than to the ecosystem functioning processes. We argue that measuring functional stability using several ecosystem processes as state variables could be more exhaustive, and would allow to study the relationship between the multiple dimensions of compositional stability and ecosystem multifunctionality (Pennekamp et al., 2018). Thus, we recommend future investigations to use ecosystem (multi)functioning processes, such as chlorophyll-a concentration, organic matter decomposition, respiration, or nutrients up-take, to quantify functional stability in a multidimensional way.

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## CONFLICT OF INTEREST

The Authors declare no conflict of interest.

## AUTHORS CONTRIBUTION

AR and FP designed the study. FP performed the statistical analysis and wrote the first draft. AR contributed substantially to the revision.

## PEER REVIEW

The peer review history for this article is available at <https://publons.com/publon/10.1111/ele.13770>.

## DATA AVAILABILITY STATEMENT

The authors state that, should the manuscript be accepted, the data supporting the results will be archived in an appropriate public repository (Dryad, Figshare or Hal) and the data DOI will be included at the end of the article.

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## SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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