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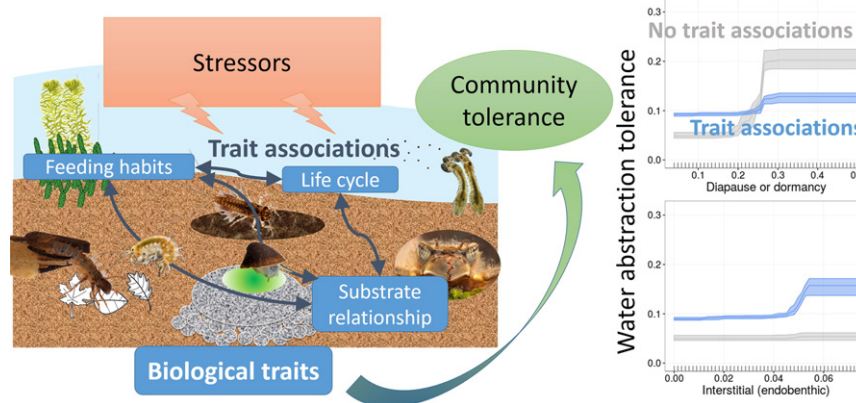
Life-history strategies constrain invertebrate community tolerance to multiple stressors: A case study in the Ebro basin

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HIGHLIGHTS

- Multiple stressors are a serious threat to aquatic ecosystems and communities.
- Life-history strategies reflect adaptation to given environmental conditions.
- Predict community tolerance using trait combinations and boosted regression trees
- Obtain efficient models where trait associations affect community tolerance
- Trait combinations and association are the basis of stressor-specific tolerance.

GRAPHICAL ABSTRACT



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ABSTRACT

Context: Multiple stressors constitute a serious threat to aquatic ecosystems, particularly in the Mediterranean region where water scarcity is likely to interact with other anthropogenic stressors. Biological traits potentially allow the unravelling of the effects of multiple stressors. However, thus far, trait-based approaches have failed to fully deliver on their promise and still lack strong predictive power when multiple stressors are present.

Goal: We aimed to quantify specific community tolerances against six anthropogenic stressors and investigate the responses of the underlying macroinvertebrate biological traits and their combinations.

Methods: We built and calibrated boosted regression tree models to predict community tolerances using multiple biological traits with *a priori* hypotheses regarding their individual responses to specific stressors. We analysed the combinations of traits underlying community tolerance and the effect of trait association on this tolerance.

Results: Our results validated the following three hypotheses: (i) the community tolerance models efficiently and robustly related trait combinations to stressor intensities and, to a lesser extent, to stressors related to the presence of dams and insecticides; (ii) the effects of traits on community tolerance not only depended on trait identity but also on the trait associations emerging at the community level from the co-occurrence of different traits in species; and (iii) the community tolerances and the underlying trait combinations were specific to the different stressors.

Conclusion: This study takes a further step towards predictive tools in community ecology that consider

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combinations and associations of traits as the basis of stressor tolerance. Additionally, the community tolerance concept has potential application to help stream managers in the decision process regarding management options.

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1. Introduction

Freshwater ecosystems are subjected to multiple human pressures and stressors, placing them among the most threatened ecosystems (Vörösmarty et al., 2010). Indeed, interactions between different types of stressors (related to water quality, hydromorphology and water scarcity), which are rather common in these ecosystems, can produce unexpected patterns and consequently make their management difficult (Ormerod et al., 2010). In Europe, the Water Framework Directive (European Council, 2000) aims to incite member states to reach “good ecological status” for all surface waters by 2027. However, such a goal appears complicated to attain within the desired time frame because the first River Basin Management Plans (EEA, 2012; ETC-ICM, 2012) have characterized >50% of the European rivers as being in a less than “good ecological status”. The main pressures acting on rivers and streams include point and diffuse pollution (e.g., increased nutrient loads that often jointly occur with fine sediment and pesticide inputs) and hydromorphological alterations (e.g., water abstraction, flash floods, weirs, straightening, removal of riparian vegetation). These co-occurring stressors obviously act on communities through complex interactions, and the understanding of such interactions is one of the many challenges that remain to be addressed (Hering et al., 2010). To respond to the increasing societal demands associated with this particular challenge, the following European research projects have been initiated in the last few years: SOLUTIONS (Brack et al., 2015), MARS (Hering et al., 2015) and GLOBAQUA (Navarro-Ortega et al., 2015). In a context of climate change, water scarcity and interactions with other human stressors are key issues, especially in sensitive areas, such as those surrounding the Mediterranean Sea (Ludwig et al., 2011). As a result, the EU-funded GLOBAQUA project aims to understand how multiple human stressors, including water scarcity, interact to modify freshwater biodiversity and ecosystem functioning (Navarro-Ortega et al., 2015).

Based on the hypothesis that environmental conditions act as a template on which evolution forges specific combinations of organism attributes (Southwood, 1977; Townsend and Hildrew, 1994), species traits have been presented as a promising tool that can provide a mechanistic interpretation of the stressor effects on communities (Statzner and Bêche, 2010). Therefore, trait-based approaches (TBA) can potentially unravel the effects of multiple stressors acting simultaneously on communities (Dolédéc and Statzner, 2008; Lange et al., 2014; Mondy and Usseglio-Polatera, 2013; Statzner and Bêche, 2010; Wooster et al., 2012). However, the concrete delivery of this promising objective is still to come (Statzner and Bêche, 2010). Most of the current studies using TBA have often lacked strong predictive power and have failed to adequately reflect species-environment relationships (Ings et al., 2009). Verberk et al. (2013) have argued that the following two main issues could explain why there is such failure in most current TBAs: (i) the lack of consideration of the co-occurrence of different traits (however, see Küster et al., 2008) and (ii) the need of more hypothesis-driven investigations (however, see Arce et al., 2014; Bonada et al., 2007a; Dolédéc and Statzner, 2008; Pollard and Yuan, 2010). As a first issue, species sorting, one of the mechanisms occurring during community assembly, does not operate on single traits but rather on whole organisms (Townsend and Hildrew, 1994; Verberk et al., 2008a). Therefore, it makes sense that using combinations of biological traits should be the next TBA development to efficiently address the issue of multiple stressors (Verberk et al., 2013), whereas many TBA studies have used multiple individual traits in their attempts to reveal response patterns of individual traits to global pressure levels (Dolédéc et al., 2006;

Mondy et al., 2012), to individual stressors (Brabec et al., 2004; Rabeni et al., 2005) or to stressor combinations (Dolédéc and Statzner, 2008; Townsend et al., 2008). Moreover, the response of a given trait to a given stressor in a community may depend on the other traits possessed by the organisms (trait association or trait context-dependence; Verberk et al., 2013), potentially masking or blurring the expected trait responses (Resh et al., 1994; Statzner et al., 1997). As a second issue, linking trait responses to multiple stressors should rely on *a priori* hypotheses based on the mechanistic effects of each stressor on organisms (Statzner and Bêche, 2010). Life-history strategies represent detailed trait combinations that reflect adaptation to given environmental conditions, e.g., general responses to disturbances (Verberk et al., 2008a). However, the number of life-history strategies to specify can rapidly grow because of the number of traits and their combinations when considering multiple stressors having different modes of action on organisms. Therefore, the definition of such life-history strategies and their adaptive value in specific environmental conditions is a challenging task (Verberk et al., 2013).

In this study, we aimed at using combinations of biological traits to predict the tolerance of invertebrate communities to specific individual stressors. We considered multiple traits and took into account trait association at the community level. We also defined *a priori* the direction (i.e., increase or decrease) of trait responses according to given stressors, based on the habitat template theory (Southwood, 1977; Townsend and Hildrew, 1994) and the literature about the responses of macroinvertebrate traits to stressors (e.g., Baird and van den Brink, 2007; Bonada et al., 2007b; Buendia et al., 2013; Dolédéc and Statzner, 2008; Lange et al., 2014; Mondy and Usseglio-Polatera, 2013; Statzner and Bêche, 2010), while limiting the complexity of manually defining the individual life history strategies corresponding to the different stressors. More specifically, we tested (i) whether combinations of biological traits constrained the community tolerance to six stressors related to water quality, hydrology and hydromorphology (Hypothesis H1); (ii) whether the consideration of trait associations can modify the stressor tolerances (H2); and (iii) whether the community tolerances and the underlying trait combinations are stressor-specific (H3).

2. Material and methods

2.1. Study area

The Ebro River catchment, the largest in Spain, has a drainage basin of 85,550 km² (Sabater et al., 2009; Fig. 1) and is subjected to multiple stressors. In particular, numerous dams and channels alter the hydrological regime and sediment loads within the river catchment (Muñoz and Prat, 1989). Ground and surface water abstraction for agriculture irrigation and industrial activities have compromised water demand and quality. Additionally, pollution from nutrients and organic compounds is a concern in the middle and lower reaches of the river that worsens during low flow conditions.

2.2. Data

2.2.1. Fauna

Macroinvertebrate data recorded during the period 2006–2013 were retrieved from the water agency ‘Confederación Hidrográfica del Ebro’ (CHE) website (<http://www.datosuperficiales.chebro.es>). The initial faunistic data set comprised 414 sites, 1508 sampling events (i.e., site × date) and covered 148 taxa. We removed sites without information about river identity or type. We also discarded some faunistic

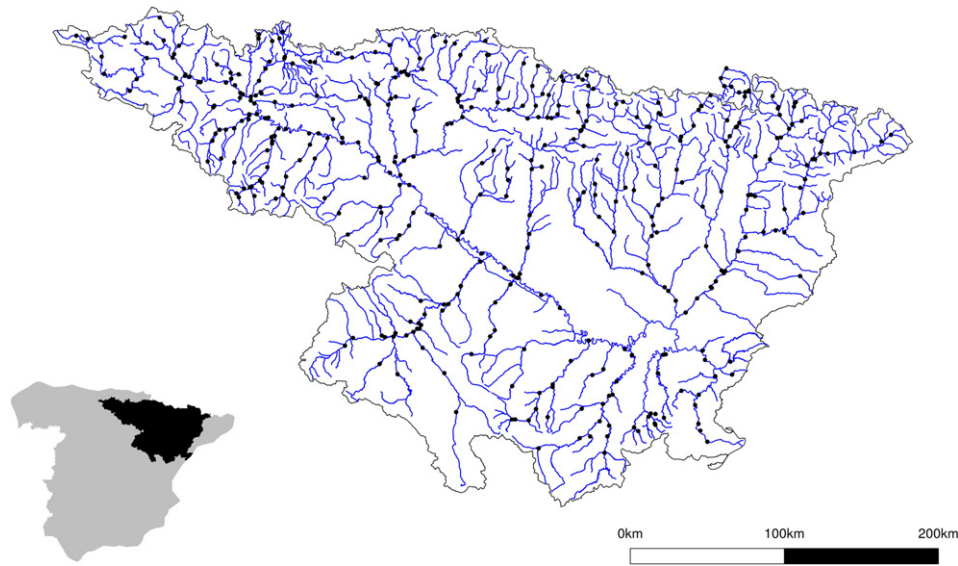


Fig. 1. Location of the study sites in the Ebro catchment.

data because they were collected using a sampling protocol different from that applied in the majority of cases. Our final faunistic data set covered 411 sites and 1505 sampling events.

Spanish monitoring of macroinvertebrates was mostly performed using family level identification and in rare cases using genus or species level or even class level identification. We harmonized the taxonomic levels to the family level and discarded 25 taxa due either to insufficient taxonomic resolution (*i.e.*, higher than the family) or to missing trait information (see below).

2.2.2. Traits

We used available information on 11 biological traits for European taxa (mainly genera; Tachet et al., 2010) with some adaptation for the Mediterranean region (Bonada and Dolédec, 2011; Bonada et al., 2007a; Statzner et al., 2007). These biological traits describe morphology, life history, feeding habits, resistance or resilience potential and respiration types. The different traits were described by 61 categories, and the affinity of each taxon for each trait category was quantified using fuzzy-coding (Chevenet et al., 1994). Taxa trait profiles were generated as frequency distributions of categories within each trait. For taxa with a higher level (*i.e.*, family) than the one given in the trait database (usually the genus), we calculated the trait profiles of each taxon by averaging the profiles of the genera reported as present in Spain.

For each sampling event, we further calculated the community-aggregated trait profile by averaging the taxa trait profiles weighted by their corresponding log₁₀-transformed abundances as commonly performed (see *e.g.*, Gayraud et al., 2003). We then applied arcsine-square root transformation to improve the normality of trait affinity distribution (Ahrens et al., 1990).

Stressors — We defined a stressor in this study as any environmental factor that exceeds natural levels of variation and causes a measurable biological or ecological response (Crain et al., 2008; Statzner and Bêche, 2010). The following six stressors were investigated: nutrients, suspended organic matter (SOM), insecticides, water abstraction, dams and physical alteration of the stream.

The water quality data retrieved from the CHE website (see fauna section) covered three stressors (nutrients, SOM and insecticides) and 37 individual parameters. Suspended organic matter concentration was directly measured, and we log₁₀-transformed values to approach a normal distribution of variables. The number and identity of the remaining parameters, which were actually measured for nutrient and insecticide stressors, were highly variable depending on the sampling sites and dates. To consolidate our data set, we synthesized the values

of the different water quality parameters in one global measure for nutrients and one for insecticides. This was performed by adapting the ‘Toxic Unit’ (TU) principle (Sprague, 1970) to a more general situation than the evaluation of toxic effects (*pseudoTU*, Eq. (1)).

$$pseudoTU = \log_{10} \left(\frac{observation}{threshold} \right) \quad (1)$$

The selected threshold depended on the stressor. For nutrients we used the High-Good ecological quality threshold obtained from the French water quality grids (SeqEau v2; Oudin and Maupas, 2003); for insecticides, we used the lowest value among (i) NOEC values from the INERIS (<http://www.ineris.fr/substances/fr/>) and (ii) LC50 from either INERIS or Malaj et al. (2014), further divided by ten to approximate NOEC values. To summarize the water quality for each stressor, we calculated the maximum *pseudoTU* among all the parameters measured at each site. The maximum was used instead of the sum because of the different number and identity of measured parameters across sites. Moreover, maximum TU has been reported to have explanatory power at least similar to the summed TU regarding community endpoints (Lies and Von der Ohe, 2005; Schäfer et al., 2011).

The available descriptors related to the hydrological regime in our study sites were derived from GIS data obtained from the CHE geoportal (<http://iber.chebro.es/geoportal/>) and GLOBAQUA partners (the Catalan Institute for Water Research, Girona, Spain and Ludwig-Maximilians-Universität, Munich, Germany). For each site we considered precipitation (mm/year), and we took into account the total area of dam reservoirs, the area of lands irrigated with different intensities (low, medium and high) and the numbers of different types of water abstraction points (spring, well, in-stream and others) within the catchment to which a site belonged. To allow a relevant comparison among sites with different catchment sizes, the data were transformed by (i) dividing the count data (water abstraction points) by the log₁₀-transformed area (in km²) of the corresponding catchment and (ii) dividing the area data (dam’s reservoirs, irrigated cultures) by the area of the corresponding catchment and then applying the arcsine-square root transformation to improve the normality of their distributions.

The transformed dam’s reservoir area was considered to be an individual stressor whereas precipitation and the other transformed hydrological variables were further processed by Principal Component Analysis (PCA) to synthesize the information into one variable describing water abstraction. PCA was performed on the largest set of sites with complete information for the hydrological variables, *i.e.*, 402 sites. We

considered the first two components that together explained 74% of the total variance. The first component reflected the number of water abstraction points, whereas the second component corresponded to a gradient of irrigation (Fig. S1). We allocated to each site its maximum score obtained on either of these two components to obtain a synthetic value of water abstraction and because these two components were positively correlated with the different descriptors of water abstraction.

The physical habitat integrity of reaches was evaluated using the Spanish river habitat index (IHF; Pardo et al., 2002) that takes into account variables such as riffle frequency, openness of the stream bed, flow velocity and depth regime, substrate diversity, heterogeneity of organic elements (e.g., presence of leaves, wood), primary producers and substrate inclusion in riffles and sedimentation in pools. Because this index describes the quality of the habitat, increasing values correspond to decreasing stress intensity. Therefore, we transformed the index values by subtracting them from the maximum potential score (100) to obtain a descriptor of the hydromorphological alteration.

2.2.3. Trait responses

Based on the habitat template theory and existing literature on invertebrate trait responses to stressors, we defined a set of model constraints for each stressor consisting of tuples of trait categories/response types (Table S1). The response types were coded by a numerical value as follows: tolerant (1) or sensitive (−1) trait categories. A tolerant trait category frequency increases in the community with stressor intensity whereas a sensitive trait category frequency decreases with stressor intensity. If the expected trait category response to increasing stressor intensity was not monotonic or if the stressor induced opposed expected effects on traits, then the response type was coded “0” and the model algorithm was allowed to find the trait response that best fit the data.

2.3. Modelling and data analysis

As a preliminary step, the stressor data were matched against the fauna data by their site codes and, eventually, the dates at which the different analyses were performed. If abiotic data were available for a site in a one-year period around the faunistic sampling event (six months before and after), we used the median of the observations over this one-year period; otherwise, we used the median of all observations made for the site over the period 2006–2013.

For each stressor, we built a boosted regression tree (BRT) model using sample stressor intensity as the response and a selection of trait categories associated with their *a priori* defined constraints (see Table S1) as predictors. BRT modelling uses the technique of ‘boosting’ to combine large numbers of rather simple tree models to adaptively optimize predictive performances of a global model. Boosting is a forward procedure where trees are iteratively fitted to the data used to build the model (i.e., training data) to increase the fit of the observations modelled poorly by the existing collection of trees. BRT models then linearly combine the results of all the trees and thus can be considered a regression model with each tree representing a regression term (Elith et al., 2008). BRT models are built using the following parameters: (i) the number of individual trees (*nt*), (ii) the fraction of the training data randomly selected to grow each tree (bag fraction: *bf*), (iii) the complexity of the trees (*tc*) that reflects the potential number of associated traits (i.e., an additive model is characterized by a *tc* fixed to 1; Elith et al., 2008) and (iv) the learning rate (*lr*) that corresponds to the weight allocated to each individual tree in the complete model. For each stressor in this application, the BRT algorithm looks for the trait category combinations that best respond to a given stressor intensity. As a result, the model output may be interpreted as a score of community tolerance to a given stressor related to specific biological trait combinations.

Because of discrepancies in data availability among the different stressors (Table 1) and the need for data with complete stressor

Table 1

Summary of the stressor values used for the community tolerance analysis. NA: sample with no stressor information.

	Range	Mean (\pm sd)	NAs
Nutrients	0.00–2.35	0.52 (\pm 0.43)	147 (10%)
SOM	0.30–2.41	0.86 (\pm 0.32)	147 (10%)
Insecticides	1.00–4.84	2.90 (\pm 1.19)	1152 (77%)
Dams	0.00–0.72	0.07 (\pm 0.09)	0 (0%)
Water abstraction	−1.29–11.63	0.55 (\pm 2.09)	21 (1%)
Hydromorphology	5.00–100.00	34.44 (\pm 8.01)	4 (<1%)

information to develop the models, we considered one data set per stressor type by selecting the largest sample collection having no missing stressor information. As a result, these stressor-specific data sets differed both in size and in the identity of the samples included. For each stressor-specific data set, we used river types and stressor intensity as strata to randomly select 90% of the sites for training the corresponding model and kept the remaining 10% of the sites for testing.

2.3.1. Model calibration and training

BRT implementations allow (i) controlling for a certain number of parameters (see above) during model construction and (ii) analysing the results in an ecologically meaningful way (Elith et al., 2008). To calibrate BRT parameters, we followed a cross-validation (CV) procedure that improved the robustness of the models (i.e., to minimize the differences between model outputs and observations for data not used in the model training) (Elith et al., 2008). We tested combinations of the following parameters: *bf* (0.5, 0.75), *tc* (1, 2, 5, 7, 10) and *lr* (0.001, 0.005, 0.01). For each combination, we developed a CV-BRT model using 30,000 trees and then pruned this model to limit over-fitting, thus obtaining the corresponding *nt* parameter value. For each stressor, the calibrated parameter values corresponded to the combination of parameter values that allows the minimum CV error.

To obtain models allowing robust predictions and estimations of model uncertainty, the tolerance models consisted of ten models, each built using 90% of the training data. For each tolerance model, the outputs were then scaled between 0 (the least tolerant) and 1 (the most tolerant) to derive the community tolerance score.

2.3.2. Model analysis

For each tolerance model, we considered the following five properties:

- (i) **Performance:** we used the Pearson's correlation coefficient between the model outputs and the corresponding stressor intensities as a measure of the fit both for training (r_{training}) and test (r_{test}) data sets;
- (ii) **Robustness:** we used the sub-model outputs to fit a quantile linear regression model (using the 2.5 and 97.5 percentiles) identifying the boundaries of the 95% confidence interval of the model outputs. The robustness of the model was then estimated as the proportion of test samples with tolerances falling outside this 95% confidence interval;
- (iii) **Local sensitivity analysis:** we investigated the marginal influence of each trait category on the model outputs. This sensitivity analysis is called local because only the values of one trait category are varied at a time and the other trait categories are fixed to their mean values. We defined the “effect” of a trait category on community tolerance as the difference between the highest and the lowest model output values obtained during the sensitivity analysis along the observed range of the trait representation in communities. We performed this analysis using models considering (global effect) or not considering (main effect; i.e., a purely additive model shaped with the same calibrated parameter values except for *tc* fixed to 1) trait associations;
- (iv) **Trait association:** we assumed that the global effect of a trait

category corresponded to the sum of its main effect and the effect of the trait associations. If the global effect was larger than the main effect, the trait associations would increase this effect. As a result, the (synergistic) association strength (α_s ; Eq. (2)) was defined as the proportion of the global effect that came from trait associations. In contrast, if the global effect was smaller than the main effect, then the main effect of the investigated trait category was compensated by the other trait categories. In that case, the (antagonistic) association strength was defined as the (negative) proportion of the main effect lost because of trait associations;

$$\alpha_s = \begin{cases} \frac{\text{global} - \text{main}}{\text{global}} & \text{if } \text{global} > \text{main} \\ \frac{\text{global} - \text{main}}{\text{main}} & \text{if } \text{global} \leq \text{main} \end{cases} \quad (2)$$

- (v) **Stressor-specificity of community tolerances:** we evaluated the specificity of the community tolerances for the different stressors by computing the Pearson's correlation coefficient (a) among community tolerances for different stressors and (b) between community tolerances and the intensity of each of the different stressors.

All analyses were performed using the R software environment (R Core Team, 2016). The data and functions used are packed together in the R source package *CommunityTolerance* provided as Supplementary material S2. The R scripts, additional results and figures are provided in the document S3.

3. Results

3.1. Model calibration

Tree complexity was rather large in the calibrated models (mean \pm standard deviation: 7.83 ± 2.48 ; Table 2). This suggests that the individual effects of traits on community tolerance were strongly influenced by the numerous other traits possessed by individuals in the community, that they did not act independently and that their responses to stressors were not solely additive.

3.2. Model analysis

3.2.1. Performance

The representation of trait categories in communities was highly influenced by all stressors. Indeed, the different predicted community tolerances were highly correlated with the corresponding target stressor (*i.e.*, the stressor for which the model was built) intensities in the training data sets ($r_{\text{training}} = 0.81 \pm 0.10$ across stressors; Fig. 2 and Table 3).

Table 2

Calibrated Boosted Regression Tree parameter values. SOM: Suspended Organic Matter. See the text for further parameter description details.

	Number of trees	Bag fraction	Tree complexity	Learning rate
	(<i>nt</i>)	(<i>bf</i>)	(<i>tc</i>)	(<i>lr</i>)
Nutrients	5300	0.75	10	0.001
SOM	4200	0.75	10	0.001
Insecticides	900	0.75	5	0.005
Dams	6400	0.75	5	0.001
Water abstraction	700	0.75	10	0.01
Hydromorphology	4100	0.75	7	0.001

The correlations between community tolerance and stressor intensity were lower when considering the test data sets ($r_{\text{test}} = 0.50 \pm 0.19$; Table 3), especially for insecticide ($r_{\text{training}} = 0.83$; $r_{\text{test}} = 0.33$) and dam stressors ($r_{\text{training}} = 0.72$; $r_{\text{test}} = 0.33$).

3.2.2. Robustness

The models were rather robust (0.11 ± 0.08 ; *i.e.*, 11% of the test samples fell outside the 95% training confidence interval; Fig. 2 and Table 3), which suggests that they could provide reasonable confidence on their predictions using new data.

3.2.3. Local sensitivity analysis

The local sensitivity analyses documented the trait categories that, individually, allowed a larger community tolerance for the different stressors.

A brief summary of the trait categories that have the most influence on the community tolerance to each stressor is as follows:

- Nutrients (Fig. S8): increasing egg protection (increasing use of ovoviviparity and/or terrestrial clutches; decreasing use of cemented eggs), resilience abilities (increasing number of life cycles per year), drift and use of atmospheric oxygen (increasing use of plastron instead of gills as breathing structures);
- SOM (Fig. S9): increasing egg protection (increasing use of ovoviviparity and/or terrestrial clutches and decreasing use of cemented eggs), escape abilities (increasing use of drift; decreasing importance of interstitial organisms), use of atmospheric oxygen (decreasing use of teguments and increasing use of plastron as breathing organs) and changes in feeding habits (increasing use of fine detritus or sediment as food sources);
- Insecticides (Figs. 3 and S10): larger organisms, increasing importance of non-aquatic stages (decreasing representation of aquatic adults), dispersal capacities (increasing use of drift and flying dispersal; decreasing use of attachment behaviour/structures), resilience capacities (short life-span, several generations per year and/or asexual reproduction) and modification of trophic structure (decreasing use of fine particles and invertebrates as food sources);
- Dams (Fig. S11): increasing resistance to high flow (increasing use of attachment of the different life stages; decreasing importance of large organisms, swimming and crawling locomotion types), resilience (use of small organisms using interstitial habitat as a refuge) and modification of trophic structure (decreasing use of fine sediments and dead leaves as food sources);
- Water abstraction (Figs. 3 and S12): increasing egg protection (ovoviviparity), resilience capacities (dormancy/diapause, short life-span, asexual reproduction and/or use of interstitial habitat as a refuge) and specialization of respiratory organs (gills); and
- Hydromorphology (Fig. S13): increasing egg protection (increasing use of ovoviviparity; decreasing use of cemented eggs and clutches), use of atmospheric oxygen (increasing use of plastron and spiracles as breathing structures) and modification of feeding habits (increasing use of fine sediment and detritus instead of dead leaf litter).

3.2.4. Trait association

The consideration of trait association in the models improved performance ($r^{\text{no-association}} = 0.59 \pm 0.13$; $r^{\text{association}} = 0.78 \pm 0.10$; Table 3, see also Figs. S2 to S7).

Across all the stressors, the trait associations were, on average, synergistic ($\alpha_s = 0.55$; *i.e.*, that 55% of the global effect came from interactions). However, depending on the stressor and trait categories, we observed a large panel of trait associations and magnitudes (Fig. 3). Trait associations could be antagonistic, *i.e.*, with a weaker global effect than the main effect ($\alpha_s < 0$; *e.g.*, large size or dormancy/diapause as resistance forms under water abstraction); additive, *i.e.*, the main and global effects were of the

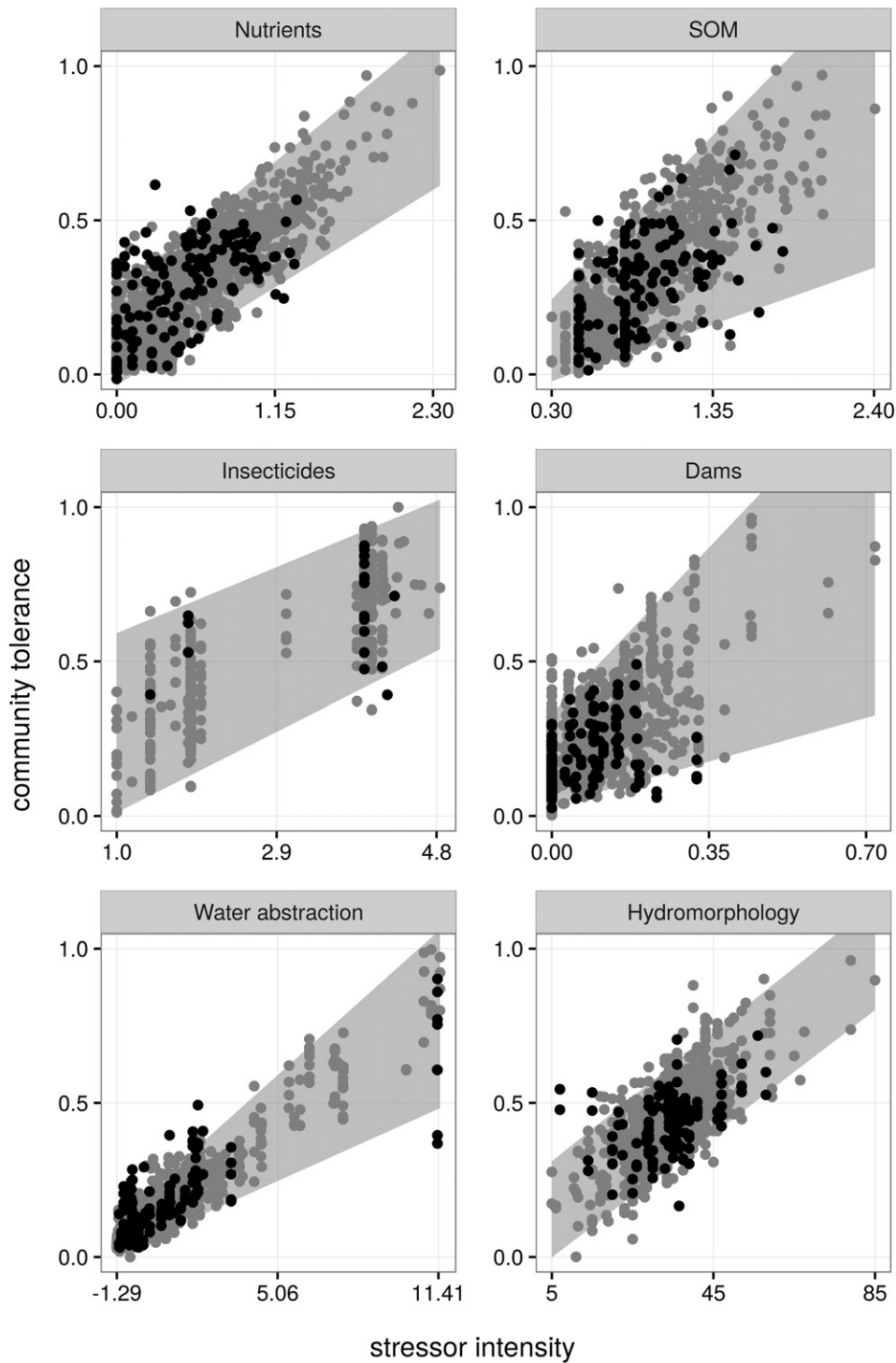


Fig. 2. Model performance. The dots represent the relationship between model outputs (tolerance score, *i.e.*, mean model predictions) and stressor intensities for the different data types (grey: training data; black: test data). The grey ribbon corresponds to the 95% training confidence interval obtained using quantile regression on sub-model outputs for the training data set.

same magnitude ($\alpha_s \approx 0$; *e.g.*, ovoviviparity under water abstraction); or synergistic, *i.e.*, with a larger global effect than the main effect ($\alpha_s > 0$; *e.g.*, interstitial organisms under water abstraction and the use of detritus or macroinvertebrates as food sources under insecticide stress).

3.2.5. Stressor-specificity of community tolerances

Globally, the community tolerances were positively correlated across the different stressors ($r = 0.32 \pm 0.45$; Fig. S17); the strongest positive correlation was obtained for nutrients and SOM ($r = 0.71$) and the largest negative correlation was obtained for insecticides and

dams ($r = -0.43$). Insecticide tolerance was the only community tolerance that was not correlated or was negatively correlated with any of the other stressor tolerances ($r = -0.25 \pm 0.16$), indicating very specific biological adaptations.

However, the strength of the correlation between community tolerance and stressor intensity was weaker for non-target stressors (*i.e.*, with no *a priori* hypotheses; $r^{\text{non target}} = 0.13 \pm 0.24$) compared with target stressors ($r^{\text{target}} = 0.78 \pm 0.10$; Fig. 4). The strongest relationship between community tolerance and a non-target stressor was found for stressor nutrients and SOM ($r = 0.58$; Fig. 4).

Table 3

Model efficiency for the different stressors. SOM: Suspended Organic Matter, r_{training} and r_{test} : Pearson's correlation coefficient between community tolerances and the corresponding stressor intensities in the training and test data sets, respectively; robustness: proportion of test samples falling outside the 95% training confidence interval (see text for further details); $r^{\text{no-association}}$ ($r^{\text{no-association}}$): Pearson's correlation coefficient between community tolerances and the corresponding stressor intensities in the training + test data set considering (or not) the associations among traits.

	r_{training}	r_{test}	Robustness	$r^{\text{no-association}}$	$r^{\text{association}}$
Nutrients	0.89	0.57	0.17	0.71	0.86
SOM	0.79	0.49	0.05	0.55	0.76
Insecticides	0.83	0.33	0.05	0.58	0.82
Dams	0.72	0.33	0.05	0.45	0.69
Water abstraction	0.94	0.84	0.25	0.77	0.92
Hydromorphology	0.68	0.44	0.07	0.46	0.66

4. Discussion

Altogether, our results support our three hypotheses. First, the combinations of biological traits can efficiently predict community tolerance to different anthropogenic stressors (H1). Second, trait association can play a major role in determining community tolerance (H2). Third, the predicted community tolerances, and therefore the underlying combinations of biological traits, are rather specific to the target stressor for which each model was developed (H3).

4.1. Combinations of biological traits shape community tolerance to stressors

The efficiency and robustness of the different models used for linking stressor intensities and combinations of biological traits support our first hypothesis (H1). However, the models corresponding to insecticide and dam stressors exhibited a lower efficiency in the test than in the training data sets. In fact, compared with the other stressors, there was a clear lack of information collected about insecticides (Table 1). This may impair model efficiency because, as for other models based on pattern recognition, the amount and quality of the training data set are critical to provide accurate and robust predictions. Moreover, because of the low amount of data and the inconsistencies of the insecticide compounds measured over the surveyed sites, we had to group all insecticide compounds in a single stressor, which might have lessened the efficiency of the insecticide model. Indeed, different types of insecticides (e.g., carbamates, pyrethroids, organophosphates) have different modes of action (Coats, 2012) and therefore may involve different biological responses. Finally, in this study, we took into account the biological traits of the invertebrates whereas the combination of traits with physiological sensitivity (obtained from laboratory experiments, e.g., Von der Ohe and Liess, 2004) could better explain changes in community composition related to pesticide exposures (as was performed in the SPEAR approach; Liess and Von der Ohe, 2005).

Low relationships between macroinvertebrate traits assessed using survey data and dam stressors have already been documented and might occur because the sampling sites are not specifically targeted towards the dam effect (Van Looy et al., 2014). Based on the distance between a dam and the sampling sites, the hydrological (Singer, 2007) and geomorphological (Brandt, 2000) changes in downstream sections of the streams are variable and it is likely that the effects on communities cannot be observed with the same intensity depending on this distance (e.g., Lessard and Hayes, 2003 but see Van Looy et al., 2014). Moreover, we assumed that hydrological variability was the main effect of dams whereas changes of the upstream and downstream fluxes can also occur (e.g., sediment, organic matter, nutrients; Muñoz and Prat, 1989) as well as changes in the thermal regime (e.g., Lessard and Hayes, 2003). The definition of dam-related stressors using more specific sources of information than those available for this study may help to better understand the dam effects on the species trait combinations in communities.

The prediction of species distribution and abundances requires the investigation of the relationships between organisms and their environment, including anthropogenic stressors (e.g., Bonada et al., 2004). These relationships, which have been fuzzy-coded in habitat requirements (Tachet et al., 2010; Usseglio-Polatera et al., 2001), have generally exhibited contrasting efficiencies to depict the responses of communities to environmental gradients, in combination or not with biological traits (Archambault et al., 2010; Dolédec et al., 1999; Mondy and Usseglio-Polatera, 2013). Moreover, coding such traits using empirical data may be observed as unrealistic for a large panel of stressors (Statzner and Bêche, 2010).

In contrast, we considered that the ecological tolerances to different stressors resulted from the combination of different biological traits that conferred to organisms some abilities to overcome adverse conditions. This approach is similar to the proposed life-history strategy approach (Verberk et al., 2008a) that is realistically applicable for general disturbances but more challenging for different specific stressors (Verberk et al., 2013). In this study, we defined *a priori* hypotheses about the trait responses to stressors and about the direction of the main response to increasing stressor levels in a much simpler way than required by the life-history strategy approach.

4.2. Trait associations influence community tolerance

Taking trait association into consideration allowed improving the correlation between community tolerance and stressor intensity by 0.36 (± 0.13) on average across the different stressors, thereby validating our second hypothesis (H2). Although we are not aware of other studies that have investigated the role of trait association on stressor tolerance, these results are congruent with the important role played by traits and their trade-offs to explain, for example, the invasion success of non-native plants (Küster et al., 2008).

The large values of the calibrated tree complexity parameter ($\bar{tc} = 7.83$) suggest that community tolerance does involve association among numerous trait categories and not only among pairs of trait categories that would be characterized by tc values close to 2 (see e.g., Fig. S14). This complexity, not only in terms of trait number but also in terms of their response types (tolerant and sensitive trait categories can be associated), prevented us from directly analysing pairs of trait categories as is usually performed. Instead, we investigated the effects of the trait association (Statzner et al., 1997; Verberk et al., 2013) by comparing the main and global effects of single trait categories on community tolerance. Our results suggest that these trait associations can be very important for community tolerance.

As expected, large body size (4–8 cm) is associated with higher water abstraction tolerance because of the release of the action of flow on the organisms (Bonada et al., 2007a). However, trait association is responsible for the loss of >90% of the response magnitude (Fig. 3). In the model, large body size is often associated with semivoltinism, sensitive to water abstraction, and dormancy/diapause, tolerant to water abstraction (Fig. S15). These two trait categories are positively ($r = 0.26$) and negatively ($r = -0.10$) correlated with large body size among macroinvertebrate families. Therefore, the detrimental effects of the low resilience of large semivoltine organisms with no specific resistance strategy are likely to outweigh the benefits of lower shear stress acting on these invertebrates. However, an increased importance of large body size was observed in the Mediterranean area (Bonada et al., 2007a) in ephemeral rather than intermittent streams (Bonada et al., 2007b). Therefore, the global effect of large body size could be more important if the intensity of the water abstraction is larger than that of our sampling domain, which highlights the potential consequences of water scarcity on community functioning.

Dormancy and diapause are physiological mechanisms occurring in most macroinvertebrates that allow them to withstand adverse

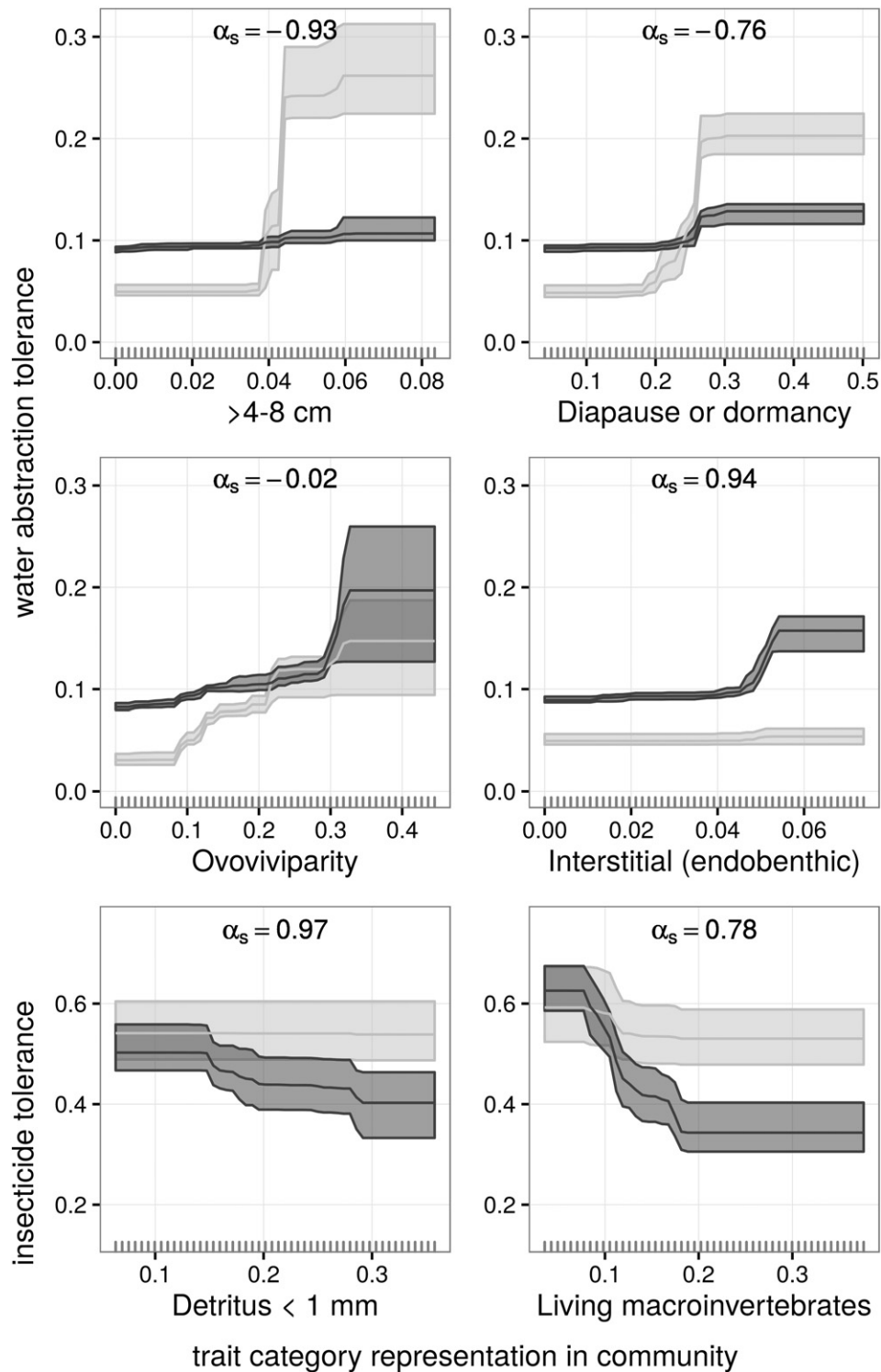


Fig. 3. Examples of local sensitivity analysis results corresponding to two community tolerance models (water abstraction and insecticides). Marginal effects on community tolerance correspond to the model outputs (y-axis) obtained when varying the affinity score of one trait category at a time (values indicated by the small vertical segments on the x-axis) while keeping the others at their mean values. Lines correspond to the mean model relationship (*i.e.*, averaged output over the 10 sub-models) between the frequency of individual biological trait categories in the community and the community tolerance. Ribbons correspond to the 95% intervals obtained from the individual sub-model analyses. Pale and dark grey correspond to the models without and with trait association, respectively. The association strength (α_s , see the text for more details) is given for each example.

environmental conditions such as river drying by lowering down the metabolic activity of one of their life stages (*e.g.*, eggs or larvae). We observed that the increasing use of these mechanisms was associated with a higher community tolerance to water abstraction in agreement with previous studies investigating intermittent streams (Bonada et al., 2007a; Harper and Hynes, 1970; Mellado Díaz et al., 2008). We also

found that the global effect of these mechanisms was reduced by 76% because of the influence of the other traits. These results suggest that the benefits of entering a low-metabolic stage are not as important as they could have been because of, for example, functional redundancy with other resistance or resilience mechanisms or trade-offs with other traits possessed by the organisms.

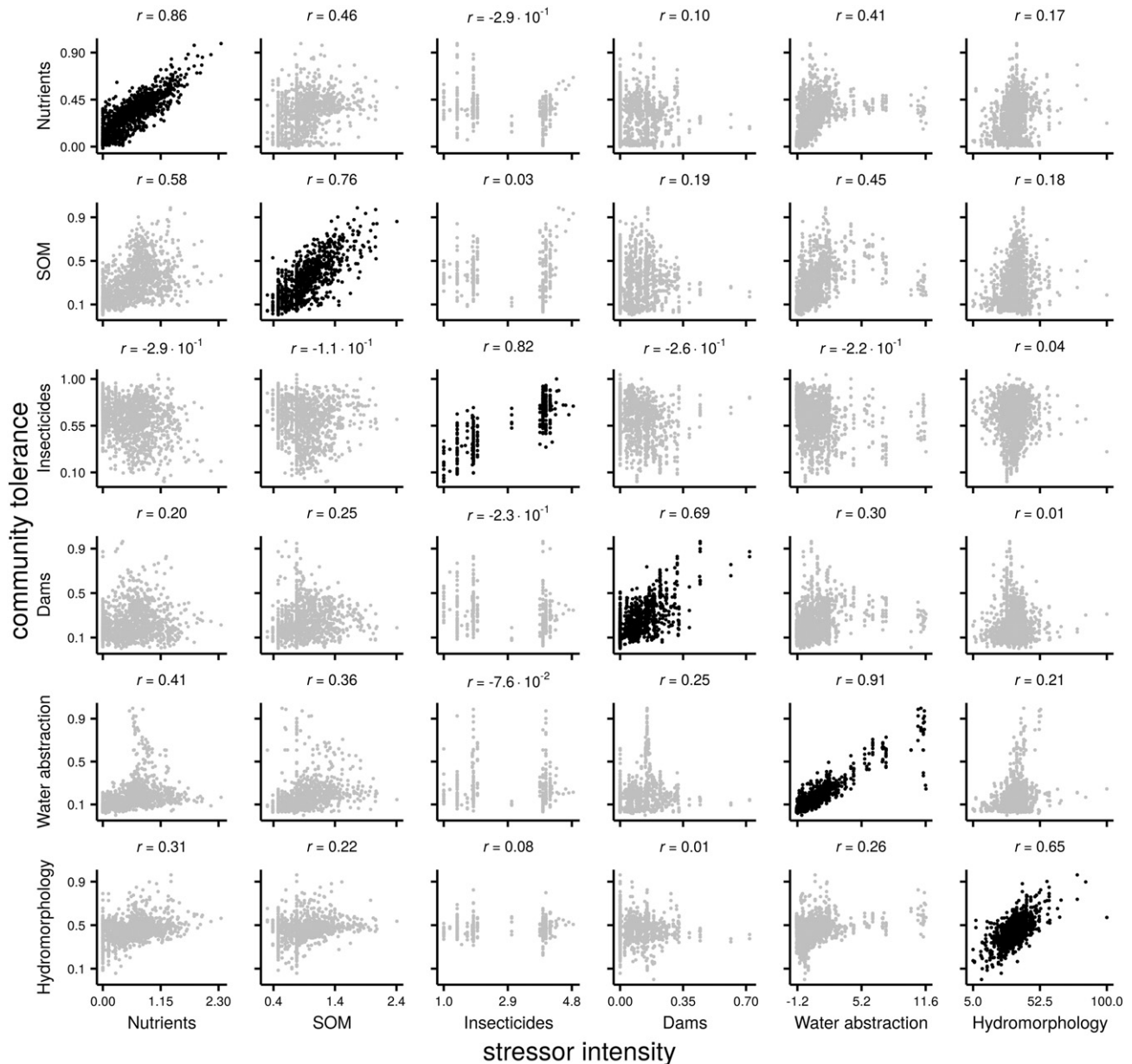


Fig. 4. Stressor-specificity of community tolerances. Scatter plots represent the relationship between the community tolerance and stressor intensity of each stressor. The strength of the relationship is given above each plot (r). Black dots represent the relationships between community tolerance and the intensity of the target stressor (i.e., the tolerance model with *a priori* hypotheses) and grey dots represent the relationships between community tolerance and non-target stressor intensities.

We observed that trait association was rather synergistic across all stressors ($\bar{\alpha}_s = 0.55$). A good example of this is the water abstraction tolerance provided by the use of interstitial spaces as a refuge (e.g., Clifford, 1966; Dewson et al., 2007). In our study, interstitial behaviour had a nearly null effect on the tolerance of communities to water abstraction when trait association was not considered. This result is in agreement with several studies that found no positive associations between interstitial behaviour and stream intermittency (Bonada et al., 2007a; Bonada et al., 2007b; Mellado Díaz et al., 2008). However, we found a substantial effect of interstitial behaviour on community tolerance when trait association was considered. Inspection of the model details showed that it was often associated with short life span and gill respiration (Fig. S16). Moreover, these two tolerant trait categories are positively correlated with interstitial behaviour across taxa ($\bar{r} = 0.17$) and even more at the community level ($\bar{r} = 0.35$). These results

suggest that the benefits of using interstitial spaces as a refuge are amplified for organisms with a short life span and gill breathing; this may explain why these benefits may have been overlooked in previous studies where trait association was not considered (e.g., Bonada et al., 2007b).

Traits related to feeding habits and food sources have been the most frequently used biological traits in the assessment of the effects of natural or human-caused stressors (Statzner and Bêche, 2010); however, their efficiency is debatable (Bonada et al., 2006), partly because of their misuse (Statzner and Bêche, 2010). Our results suggest that not considering trait association can also blur the response of these functional traits to stressors, even when *a priori* hypotheses exist (e.g., Mondy and Usseglio-Polatera, 2013). For example, the effect of two sensitive feeding habit traits (the use of detritus and living macroinvertebrates as food sources) on community tolerance to insecticides was

limited when not considering trait association but significantly increased when these associations were taken into account (Fig. 3).

4.3. Community tolerances and underlying trait combinations are stressor-specific

Our results support the hypotheses that different trait combinations are specific to different stressors (Hypothesis H3; Dolédec and Statzner, 2008; Mondy and Usseglio-Polatera, 2013; Statzner and Bêche, 2010) and that the stressor intensity is also more or less tightly related to different adaptive strategies (Archaibault et al., 2010; Dolédec et al., 2006; Southwood, 1977; Townsend and Hildrew, 1994; Verberk et al., 2008b).

There is a long history of applying community ecology principles to aquatic ecosystem monitoring. However, most of the methods developed still lack sufficient specific responses to different stressors to be completely satisfying for environmental managers (Bonada et al., 2006). Although some studies using multiple traits have provided promising results (Statzner and Bêche, 2010), the specificity of the obtained responses has often been tested against only one non-target stressor (Dolédec and Statzner, 2008; Lange et al., 2014; Townsend et al., 2008). Even trait-based indicators designed to be specific to a given stressor may respond to a large range of stressors, e.g., the number of taxa considered 'at risk' regarding pesticide contamination (SPEAR; Liess and Von der Ohe, 2005) responded to eight stressors related to water quality and six related to habitat degradation (Mondy et al., 2012).

In contrast, the use of combinations of trait-based metrics allowed the classification of sites efficiently and specifically as impaired or not for 16 different anthropogenic stressors (Mondy and Usseglio-Polatera, 2013). The present study is conceptually similar to this previous work and similarly exhibits the strongest correlation among model outputs for nutrients (nitrates in Mondy and Usseglio-Polatera, 2013) and SOM (clogging risk). This correlation is probably more related to the common origin of the two stressors (intensive agriculture; Sarriquet et al., 2007) than to similar adaptive responses (Mondy and Usseglio-Polatera, 2013). The high co-occurrence observed between the two stressors both in France (Mondy and Usseglio-Polatera, 2013) and in the Ebro catchment in Spain (this study) supports this hypothesis.

5. Concluding remarks

Our study provides evidence that trait combinations, including the effects of trait associations, define stressor-specific community tolerance. In this sense, our study enables improvement in predictive ability in community ecology, which is a feat claimed as doubtful until recently (Lawton, 1999; Scheiner and Willig, 2008).

Moreover, the models proposed in our study have implications for stream managers. For example, a conceptually similar diagnostic tool developed in France (Mondy and Usseglio-Polatera, 2013) is now included in a toolbox that helps stream managers identify the most probable stressors impairing benthic communities. The models presented in our study could be used to identify the stressors for which given communities are the least tolerant and thus to select the management options minimizing the risks. The model application to the Ebro basin benefits the understanding and prediction of the effects of water scarcity and other concurrent stressors in a Mediterranean basin. As part of the GLOBAQUA European project, the result of our study should contribute to minimizing anthropogenic impacts on biological communities and ecosystem functioning, adapting to oncoming changes and improving management and policies of particular importance for proposing cost-effective Programmes of Measures at the basin level in the context of the future revision of the WFD.

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