Non-interactive effects drive multiple stressor impacts on the taxonomic and functional diversity of atlantic stream macroinvertebrates

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ABSTRACT

Freshwaters are considered among the most endangered ecosystems globally due to multiple stressors, which coincide in time and space. These local stressors typically result from land-use intensification or hydroclimatic alterations, among others. Despite recent advances on multiple stressor effects, current knowledge is still limited to manipulative approaches minimizing biological and abiotic variability. Thus, the assessment of multiple stressor effects in real-world ecosystems is required.

Using an extensive survey of 50 stream reaches across North Portugal, we evaluated taxonomic and functional macroinvertebrate responses to multiple stressors, including marked gradients of nutrient enrichment, flow reduction, riparian vegetation structure, thermal stress and dissolved oxygen depletion. We analyzed multiple stressor effects on two taxonomic (taxon richness, Shannon-diversity) and two trait-based diversity indices (functional richness, functional dispersion), as well as changes in trait composition.

We found that multiple stressors had additive effects on all diversity metrics, with nutrient enrichment identified as the most important stressor in three out of four metrics, followed by dissolved oxygen depletion and thermal stress. Taxon richness, Shannon-diversity and functional richness responded similarly, whereas functional dispersion was driven by changes in flow velocity and thermal stress.

Functional trait composition changed along a major stress gradient determined by nutrient enrichment and oxygen depletion, which was positively correlated with organisms possessing fast-living strategies, aerial respiration, adult phases, and gathering-collector feeding habits.

Overall, our results reinforce the need to consider complementary facets of biodiversity to better identify assembly processes in response to multiple stressors. Our data suggest that stressor interactions may be less frequent in real-word streams than predicted by manipulative experiments, which can facilitate mitigation strategies. By combining an extensive field survey with an integrative consideration of multiple biodiversity facets, our study provides new insights that can help to better assess and manage rivers in a global change context.

1. Introduction

The Anthropocene is characterized by an unprecedented impact of human activities on ecosystems (Tong et al., 2022). Freshwater ecosystems are considered among the most endangered globally (Mouton et al., 2020), as evidenced by the disproportionate percentage of endangered species inhabiting freshwaters (WWF et al., 2020). There is a growing consensus that freshwater degradation is caused by multiple co-occurring impacts (hereafter multiple stressors), which coincide in time and space (Jackson et al., 2021; Birk et al., 2020). These local stressors typically result from land-use intensification, hydroclimatic alterations or invasive species, among other pressures (Reid et al., 2019). Despite increased advances in multiple stressor research (Orr et al., 2020), current knowledge on multiple stressor effects is still

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limited because the focus on manipulative approaches constrains the realism (Spears et al., 2021) and the lack of consistent responses across stressor combinations (Jackson et al., 2016). To address this challenge, recent calls highlighted the urgency of intensifying efforts to capture multiple stressor effects in real-world ecosystems (Gutiérrez-Cánovas et al., 2022).

When aquatic organisms are simultaneously exposed to various stressors, their responses are difficult to predict from individual stressor effects (Feld et al., 2016). This occurs because stressor effects can add to each other (additive effect) or interact, resulting in combined effects that are larger (synergism) or smaller (antagonism) than the additive effect (Côté et al., 2016; Piggott et al., 2015a). To identify combined stressor effects on aquatic organisms, freshwater ecologists have conducted both manipulative and observational studies, with a clear dominance of the former (e.g., Jackson et al., 2016; Morris et al., 2022). Recent works synthesizing multiple stressor effects across published literature showed that additive and antagonistic interactions tend to be more common in freshwaters, but authors found a strong variability across types of experimental design, organisms and spatial scales (Birk et al., 2020). For example, interactive effects tend to be more common for invertebrates than for primary producers (Jackson et al., 2016) in manipulative or small-scale studies in comparison with those observational or performed at larger scales (Gutiérrez-Cánovas et al., 2022). For these reasons, there is a need to validate experimental findings with those obtained from naturally assembled communities, which offer more realism albeit at the cost of losing causality (Stewart et al., 2013). Under these circumstances, identifying consistent effects of stressor combinations across organisms and scales will help targeting more appropriate solutions to halt biodiversity loss and stream ecosystem degradation (Kelly et al., 2017; Spears et al., 2021).

Macroinvertebrates have been traditionally used to assess stream ecosystem health (Piggott et al., 2015b; Calapez et al., 2018) due to their diversity, ecological importance and contrasting tolerance to human impacts (Bonada et al., 2006). A myriad of indices has been developed to assess stream ecosystem health, mostly based on the taxonomic composition of the macroinvertebrate community (Birk et al., 2012). However, taxonomic-based assessments are bound to the regional species pool reducing their potential for generalization (Juvigny-Khenafou et al., 2021). To overcome this limitation, functional trait-based approaches are receiving increasing attention, since traits are broadly shared across different areas and organisms (Kuzmanovic et al., 2017; Juvigny-Khenafou et al., 2021). Functional traits and their diversity are also useful to better understand mechanisms underlying organism responses to stressors (Cadotte et al., 2009; Voß and Schäfer, 2017) and their effects on ecosystem functioning (Gutiérrez-Cánovas et al., 2021). Despite these promising advantages, functional diversity can show different responses to stressors ranging from situations where responses are similar to taxonomic diversity, to others where they are different (Flynn et al., 2009; Bruno et al., 2016), highlighting the complementarity of information these metrics supply (Legras et al., 2018). The similarity in response of taxonomic and functional diversity strongly depends on functional redundancy (Baker et al., 2021). This prediction lies in the idea that communities with many functionally similar taxa (high functional redundancy) may show taxonomic declines but remain functionally diverse (Biggs et al., 2020). In contrast, communities dominated by functionally unique taxa may display similar rates of taxonomic and functional diversity loss in response to stressors; but note that more scenarios are possible (Flynn et al., 2009). However, it still remains unclear if stressor accumulation gives rise to similar taxonomic and functional diversity responses, and if traits that confer resistance to a major stressor (e.g., nutrient enrichment) enable the tolerance to additional stressors (Pallarés et al., 2017).

By following an empirically-based approach, this study aims to evaluate macroinvertebrate responses to multiple stressors. To do this, we gathered taxonomic and functional trait information of macroinvertebrates for 50 Atlantic stream sites covering realistic stress gradients across North Portugal (Minho region). This comparison allowed us to test if functional diversity can provide complementary information compared to that obtained by taxonomic diversity as a result of directional trait filtering, as expected by ecological theory (Poff et al., 2006; McGill et al., 2006). We first investigated the consistency of combined stressor effects on both taxonomic (taxon richness, Hill Shannon diversity) and trait-based diversity metrics (functional dispersion, functional richness). Second, we explored major changes in functional trait structure of macroinvertebrate over the stressor gradients to identify which traits are more likely to be lost due to multiple stressors. Our overarching hypothesis is that multiple stressors will give rise to different combined effects on taxonomic and functional metrics due to functional redundancy. Specifically, we expect that taxonomic diversity will show additive or synergistic responses as a result of the rapid loss of vulnerable taxa, whereas functional diversity would show antagonistic responses due to the stabilizing effects of functional redundancy. We also hypothesize that multiple stressors will drive changes in functional traits fostering strategies with increased resistance (e.g., aerial respiration, multivoltinism, less specialized feeding) and resilience (e.g., short lifespan, ovoviviparous reproduction) to disturbance.

2. Materials and methods

2.1. Stressor characterization

We surveyed 50 stream reaches in four river basins across the Minho region in the Northwest of Portugal (Appendix S1), during summer conditions (July–September 2020) to characterize five stressor gradients: 1) Nutrient enrichment, characterized by total dissolved inorganic nitrogen (DIN) and soluble reactive phosphorus (SRP). 2) Flow reduction, characterized by flow velocity and discharge. 3) Riparian vegetation structure, characterized by three indicators outlined in Pace et al., (2022): the riparian quality index (QBR); the mean riparian forest buffer width (BUFF) and the percentage of stream channel shading (shadow). 4) Thermal stress, characterized by the maximum daily temperature (TMAX) and 5) dissolved oxygen levels as the minimum daily dissolved oxygen (DOMin). More details on stressor characterization are reported in Table 1 and Appendix S2.

To overcome the incapacity of manipulating environmental variables and to reach extreme 'stressor' levels as in manipulative studies, our approach was to select sites with a wide distribution of environmental variables across the study area (i.e., including some of covariates related to different sources of natural variation (e.g., elevation). In addition, to

Table 1

Mean values and range (minimum and maximum) and relative stressor categories for the environmental variables used in the study.

Stressor category	Variables	Code	Range	Units
Nutrient enrichment	Dissolved inorganic nitrogen	DIN	1.12 (0.03–4.69)	mg L^{-1}
	Soluble reactive phosphorus	SRP	0.32 (0–2.82)	mg L^{-1}
Flow reduction	Discharge	Q	0.170 (0.001–1.731)	m^3 s^{-1}
	Average current velocity	mean_V	0.1 (0–0.3)	m s^{-1}
Riparian vegetation structure	Riparian vegetation width	BUFF	32.80 (0–272.5)	m
	Riparian quality index	QBR	54.80 (0–95)	–
	Stream channel shading	shadow	47.30 (0–100)	%
Thermal stress	Maximum daily temperature	TMAX	21.67 (13.9–28)	°C
Dissolved oxygen levels	Minimum daily dissolved oxygen	DOMin	6.92 (1.58–9.10)	mg L^{-1}

clarify if some of the stressors here considered (e.g. thermal stress; dissolved oxygen levels and flow reduction) were not related to natural differences among stream reaches (e.g. altitude, river types), reflecting a stressor effect as applied in manipulative studies, we tested for the effect of spatial covariation (e.g. altitude) on stressors types. We reported in Fig. S3 (Supplementary Material) the results of analysis of variance between stressors and categories of altitude, followed by Tukey post hoc comparisons (when significant differences occurred).

2.2. Macroinvertebrate data collection and calculation of diversity metrics

Macroinvertebrate communities were sampled during late summer (September–October 2020) using kick-net samples (mesh size: 500 μm) and a multi-habitat standardized protocol (INAG, 2008). All samples were preserved in 96% ethanol. We identified macroinvertebrate taxa to the genus level, whenever possible, and count individuals. In some cases, taxa were identified to family or subfamily level (e.g., some Diptera), or subclass (e.g., all Oligochaeta). This resulted in a total of 107,190 individuals identified encompassing 172 taxa (8 classes, 18 orders, 84 families, 152 genera).

To characterize the taxonomic diversity, we calculated two measures: taxon richness (Rich), representing the number of taxa in the community, and the Hill Shannon's diversity index (Hill H), indicating how abundance is distributed across taxa (Jost, 2007; Maturo, 2018). We also characterized two measures of functional-trait diversity (Appendix S3), including functional richness (FRic) that estimates the range of variation of traits within a community (Villéger et al., 2008), and functional dispersion (FDis), representing abundance-weighted variability in trait composition within a community (Laliberté and Legendre, 2010).

2.3. Statistical analysis

To evaluate multiple stressor effects and their relative influence on macroinvertebrate metrics, we adopted a multi-model inference approach based on linear regression models using a two-step approach (Burnham and Anderson, 2002; Grueber et al., 2011). First, for each response variable, we construct five alternative models, the first one including only additive terms (five predictors; Appendix S4), and the other considering an additional pairwise interaction (five predictors plus the interaction). We considered five plausible interactions (Jackson et al., 2016; Birk et al., 2020), where nutrient effects were modulated by temperature (DIN x TMAX) or shading (DIN x shading), oxygen depletion interacts with thermal stress (DOMin x TMAX) and flow velocity interacts with temperature (flow velocity x TMAX). After visual exploration of scatterplots, we added and tested a quadratic term of TMAX for all response variables. We retained the model with the lowest Akaike Information Criterion for small sample sizes (AICc), which was subsequently used in the second step.

Second, we used dredge(z) function (MuMIn R package, Barton, 2020) on the retained model, to produce a set of models including all potential predictor combinations (single and interactive terms, when apply), which were ranked based on their AICc values. We then retained a subset of those models, with the criteria ΔAICc ≤2 relative to the model ranked first (Zuur et al., 2010). For each model, we also derived the explained variance (R2) and Akaike weights to inform on the explanatory power and the relative likelihood of each model of being the best model within the dataset. As a measure of importance, we calculated the variance explained by each stressor (and stressor interactions) for each response variable. To do this, using model's Akaike weights, the mean-weighted partitioned variance was calculated for each model term (Hoffman and Schadt, 2016). In all models, we evaluated the combined effects based on model coefficients and visual interpretation (Feld et al., 2016; Segurado et al., 2018). For the visual interpretation, we plotted the combinations of the first three stressors with the higher relative explained variance of the diversity metrics (Figs. 2 and 3).

Finally, to understand if macroinvertebrate trait composition respond to one single gradient or to multiple stressor gradients, we used a redundancy analysis RDA (Appendix S5).

To reduce distribution skewness, functional richness, dissolved inorganic nitrogen, soluble reactive phosphorus and average current velocity were squared-root-transformed; taxon richness, stream discharge and riparian vegetation width were log-transformed, whereas the stream channel shading was logit-transformed before the analyses. All variables were z-standardized (mean = 0, SD = 1) to allow the comparison of model coefficients. In all cases, model residuals were visually assessed to verify linear regression model assumptions (Zuur et al., 2009). All statistical analyses were executed in R 3.5.1 (R Core Team, 2020).

3. Results

3.1. Responses of macroinvertebrate taxonomic and functional diversity to multiple stressors

Taxon richness (Ric), Hill Shannon diversity (Hill H) and functional richness (FRic) showed similar responses to the multiple stressor gradients, whereas functional dispersion (FDis) exhibited a different pattern (Fig. 1). Nutrient enrichment (DIN) was the stressor that best explained taxon richness (r^2 = 36.7%), Hill Shannon diversity (r^2 = 42.1%) and functional richness (r^2 = 27.7%). Thermal stress, which had a non-linear effect, was the second best explanatory variable for those diversity metrics (taxon richness r^2 = 20.5%; Hill Shannon r^2 = 9.5%; functional richness r^2 = 13.5%), followed by minimum daily dissolved oxygen (taxon richness r^2 = 9.4%; Hill Shannon r^2 = 7.2%; functional richness r^2 = 7.3%). Functional dispersion (FDis) was better explained by flow reduction (r^2 = 20%), followed by thermal stress (r^2 = 8.4%) and nutrient enrichment (DIN; r^2 = 6.3%). The ranking of retained linear models (ΔAICc ≤2) to explain each diversity (taxonomic and functional) metric is in Table A4.

The analysis of variance between stressor variables and classes of altitude revealed only marginal effect of stream type (altitude) on DIN and TMax (Fig. S3, Supplementary Material).

Multiple stressors showed additive effects on diversity metrics, with pairwise interactions having low statistical support. All diversity variables decreased with increasing dissolved inorganic nitrogen concentrations and decreasing dissolved oxygen (Figs. 2 and 3, Table A4, Supplementary Material). Whereas taxonomic and functional richness increased with flow reduction (Table A4, Supplementary Material), Hill Shannon diversity and functional dispersion showed a negative response. Shadow had a positive effect on taxonomic richness, Hill Shannon diversity and functional richness, but it was less frequently included in the retained models. All diversity metrics displayed a hump-shape responses to maximum daily temperature (Fig. 2c,f; Fig. 3c,f).

3.2. Changes in macroinvertebrate trait structure along a gradient of multiple stressors

The derived stressor gradient (RDA axis 1; Fig. 4 and Appendix S5) favored fast-living strategies (PLURIVO), aerial respiration (SPIR), adult phases (IMAGO), and gathering-collector feeding habits (GAT). Conversely, univoltinism (UNIVO), crawling mobilities (CRAW), shredder feeding (SHR), aerial active dispersion (AERACT) and reproduction by cemented or fixed clutches (CEMCLU) and cemented isolated eggs (CEMEGG), were favored by low nutrient concentration, higher dissolved O_2 and by shadow.
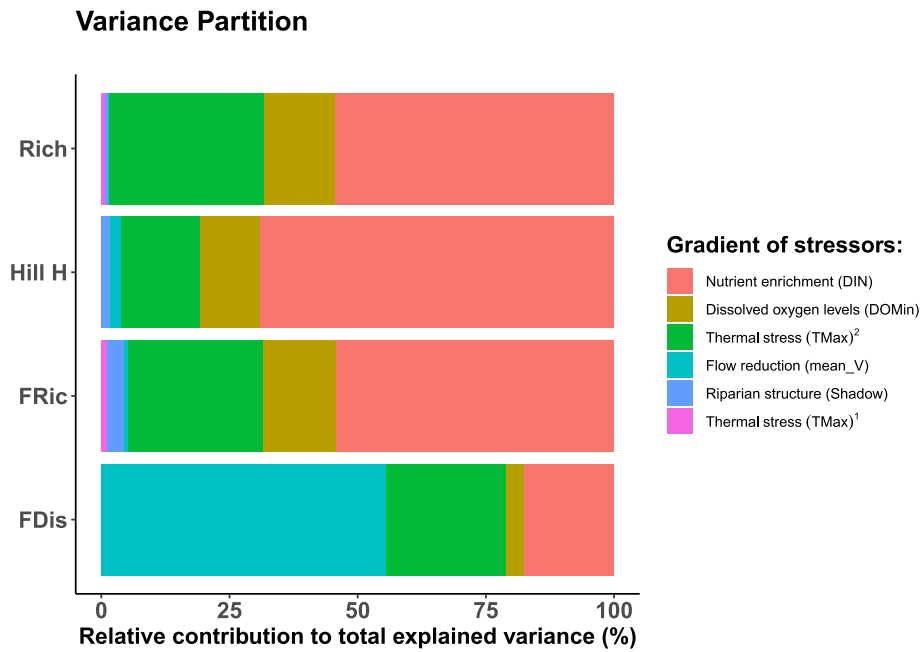
Variance Partition

Fig. 1. Relative contribution of multiple stressors to explain macroinvertebrate taxon richness (Ric), Hill Shannon diversity (Hill H), functional richness (FRic) and functional dispersion (FDis). Stressor labels: DIN = dissolved inorganic nitrogen concentration; DOMin = minimum daily dissolved oxygen; mean_V = flow velocity; shadow = stream channel shading percentage; Tmax[1] = maximum daily temperature - linear term; Tmax[2] = maximum daily temperature – quadratic term.
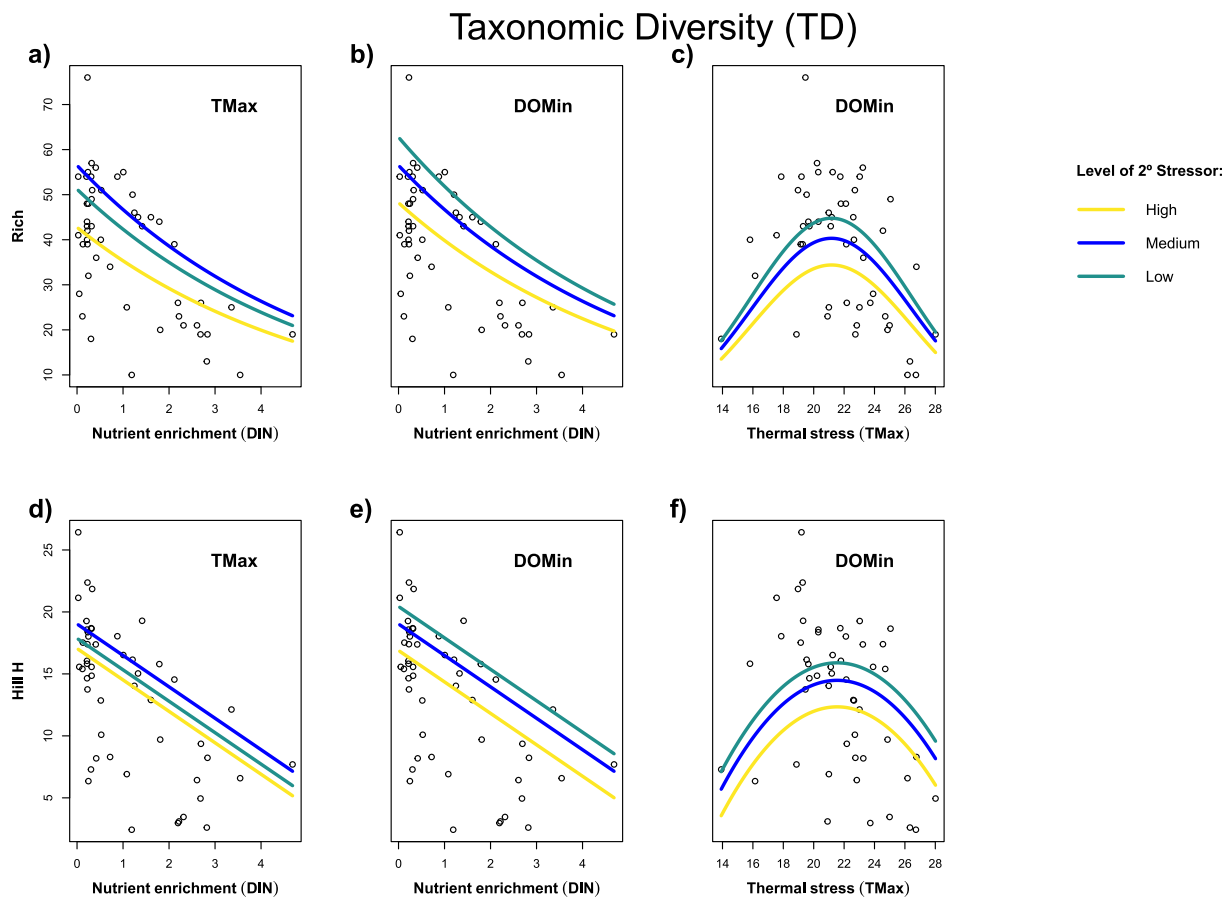
Taxonomic Diversity (TD)

Fig. 2. Responses of macroinvertebrate taxonomic richness (Rich) and Hill Shannon diversity (Hill H) to pairwise combinations of DIN, DOMin and TMax, as second stressor indicated in the top right corner. Coloured lines represent responses to the stressor showed at x axis in relation with low (Q10), intermediate (Q50) and high levels (Q90) of a second stressor. Relative to DOMin the highest stress values are reached when the dissolved oxygen values are lower. Response type distribution to the main stressor: Exponential-Logarithmic distribution (a) and (b); Quadratic distribution (c) and (f); Linear distribution (d) and (e).
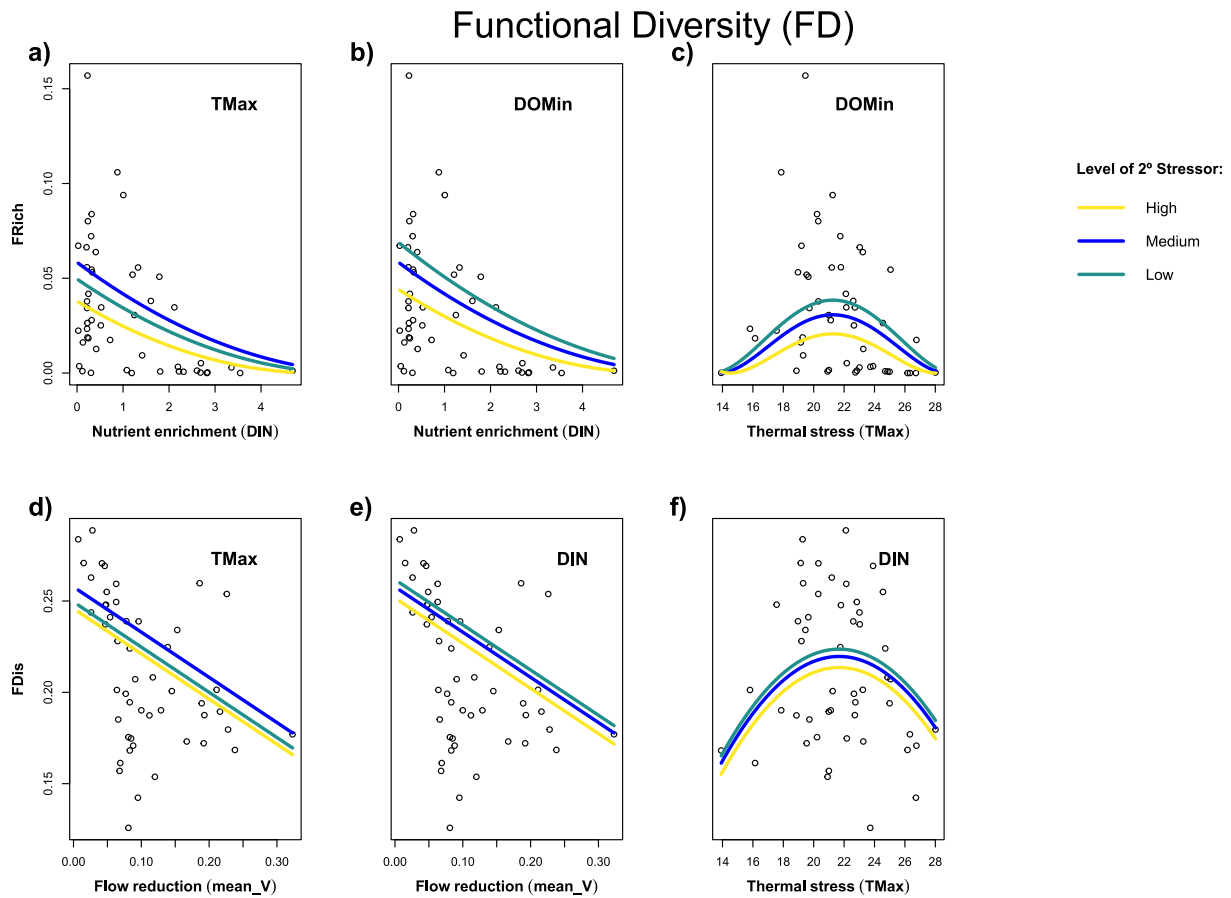
Functional Diversity (FD)

Fig. 3. Responses of macroinvertebrate functional richness (FRic) and functional dispersion (FDis) to pairwise combinations of mean_V; DOmin, DIN and TMax, as second stressor indicated in the top right corner. Coloured lines represent responses to the stressor showed at x axis in relation with low (Q10), intermediate (Q50) and high levels (Q90) of a second stressor. Relative to DOmin the highest stress values are reached when the dissolved oxygen values are lower. Response type distribution to the main stressor: Exponential-Logarithmic distribution (a) and (b); Quadratic distribution (c) and (f); Linear distribution (d) and (e).
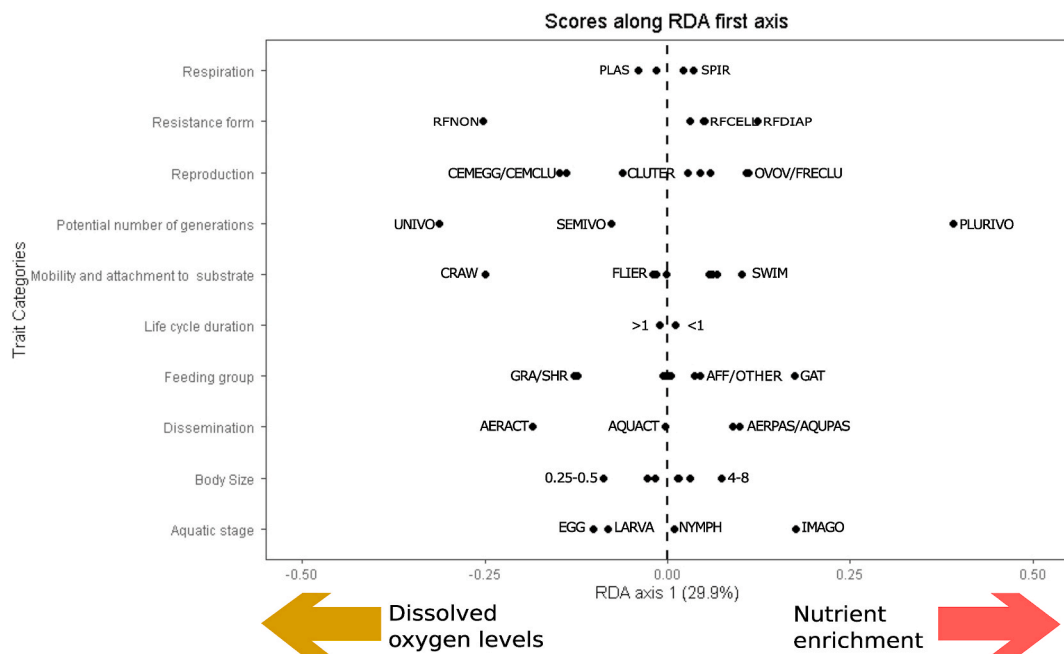

Fig. 4. Scores of trait categories over the first RDA axis. Positive correlations with RDA axis 1 indicate those traits associated with nutrient enrichment and oxygen depletion (details in Appendix S5). For trait category labels, see Table A2.

4. Discussion

4.1. Comparison of taxonomic and functional responses to multiple stressors

We found that taxonomic and functional richness responded similarly to multiple stressors, whereas functional dispersion displayed a different pattern. In all cases, we found that stressors caused additive effects in all diversity metrics, not supporting our initial hypothesis. These patterns indicate similar taxonomic and trait losses in response to multiple stressors, and suggest low functional redundancy (Flynn et al., 2009). This may imply that, in our study area, macroinvertebrates tend to have unique trait combinations. These low functional overlap among taxa can reduce community's capacity to functionally compensate taxon losses (Coccia et al., 2021). Similar patterns were observed in Northern Europe (Heino, 2005; Eros et al., 2009), in which freshwater communities were represented by a few conspecific species and showed a limited functional redundancy. However, our results strongly contrast to those found in Central Europe (Schmera et al., 2012; Voß and Schäfer, 2017) and in Tropical rivers (Liu et al., 2021; Espinoza-Toledo et al., 2021), where taxonomic diversity indexes showed higher sensitivity to stressors than functional diversity measures, suggesting higher functional redundancy. Therefore, further inter-regional studies are needed to investigate how stressors and other natural drivers shape functional redundancy, and thus the similarity between taxonomic and functional responses across biogeographic contexts. Future studies should address how trait selection can influence the identification of responses to multiple stressors.

As opposed to general trends (Jackson et al., 2016), our study showed little support for interactive effects between multiple stressors. Recent synthesis showed that non-interactive responses tend to be more common in observational than in experimental (manipulative) studies (Birk et al., 2020; Gutiérrez-Cánovas et al., 2022). The spatial temporal modes of stressor action in short-term experiments *vs* real-word conditions, and the greater capacity to recover of naturally-assembled communities could explain discrepancies between manipulative and observational studies. For example, treatments may be applied at a constant rate and over a short term (e.g., Piggott et al., 2015b), whereas in observational studies, stressors may have different spatial temporal effects (Lake, 2000; Jackson et al., 2021). In addition, acute effects can be more common in experimental designs where responses are measured after a short-term exposure (Spears et al., 2021), whereas organisms are able to recolonize habitats after pulse stressor effects in naturally-assembled communities (Schäfer et al., 2011). Conversely, the behavior of organisms at experimental scales does not fully account for the spatial temporal heterogeneity in natural habitats, where organisms can find small-scale refuges (Mac Nally, 2000). In addition, stressors are applied simultaneously in experiments, whereas they might not overlap in time in natural communities (Côté et al., 2016; Jackson et al., 2021), giving room for recovery. Another possible explanation for the lack of interactive observed effects, could be related to the effect of taxonomic resolution and to the nature of metrics/indices tested in our study (i.e., community-level indices; Kath et al., 2018). Considering that inside the macroinvertebrate community there is a high diversity of inter- and intra-family life-history traits, the interactive effects might have occurred at finer taxonomic or population scales, which we were unable to detect, but this should be addressed in future studies.

Besides the usual impact of nutrient enrichment (Grizzetti et al., 2017; Lemm et al., 2021), our study demonstrates that thermal stress and dissolved oxygen depletion also play a relevant role in taxonomic diversity and functional richness. Because of the ectothermic nature of macroinvertebrates, temperature may induce pronounced impact on life cycle characteristics, such as embryonic development, growth, diapause and emergence (Bonacina et al., 2022). Oxygen depletion tends to extirpate macroinvertebrates with aquatic respiration (e.g., tegument, gills), unless they have adaptations to cope with hypoxia (e.g.,

Chironomus plumosus; Nagell and Landahl, 1978). These results indicate that taxonomic declines are accompanied by the loss of extreme trait combinations affecting trait hypervolumes, on which functional richness depends.

In contrast, functional dispersion tends to provide a complementary description of community changes in mean trait profiles (Laliberté and Legendre, 2010). Small rivers, with low and intermediate flow velocities can offer a richer range of habitats, which foster a wider trait diversity. In contrast, high flow velocities can reduce habitat diversity (e.g. loss of pools), being less suitable for organisms with diving and skating mobility (Dolédec et al., 2007; Buffagni, 2021). Nonetheless, extremely low velocities can also constrain functional diversity by reducing habitat diversity (Bae and Park, 2016) and by extirpating taxa that depends on flowing conditions (Piano et al., 2020; Arias-Real et al., 2021).

Overall, our results reinforce the need to consider different facets of biodiversity (taxonomic and functional) to better identify assembly processes in response to multiple stressors (Boersma et al., 2016). Here, we show that this complementary exists even within functional diversity metrics, representing different aspects of the trait space.

4.2. Changes in trait profiles over gradients of multiple stressors

We observed that changes in functional trait structure of macroinvertebrate communities occurred over a major stress gradient, associated with nutrient enrichment and oxygen depletion (Kuzmanovic et al., 2017), suggesting similar effects on trait structure (Appendix S5). As expected, RDA ordination plot showed that the increase in the major stress gradient tended to select organisms with fast-living strategies, aerial respiration, imago (aquatic adult stage), and gathering-collector feeding habits (Fig. 4 and Fig. S2). Plurivoltine organisms, with high reproductive outcomes, frequently increases with human impacts because they can easily recover from population declines (Walsh et al., 2005) and often have trait combinations that allow them to resist to stressful conditions (Cooke et al., 2019). In addition, aerial respiration confers advantages in highly impacted habitats with episodic or chronic hypoxic conditions (Calapez et al., 2018). Adverse conditions may also induce facultative dispersal capacities, favouring taxa with adult aquatic stages (i.e., coleopterans and hemipterans), which have high colonization ability (Coccia et al., 2021), desiccation resistance (Pallarés et al., 2016) and physiological plasticity (Botella-Cruz et al., 2021). The increase of gatherer and filterer feeding modes over the stress gradient can be related to the higher amounts of deposited and suspended fine particulate organic matter in impacted streams (Yadamsuren et al., 2020; Erasmus et al., 2021). Furthermore, our results showed that the stress gradient was associated with organisms with larger body sizes, resistance forms, swimming mobility and passive dispersal. However, this can be a result of trait syndromes that co-occur with other traits that confer resistance to nutrient enrichment and oxygen depletion (Meyer et al., 2022).

4.3. Implications for biomonitoring and management

Our findings reinforce the need to consider multiple stressors in river biomonitoring and management. We showed that, besides classical target stressors such as nutrient enrichment, variations in thermal regimes, oxygen content and flow velocity could alter different facets of taxonomic and functional diversity of river macroinvertebrates. This is particularly important given the predicted increases in temperature and hypoxic conditions due to global change, that can potentially affect the current stream assessment systems (Haubrock et al., 2020). In addition, considered together, our results imply that biomonitoring indexes, mostly relying on diversity metrics, can be able to detect cumulative stressor effects when they act additively (Herrero et al., 2018). Thus, further effort is needed to develop stressor-specific metrics that help to diagnose the major cause of degradation and disentangle the relative effects of specific stressors (Lemm et al., 2019). Specifically, our results

highlight that functional diversity metrics can provide complementary information to taxonomic diversity, which is typically used in biomonitoring programmes. Based on our results, functional dispersion can be considered as a complementary biomonitoring metric, which can be more responsive to hydrological changes than functional richness and taxonomic metrics (Laini et al., 2022).

Also, our study is novel in demonstrating that the cumulative effects of multiple stressors captured with observational studies contrast with the results obtained in manipulative studies, urging caution when scaling results up to the real world. However, it is important to note that variability in the abiotic factors considered in this study can be attributed to both natural (e.g. climate and elevation) and anthropogenic drivers (e.g. land-use intensity). Despite this limitation, our approach allowed us to capture wide gradients of abiotic factors, which allows for a greater capacity to identify potential combined effects on stream biota (Schäfer and Piggott, 2018; Segurado et al., 2022). In addition, the observational nature of our approach allows us to consider wider spatiotemporal dynamics in stressor effects and macroinvertebrate communities (Jackson et al., 2021), which might be overlooked in short-term experiments, that allows for a more integrative assessment of multiple stressors hierarchy (Markert et al., 2022).

From a management perspective, our results suggest that the studied stressors can be addressed individually, in our study area, without a risk of ecological surprises. In this case, restoration efforts should prioritize reducing nutrient enrichment (Birk et al., 2020), whenever possible, but increasing dissolved oxygen and reducing extremely high temperatures can also provide benefits when necessary. In this sense, riparian restoration in small streams seems to be a good strategy to reduce nutrient inputs and to buffer thermal extremes (Feld et al., 2018).

Finally, our integrative analysis suggests that climate change can further degrade rivers exposed to land-use intensification through warming (Bonacina et al., 2022), oxygen depletion (Calapez et al., 2018) and hydropeaking (Leiva et al., 2022), so that concerted and additional efforts is required to maintain rivers within a good ecological status, especially in a global change context.

Statement on inclusion

Our study brings together authors from a number of different countries, including scientists based in the country where the study was carried out. All authors were engaged early on with the research and study design to ensure that the diverse sets of perspectives they represent was considered from the onset.

Credit author statement

J. Lourenço: Conceptualization, Data collection, Invertebrate identification, Data curation, Formal analysis, Methodology, Writing – original draft, Writing – review & editing. C. Gutiérrez-Cánovas: Conceptualization, Data collection, Data curation, Formal analysis, Methodology, Writing – original draft, Writing – review & editing. F. Carvalho: Conceptualization, Writing – original draft, Writing – review & editing. F. Cássio: Conceptualization, Supervision, Writing – review & editing. C. Pascoal: Conceptualization, Supervision, Writing – original draft, Writing – review & editing, funding. G. Pace: Conceptualization, Data collection, Invertebrate identification, Data curation, Formal analysis, Methodology, Writing – original draft, Writing – review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envres.2023.115965.

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