# Title: Combined effects of land-use- and climate-driven stressors on stream fungi and organic matter decomposition

**Authors:** Aida Viza[1], Encarnación Fenoy[2,3,4], Meritxell Abril[5], Mario Álvarez-Cabria[6], Rebeca Arias-Real[7], Christian Arnanz[8], José Barquín[6], Andreas Bruder[9], Red Calore[9], Íñigo Donázar[10], Isabel Fernandes[11,12], Antonio J. García-Meseguer[13], Anna Lupon[14], Itxaso Martínez-Sanz[1], Margarita Menéndez[1], Isabel Muñoz[1], Cláudia Pascoal[11,12], Marina Tomás-Martín[8] and Cayetano Gutiérrez-Cánovas[15,16]

Affiliations:

1. Departament de Biologia Evolutiva, Ecologia i Ciències Ambientals, Facultat de Biologia, Universitat de Barcelona, Barcelona, Spain
2. Department of Biology and Geology, University of Almería, Almería, Spain
3. Centro Andaluz para el Cambio Global—Hermelindo Castro (ENGLOBA), Almería, Spain
4. Department of Functional and Evolutionary Ecology, Estación Experimental de Zonas Áridas (EEZA), CSIC, Almería, Spain
5. BETA Technological Centre- University of Vic- Central University of Catalunya (BETA-UVIC- UCC), Vic, Spain
6. IHCantabria—Instituto de Hidráulica Ambiental de la Universidad de Cantabria, Santander, Spain
7. Museo Nacional de Ciencias Naturales, Consejo Superior de Investigaciones Científicas, Madrid, Spain
8. Department of Ecology, Facultad de Ciencias, Universidad Autónoma de Madrid, Spain
9. Institute of Microbiology, University of Applied Sciences and Arts of Southern Switzerland, Mendrisio, Switzerland
10. Laboratorio de Biología Marina, Departamento de Zoología, Facultad de Biología, Universidad de Sevilla, Sevilla, Spain
11. Centre of Molecular and Environmental Biology (CBMA), Aquatic Research Network (ARNET) Associate Laboratory, Department of Biology, University of Minho, Campus of Gualtar, Braga, Portugal
12. Institute for Science and Innovation for Bio-Sustainability (IB-S), University of Minho, Campus of Gualtar, Braga, Portugal
13. Department of Ecology and Hydrology, University of Murcia, Spain
14. Centre of Advanced Studies of Blanes (CEAB), CSIC, Blanes, Spain
15. Area of Biodiversity and Conservation, Universidad Rey Juan Carlos, Móstoles, Spain
16. Instituto de Investigación en Cambio Global (IICG-URJC), Universidad Rey Juan Carlos, Móstoles, Spain

## Abstract

1. Freshwater microbial communities are essential for maintaining ecosystem functions and services, with aquatic fungi playing a particularly critical role in decomposing terrestrial organic matter entering streams and converting it into energy and nutrients that sustain higher trophic levels. However, freshwater ecosystems face growing threats from multiple stressors. The combined effects of these stressors on fungal biodiversity and functioning in streams remain poorly understood. This study examines the joint effects of land-use and climate-driven stressors on fungal biodiversity and two key functions—fungal biomass accrual and organic matter decomposition—across two stream compartments: the water column and streambed sediments.

2. We conducted an extensive two-year survey across 62 Iberian stream sites in southwestern Europe. To assess stressor impacts, we analysed responses from both the water column and streambed sediments, examining compartment-specific differences. Models were developed to evaluate six stressor types - organic matter and inorganic nutrient enrichment, oxygen depletion, thermal stress, drying stress and riparian degradation- and hydrology. Fungal responses were evaluated across multiple biodiversity dimensions, including taxonomic diversity, functional diversity, and community composition.

3. Results revealed that organic matter enrichment positively influenced fungal biodiversity and functioning, while thermal and drying stresses, along with riparian degradation, had significant negative effects. Stressor effects were predominantly additive, with limited interactions observed. Inorganic nutrient enrichment showed weak effects, likely reflecting minimal nutrient limitation in Iberian streams. Differences between stream compartments emerged, with streambed sediments buffering the negative impacts of thermal and drying stresses on fungal biodiversity and functions.

4. Our findings underscore the influence of multiple stressors on fungal biodiversity and functioning, which are expected to intensify under climate change. The predominance of additive stressor effects suggests that individual stressors can be targeted independently, simplifying management strategies. The buffering role of streambed sediments highlights their importance in providing resilience against global stressors, particularly thermal and drying stresses.

5. This study emphasises the urgent need to safeguard conditions that support fungal biodiversity and ecosystem functions in streams to ensure the continued provision of vital ecosystem services in the face of global change.

# 1.Introduction

Stream ecosystems are vital for sustaining biodiversity, regulating biogeochemical cycles, and promoting human well-being (Aufdenkampe et al., 2011; Lynch et al., 2023; Tickner et al., 2020). However, freshwater ecosystems are increasingly threatened by multiple stressors that adversely affect riverine biota and ecosystem functioning (Carpenter et al., 2011; Reid et al., 2019). A stressor is defined as any abiotic variable that, when altered by human activities, results in significant changes in riverine biota or ecosystem functioning (Birk et al., 2020;

Sabater et al., 2019). Simultaneous alterations in multiple abiotic factors can produce cumulative ecological impacts that are challenging to predict when each factor is considered individually (Birk et al., 2020; Rillig et al., 2023; Sabater et al., 2019), especially at the scale of food-webs or ecosystems (Bruder et al., 2019). Consequently, there has been an increasing focus on studies investigating the combined effects of co-occurring stressors (e.g., David et al., 2024; Graça et al., 2024). However, most of these studies are limited to small-scale observational or experimental settings, limiting our ability to identify general patterns that could inform biomonitoring and restoration efforts (Gutiérrez-Cánovas et al., 2022; Orr et al., 2024; Rillig et al., 2023). Therefore, it is essential to develop studies that address realistic gradients of multiple stressors and incorporate large spatial scales to better understand general responses of stream biodiversity and functions to global change (Johnson & Penaluna, 2019).

On a global scale, stream ecosystems receive, process, and transport approximately 1.9 Pg of terrestrially derived carbon annually (Cole et al., 2007). Many headwater streams rely on terrestrial carbon inputs to sustain their food webs and key ecosystem functions, such as organic matter (OM) decomposition and the transfer of energy to higher trophic levels (Gessner et al., 2010; Vannote et al., 1980). Microbial communities, particularly fungi, are key biotic drivers of these processes, playing a central role in carbon cycling by decomposing large amounts of OM entering streams (Gessner et al., 2007). The functional importance of fungi in streams is supported by strong positive associations between fungal biodiversity, reproduction (e.g., conidial production), biomass production, and OM decomposition rates, particularly for coarse particulate OM, such as leaves and wood (Arias-Real et al., 2022; Fenoy et al., 2021; Gessner, 1997). These positive biodiversity effects arise through increased efficiency due to wider niche representation and complementarity in diverse fungal communities or due to the presence of highly performing species (Duarte et al., 2006; Geraldes et al., 2012).

The pivotal role of aquatic fungi in stream ecosystems can be compromised by global change drivers, including land-use intensification and the climate crisis. Agriculture and urbanisation impact streams through organic and inorganic nutrient enrichment, oxygen depletion, and riparian degradation, among other impacts (Bruno et al., 2016; Carpenter et al., 1998; Colls et al., 2024). While the links between catchment management and stream biodiversity are well-documented for organisms commonly used in biomonitoring (e.g., macroinvertebrates, diatoms, macrophytes; Feld et al., 2018; Hering et al., 2006), there is limited understanding of how land-use stressors interact with climate-driven impacts to affect fungal biodiversity and functions in streams. For example, rising thermal and drying stresses in stream ecosystems may amplify the impact of nutrient enrichment and riparian degradation (Birk et al., 2020; Graça et al., 2024; Soria et al., 2020), leading significant challenges for ecological management and mitigation.

Previous research has revealed contrasting effects of individual stressors on fungal communities and associated functions. For instance, moderate inorganic nutrient enrichment and warming often enhance fungal activity and OM decomposition (Biasi et al., 2017; Fenoy et al., 2016, 2024; Fernandes et al., 2012; Ferreira & Graça, 2016; Martínez et al., 2014) or show no response (Bruder et al., 2016). However, studies addressing broader stressor gradients suggest more complex, non-linear relationships. For example, a non-linear relationship between nutrients and OM decomposition, with decomposition rates peaking at intermediate inorganic nutrient levels (Pereira et al., 2016; Woodward et al., 2012). Similarly, when considering wider water temperature gradients (4-22ºC), fungal functional diversity declined at higher temperatures (Fenoy et al., 2021). In addition, dissolved organic carbon enrichment can stimulate fungal biomass and growth in the water column (Jørgensen & Stepanauskas, 2009; Wurzbacher et al., 2014). In contrast, stressors such as oxygen depletion and drying reduce fungal diversity and inhibit fungal production, biomass, and reproduction (Abril et al., 2016; Arias-Real et al., 2022; Gomes et al., 2018; Graça et al., 2024). Since aquatic hyphomycetes rely on dissolved oxygen and water availability for OM decomposition, their activity declines monotonically under hypoxic and drying conditions (Bruder et al., 2011; Medeiros et al., 2009).

Riparian degradation, including the loss of plant diversity and cover, also influences fungal community composition and activity by reducing the quality and quantity of OM inputs (Fernandes et al., 2013; Laitung & Chauvet, 2005; Tonin et al., 2018). However, the effects of increased light availability due to riparian canopy loss are mixed, with studies reporting both positive and negative outcomes (Ashberry et al., 2021; Danger et al., 2013; Tonin et al., 2018). Thus, addressing the combined effects of multiple stressors under realistic ecological conditions is essential to understanding their cumulative impacts on stream ecosystems.

The effects of stressors also vary across stream compartments, reflecting differences in their stability and vulnerability to human impacts (Burrows et al., 2017; Gonçalves et al., 2019; Solagaistua et al., 2015). For instance, previous studies suggest that microbial biodiversity and functions within streambed sediments generally show greater resistance to stressors than those in the water column. This “insurance capacity” may be attributed to the reduced exposure of sediments to environmental extremes, such as solar radiation and drying (Arias-Real et al., 2022; Gionchetta et al., 2024), and could enable stream ecosystems to face human impacts more effectively than expected. However, under global change scenarios where multiple stressors co-occur, little is known about how their combined effects influence ecosystem functions across different stream compartments, such as the water column and streambed sediments. Understanding these dynamics is critical to informing management and restoration actions that aim to enhance stream resilience and resistance to anthropogenic impacts.

In this study, we explore the combined effects of land-use and climate-driven stressors on fungal biodiversity and two key functions — fungal biomass accrual and OM decomposition (Abril et al., 2021; Ferreira & Graça, 2006)— across different stream compartments. Using data from an extensive two-year survey of 62 Iberian streams sites (southwestern Europe), we investigated these functions in both the water column and streambed sediments to capture potential differences in stressor impacts between ecosystem compartments. Our models considered the effects of six types of stressors — organic and inorganic nutrient enrichment, oxygen depletion, thermal stress, drying stress, riparian degradation—and hydrology, a critical factor for stream biodiversity and functioning (Abril et al., 2021; Ferreira & Graça, 2006). Fungal responses were evaluated across multiple biodiversity facets, including taxonomic and functional diversity and community composition.

# 2. Methods

## 2.1 Study area and sampling design

The study was conducted in 62 stream sites across the Iberian Peninsula between 2022 and 2023. Thirty-six sites were surveyed twice within this period, whereas the remaining 27 sites were surveyed once. The surveyed watercourses were in seven regions representing contrasting climatic conditions, differing primarily in annual aridity and mean air temperature

(Figure 1; Table S1). The Andalusia, Catalan, and Segura regions are characterized by arid and warm climatic conditions, while the Cantabrian Mountain Range and North Portugal exhibit more humid and cooler climates. The Mid Tagus and South Douro regions experience intermediate levels of aridity, with the South Douro region having the lowest mean air temperatures among the studied areas. Lithology varied across the regions: the Cantabrian, Catalonian, and Segura areas were predominantly calcareous, whereas siliceous bedrocks dominated in the Mid Tagus, North Portugal and South Douro regions. Andalusia presented a mix of sedimentary and siliceous lithologies.

In each region, we selected nine stream sites covering crossed gradients of land-use intensification and reach-scale riparian vegetation cover to capture a wide range of organic and inorganic nutrient enrichment, oxygen depletion, thermal stress, riparian degradation, drying stress and stream hydrology (Table S2). The sampling design was intended to represent realistic gradients of multiple abiotic factors that could act as stressors for stream ecosystems (Brauns et al., 2022; Gutiérrez-Cánovas et al., 2022; Lourenço et al., 2023). We define stressors as any abiotic variable that, when altered by human intervention, results in significant changes in riverine biota or ecosystem functioning (Birk et al., 2020; Sabater et al., 2019).

By capturing different land-use intensifications and climate settings, our sites cover long gradients of abiotic stress, including dissolved nitrate (DN; 0.01–28.81 mg L$^{-1}$), dissolved

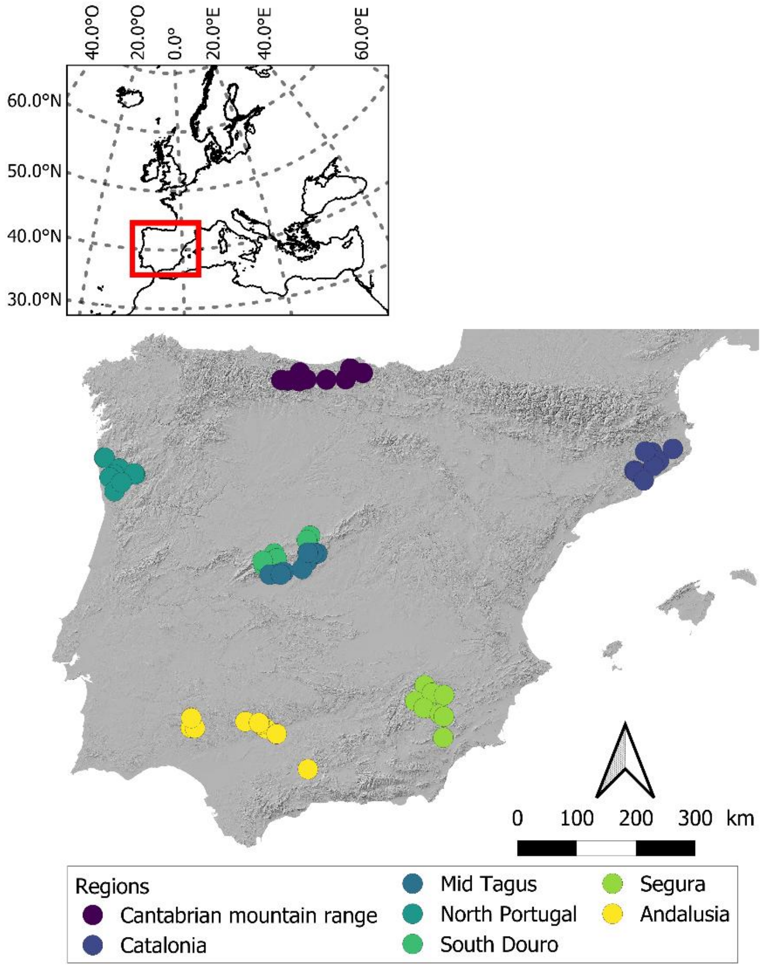

**Figure 1.** Locations of the study sites within the Iberian Peninsula and their context within Europe (red square).

reactive phosphorous (SRP; 0.4-2138.2 µg $L^{-1}$), dissolved organic carbon (DOC; 0.9-233.9 mg $L^{-1}$) and dissolved oxygen (DO) concentration deficit (-1.68–6.35 mg $L^{-1}$). Variations in site elevation (10–1.537 m.a.s.l.), riparian canopy openness (0-96%) and local climate (annual mean air temperature: 9.4–18.5°C) permitted us to capture a wide range of daily mean water temperatures (10.7–28.3°C). Hydrological conditions also varied widely, including gradients of both discharge (0–914 L $s^{-1}$) and drying stress (0-134 dry days). These gradients represent a spectrum of stress intensities and combinations, simulating realistic global change scenarios related to land-use intensification and climate change (Table S1), and are comparable to those used in manipulative experimental studies (e.g., Cross et al., 2022; Matthaei et al., 2010; Romero et al., 2018).

We conducted a field experiment at each stream, starting in late spring and continuing through summer. Initially, we characterised the stressors and fungal communities (see sections 2.2 and 2.3, respectively) and deployed organic matter substrates (wooden sticks). Subsequently, in the following autumn or winter of each year, the wooden sticks were retrieved to assess OM decomposition and fungal biomass accrual (see section 2.4). This strategy allowed us to capture the period of maximum biological activity and stressor levels (e.g., thermal stress, oxygen depletion and dry stress, which tend to peak during summer).

## 2.2 Stressor and environmental gradients

At each sampling site, we characterised indicators of organic and inorganic nutrient enrichment, oxygen depletion, thermal stress, riparian degradation, drying stress, and stream hydrology (Table S1). To assess gradients of organic matter and inorganic nutrients, water samples were filtered in the field through pre-washed glass fibre filters with a 0.7-µm pore size (Whatman, UK). Dissolved nitrate (DN) and soluble reactive phosphorus (SRP) were used as proxies for inorganic nutrient enrichment. The filtered water samples for inorganic nutrient analysis were stored in plastic vials and kept frozen until laboratory analysis. Inorganic nutrient concentrations were determined using standard colourimetric methods (APHA, 1995; FUTURA Autoanalyzer, Frepillon, France). Dissolved organic carbon (DOC) was used as a proxy for organic matter enrichment. DOC samples were acidified, stored in glass vials, and kept cold until analysis. DOC concentrations were measured using high-temperature catalytic oxidation (TOC/TN analyser, Shimadzu Corp., Kyoto, Japan) after sparging to remove dissolved inorganic carbon.

We calculated mean daily dissolved oxygen (DO) deficit in the water column as an indicator of oxygen depletion and hypoxic conditions, which can enhance anaerobic respiration (Blaszczak et al., 2023; Gómez-Gener et al., 2020). DO was recorded every 10 minutes over at least 24 hours using miniDOT loggers (PME, USA) placed at a depth of 10–30 cm and secured to the streambed with iron bars. The DO deficit was calculated as the difference between the saturation concentration and the mean daily DO concentration.

Mean water temperature was recorded continuously during the study periods (89–212 days) using HOBO MX2201 loggers (Onset, USA) and used as an indicator of thermal stress. To characterise drying stress, we measured the drying duration (dry days), which was inferred from variations in the diel range of water temperature and field visits following Arias-Real et al., 2020. Abrupt increases in diel temperature range indicated streambed drying, allowing us to capture the duration of the dry period.

To determine riparian canopy openness, indicative of riparian degradation, we used the Gap Light Analysis Mobile Application (GLAMA) from five hemispheric photographs taken along a

100-m site. To characterise hydrology and control for catchment and stream size, we measured discharge. Discharge was calculated as the product of mean width, mean depth, and mean current velocity across five transects over a 100-m site. For each transect, we recorded at least five measures of depth and current velocity using a flow meter and graduated sticks.

Our environmental dataset included some missing values for DN (n=4), SRP (n=5), DOC (n=13), DO deficit (n=8), water temperature (n=33) and drying stress (n=33) due to absence of water (stream desiccation), equipment malfunctions, or sample loss. Missing values for DN, SRP, DOC, DO deficit, and water temperature were imputed using linear-mixed effects models, except for DOC, where a linear regression model was statistically better supported. Methodological details and results are available in Appendix 1. In brief, for each response variable and based on previous knowledge (nutrients: Dodds & Oakes, 2004; Shen et al., 2020; DOC: Catalán et al., 2018; Granados et al., 2022; DO deficit: Blaszczak et al., 2023; Gutiérrez-Cánovas et al., 2024; water temperature: Kamarianakis et al., 2016; Segura et al., 2015), we produce a full model including spatial (latitude and longitude), temporal (sampling year), land-use (percentage of agricultural and urban cover), climatic (mean annual air temperature), riparian degradation (riparian canopy openness), and/or hydrological (discharge) factors. We evaluated each model's performance using explained variance by fixed factors ($r^2_m$) and a 10-fold cross-validation. Models explained moderate to high amounts of variance: 37.2% for DN, 38.8% for SRP, 69.1% for DOC, 48.4% for DO deficit, and 80.1% for water temperature. Missing data for drying days were imputed using field information about water presence from pre- and post-summer field visits conducted between 2022 and 2024.

## 2.3 Fungal biodiversity

To assess the biodiversity of aquatic fungal communities, foam samples for the identification of aquatic fungi (mainly aquatic hyphomycetes) were collected during the summers of 2022 and 2023. Fresh foam from the surface of stream water was collected using a spoon, transferred to plastic tubes, and fixed with 96% ethanol. An aliquot was filtered through 5 µm pore-size cellulose nitrate filters (Merck, Germany). The retained conidia were stained with a 0.1% trypan blue solution in lactic acid and examined under a light microscope (400x). Spores of aquatic hyphomycete species were identified to the lowest possible taxonomic level and counted based on conidial morphology (Gulis et al., 2020). In total, 122 taxa were identified, representing 57 genera, 22 families, and 16 orders. Twenty taxa could not be identified and were therefore included only in the taxonomic analyses but excluded from the functional ones.

Functional traits of fungal species were compiled, including six traits based on previous efforts (Arias-Real et al., 2023; Table 1). Specifically, we characterised traits such as primary lifestyle, decay substrate, habitat, capacity to inhabit tree-holes, endophyte capacity, and conidial morphology. These traits encompassed 20 categories for 98 species out of the 122 taxa identified.

**Table 1**. Fungal traits, their corresponding categories, and the references used for their definition. The table is adapted from Arias-Real et al. (2023) with additional references included.

| Trait | Category | References |
|---|---|---|
| Primary lifestyle | Litter saprotrophic<br>Wood saprotrophic<br>Plant pathogenic<br>Mycoparasite | Bärlocher, 1992; Gönczöl & Révay, 2006; Magyar, 2008; Põlme et al., 2020; Sampera-Calbet et al., 2017 |

| | | |
|---|---|---|
| Decay substrate | Litter<br>Root<br>Wood | |
| Habitat | Aquatic<br>Non-aquatic | Boonmee et al., 2021; Cai et al., 2006; Gönczöl & Révay, 2006; Kodsueb et al., 2016; Põlme et al., 2020; Sridhar & Kaveriappa, 1987 |
| Tree-holes | Yes<br>No | Gönczöl & Révay, 2003; Karamchand & Sridhar, 2008; Kaufman et al., 2008; Kitching, 1971; Osono & Hirose, 2009; Sridhar, 2009; Sridhar et al., 2013; Sudheep & Sridhar, 2010 |
| Endophyte capacity | Yes<br>No | Chauvet et al., 2016; Koivusaari et al., 2019; Koranga & Sati, 2023; Leroy et al., 2011; Marvanová & Laichmanová, 2014; Põlme et al., 2020; Porras-Alfaro & Bayman, 2011; Rashmi, 2019; Seena & Monroy, 2016; Selosse et al., 2008; Sridhar, 2009; Sridhar et al., 2006; Sridhar & Bärlocher, 1992; Vendramin et al., 2010 |
| Conidia or spore morphology | Branched<br>Tetraradiate<br>Filiform<br>Sigmoid<br>Compact<br>Clove-shaped<br>Ascospores | Bärlocher, 2009, 2020; Chauvet et al., 2016; Chaverri et al., 2011; Cornut et al., 2014; Fiuza & Gusmao, 2013; Fiuza et al., 2015; Ghate & Sridhar, 2015; Marvanová et al., 2003; Patil et al., 2014; Ramesh & Vijaykumar, 2005; Webster & Davey, 1984 |

To characterise the taxonomic diversity of fungal communities, we calculated taxon richness, representing the number of taxa in each community. Additionally, we characterized two measures of functional-trait diversity: functional richness (FRic), which estimates the range of trait variation within a community (Villéger et al., 2008), and functional dispersion (FDis), which represents the variability in trait composition within a community (Laliberté & Legendre, 2010).

To calculate FRic and FDis, we built a principal coordinate analysis (PCoA) based on a fuzzy-coded adapted Gower dissimilarity matrix (Pavoine et al., 2009) derived from the six fungal traits, including all their categories. The functional space generated from this analysis represented trait variation across fungal species. The first five axes, explaining 81.7% of the Gower dissimilarity matrix, were retained to ensure adequate representation of the five-dimensional space (Maire et al., 2015). To quantify functional composition, we estimated the community centroids for the first two axes by calculating the mean position of the occurring species along these axes.

## 2.4 Organic matter decomposition and fungal biomass accrual

To quantify the decomposition of OM in the different stream compartments five tongue depressor sticks (15 × 2 × 0.2 cm) made of *Populus x canadensis* wood were placed on top of the streambed to characterise decomposition in the water column, while another five sticks were buried vertically beneath the streambed to characterise decomposition in the streambed sediments (Arroita et al., 2012). All wooden sticks were pre-marked and weighed. Wooden sticks provide a standardised and chemically untreated material, making them ideal for decomposition experiments (Arroita et al., 2012).

In the water column, each group of sticks was inserted into the holes of a brick to prevent exposure to sunlight and algae growth. The brick was secured to metal bars using nylon threads, branches, or roots to ensure its position within the lotic habitat. During installation, we ensured that the sticks were fully submerged in the water. In the streambed sediments, each group of sticks was buried in the sediment and similarly tied to metal bars with nylon thread. The sticks were placed at each site in late spring or summer of 2022 and 2023, an average time of 147 days (range: 89-212 days).

To prevent ergosterol degradation and potential weight changes during wooden stick collection, the sticks were stored in zip-lock bags, transported in dark, refrigerated containers, and processed immediately upon arrival at the laboratory. Each stick was brushed to remove adhered material and washed with distilled water. A 1-cm section was cut from each stick and frozen at −80 °C for later ergosterol determination as a proxy for fungal biomass accrual (Gessner, 2020). The remaining portion of each stick was dried at 70 °C for 72 hours, weighed, and summed to the frozen aliquot fraction for ergosterol measurement (see below).

Decomposition rates were calculated using the negative exponential equation $M_t = M_0 \cdot e^{-kt}$, where $M_0$ is the initial dry mass, $M_t$ is the remaining dry mass at time $t$, and $k$ is the decay rate (Bärlocher, 2020). Decomposition rates were not corrected for temperature to assess the impact of thermal stress in our models. Additionally, the percentage of remaining mass, the decay rate, and the temperature-corrected decay rate exhibited a strong positive correlations for each compartment ($r_p$=0.88-0.97), indicating minimal influence of the methodological choice.

To calculate the fungal biomass accrual, we measured the ergosterol concentration at each stick. First, frozen aliquots of sticks were lyophilised and weighed. Second, lipid extraction and saponification were performed using 0.14 M KOH in methanol (8 g L−1) in a shaking bath at 80 °C for 30 minutes. Solid-phase extraction cartridges (Waters Sep-Pak® Vac RC, 500 mg tC18, Waters Corp, Milford, MA, USA) were used to purify lipid extracts, with ergosterol eluted using isopropanol. High-pressure liquid chromatography (HPLC; Jasco HPLC system, USA) was used to detect and quantify ergosterol at 282 nm with a Gemini-NX 5 µm C18 250 × 4.6-mm column (Phenomenex, UK). Finally, ergosterol was converted into fungal biomass using a conversion factor of 5.5 mg of ergosterol per gram of fungal mycelium (Gessner & Chauvet, 1993). Results were expressed as mg of fungal biomass per gram of OM dry mass.

To examine whether the effects of multiple stressors on fungal decomposition and biomass accrual differ across compartments, we calculated the ratio of decomposition and fungal biomass accrual between the streambed sediment and water column.

## 2.5 Data analysis

To explore the effects of multiple stressors on fungal biodiversity metrics and functions, we used linear mixed-effects models (LMMs) and linear regression models (LMs), following a multi-model inference approach (Burnham & Anderson, 2004). All models included all eight predictors representing the six stressor gradients and hydrology (see before): DN, SRP, DOC, oxygen deficit, thermal stress (mean water temperature), riparian canopy openness, drying stress and discharge.

A three-step process was employed for multi-model inference. First, we evaluated whether to include a random intercept (site) to account for the dependence structure, as 36 sites were surveyed twice during the study period. Using the functions *lmer*() from the *lme4* R package and *lm*(), we fitted two models for each response variable, one with and one without the random factor. We calculated the Akaike information criterion for small sample sizes (AICc), the explained variance by the random factor (Nakagawa & Schielzeth, 2013), and evaluated the occurrence of singular fits. Random factor was retained if they improved model fit (lower AICc values, higher variance explained, and no singular fits). As a result, we fitted mixed-effects models for all variables except for fungal biomass accrual in streambed sediments, decomposition in streambed sediments, FDis and functional community centroids, for which linear regression models were applied (Appendix 2).

Second, AICc was used to determine whether global models for each response variable should include pairwise interaction terms besides the additive stressor terms. When a model including an interaction has a lower AICc value than the pure additive model, we used the *anova*() function to evaluate if retaining the model with the interaction leads to a significant gain of explanatory capacity. A recent synthesis revealed that inorganic nutrient enrichment is an overarching stressor for riverine ecosystems, which can interact with light, thermal and hydrological stressors across spatial scales (Birk et al., 2020). Thus, we tested three related interactive terms where inorganic nutrient enrichment effects can be modulated by thermal stress (DN × water temperature), riparian degradation (DN × riparian canopy openness) or drying (DN × drying). Additionally, we tested the interaction between drying and canopy openness to evaluate whether higher riparian cover mitigates the impacts of drying by retaining moisture and maintaining fungal activity (Gionchetta et al., 2020).

Third, for each response variable, we quantified stressor coefficients, statistical support, and importance using the function *dredge*() from the *MuMIn* R package (Bartoń, 2024). This function generates models for all potential combinations of predictors included in the global selected model. Models were ranked based on AICc, and those within delta≤7 of the top-ranked model were retained (Burnham et al., 2011). We calculated explained variance by fixed factors ($r^2_m$) and by fixed and random factors ($r^2_c$). For each response variable and model, we derived Akaike weights to determine the explanatory power and the relative likelihood of each model (statistical support), respectively. Akaike weights were also used to calculate mean-weighted variance explained by each predictor (Hoffman & Schadt, 2016). To visualise the overall response of these models and using model's Akaike weights, we calculated a weighted-average of their standardized regression coefficients and predictions across the retained models (ΔAICc ≤7). Model residuals were visually assessed to verify linear model assumptions. All statistical analyses were performed using R (v4.2.2, R Development Core Team, 2022).

# 3. Results

## 3.1 Multiple stressor effects on fungal biodiversity

The three most common taxa were *Alatospora acuminata* Ingold 1942 (63.0% of sites), *Hymenoscyphus tetracladius* Abdullah, Descals & Webster 1981 (53.7%), and *Tetracladium marchalianum* De Wild. 1893 (51.9%; Table S3). Fungal assemblages in northern Portugal exhibited the highest taxonomic richness (Ric; mean ± SE: 14.4 ± 1.46 taxa), whereas the Segura and Andalusia regions showed the lowest values (8.9 ± 1.8 and 8.3 ± 0.9 taxa, respectively; Figure 2a & Table S4). For functional richness (FRic), the Segura, Tagus and Douro regions displayed the highest mean values (FRic; 0.67 ± 0.08, 0.62 ± 0.07, and 0.61 ± 0.04, respectively; Figure 2b), while the Andalusia and Catalonia regions exhibited the lowest mean functional richness (0.32 ± 0.09 and 0.26 ± 0.00, respectively). In contrast, functional dispersion (FDis) showed similar mean values across regions, with the highest value in the Douro region (0.36 ± 0.03; Figure 2c) and the lowest in the Catalonia region (0.20 ± 0.04).

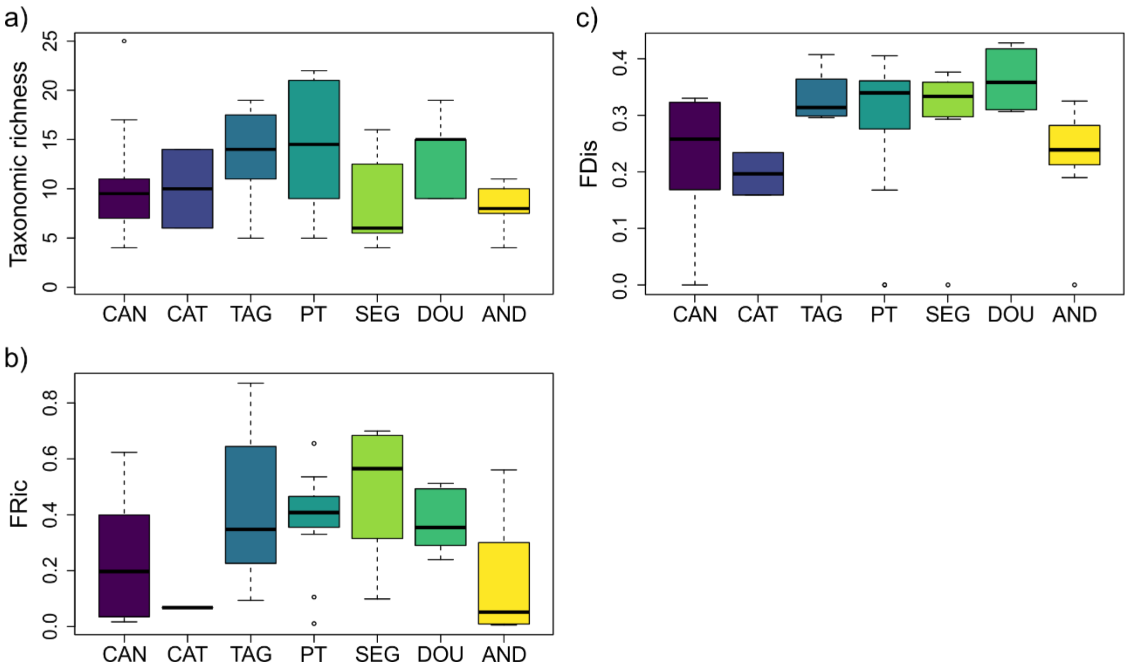

**Figure 2.** Boxplots showing the variation in (a) taxonomic richness, (b) functional richness (FRic), and (c) functional dispersion (FDis) across the study regions. Region abbreviations: CAN – Cantabrian Mountain Range, CAT – Catalonia, TAG – Mid Tagus, PT – North Portugal, SEG – Segura, DOU – South Douro, and AND – Andalusia.

The first and second axes of the functional space for fungal communities explained 30% and 23% of the variance, respectively. The first axis represents a trade-off between wood and leaf decomposers (Table S5). This axis was positively correlated with primary lifestyle as wood saprotrophic ($r_P$=0.69), wood as the decay substrate ($r_P$=0.60), and primary lifestyle as plant pathogenic ($r_P$=0.59). In contrast, it was negatively correlated with primary lifestyle as litter saprotrophic ($r_P$=-0.89) and litter as the decay substrate ($r_P$=-0.69). The second axis represented differences in habitat preferences and conidial shape, showing a positive correlation with the capacity to inhabit tree-holes ($r_P$=0.53) and branched conidial shape ($r_P$=0.51). Conversely, it was negatively correlated with non-aquatic habitat ($r_P$=-0.80) and tetraradiate conidial morphology ($r_P$=-0.58).

Our models predicting fungal biodiversity responses to multiple stressors generally indicated additive responses (Figure 3). Trait-based metrics were generally more responsive than taxonomic richness (Tables S6 & S7).

Taxonomic richness was negatively associated with water temperature ($R^2$ = 1.7%). Functional richness (FRic) was positively related with discharge ($R^2$=2.5%), SRP ($R^2$=2.2%), DOC ($R^2$=1.8%), and DO deficit ($R^2$=1.6%). Functional dispersion (FDis) showed a positive correlation with DO deficit ($R^2$=6.7%) and DOC ($R^2$=4.5%), but a negative response to water temperature ($R^2$=5.5%) and drying duration ($R^2$ = 1.0%). The mean centroid of the first functional axis (wood vs leaf decomposers) was positively related with SRP ($R^2$=8.3%) and water temperature ($R^2$=7.2%), but correlated negatively with DO deficit ($R^2$=1.3%). Functional axis 2 (habitat preferences and conidial morphology) had a positive relationship with discharge ($R^2$=2.6%) and drying duration ($R^2$=2.5%), while it responded negatively to riparian canopy openness ($R^2$=5.1%).

### 3.2 Multiple stressor effects on fungal ecosystem functioning

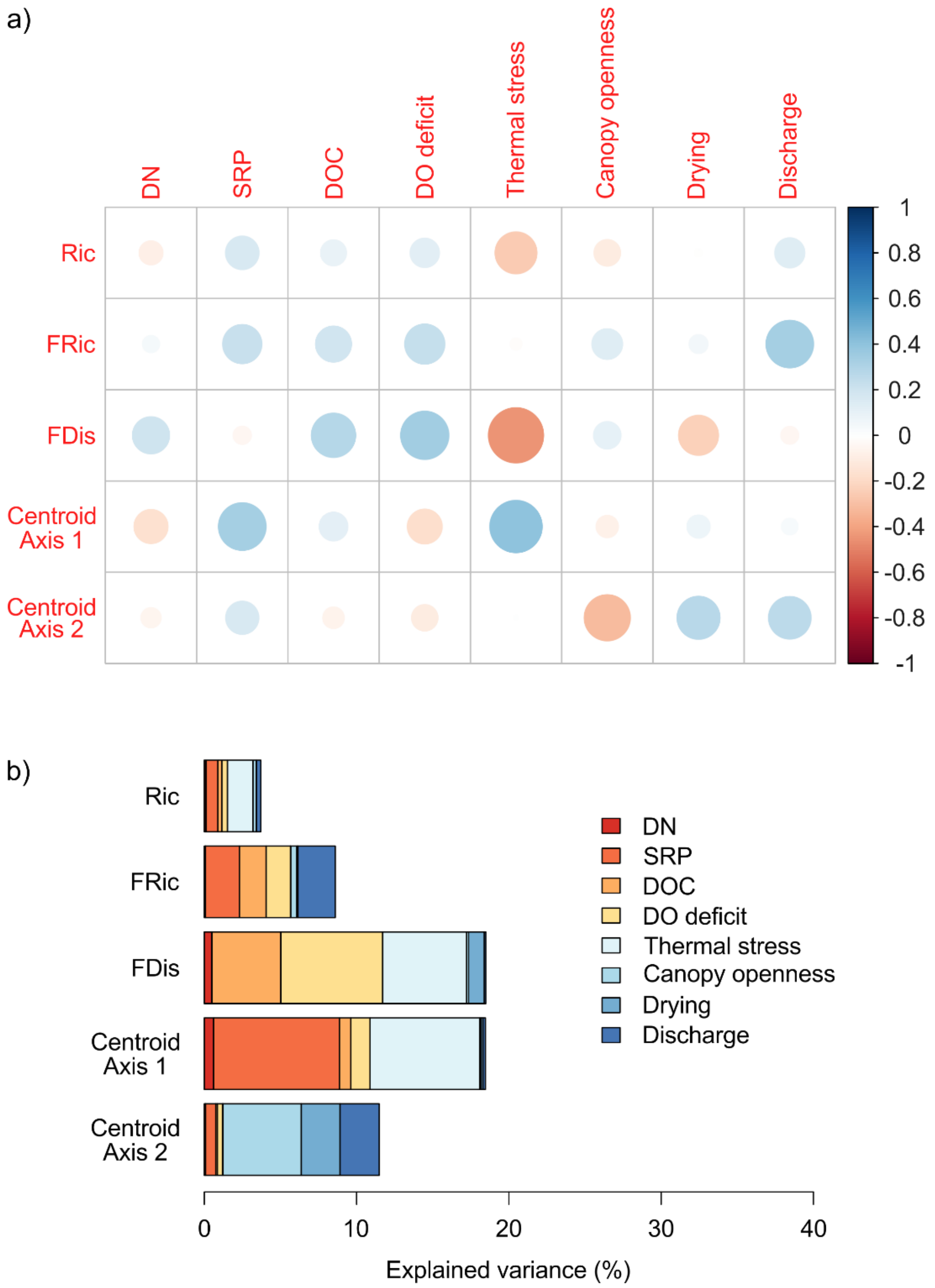

**Figure 3.** (a) Averaged and standardised model coefficients for the different fungal biodiversity facets, including taxonomic richness (Ric), functional richness (FRic), functional dispersion (FDis), and centroids of the first and second trait axes (Axis 1 and Axis 2). Red tones represent negative predictor effects, while blue tones indicate positive effects. (b) Mean explained variance of stressors and hydrology for the different fungal biodiversity facets, including taxonomic richness (Ric), functional richness (FRic), functional dispersion (FDis), and centroids of the first and second trait axes (Axis 1 and Axis 2). The random factor importance is not shown for simplicity (see further details in Table S7).

The greatest decomposition rates in the streambed sediment and water column compartments were found in the Cantabrian Mountains (mean ± SE: 0.0048 ± 0.0006 day$^{-1}$, 0.0038 ± 0.0006 day$^{-1}$, respectively) and Catalonian regions (0.0045 ± 0.0006 day$^{-1}$, 0.0034 ± 0.0006 day$^{-1}$), while the other regions had lower mean values (Figure 4 & Table S8). Decomposition rates were greater in the water column (0.0033 ± 0.0002 day$^{-1}$) compared to the sediment (0.0029 ± 0.0002 day$^{-1}$). In the water column, the Tagus and Cantabrian regions showed the highest

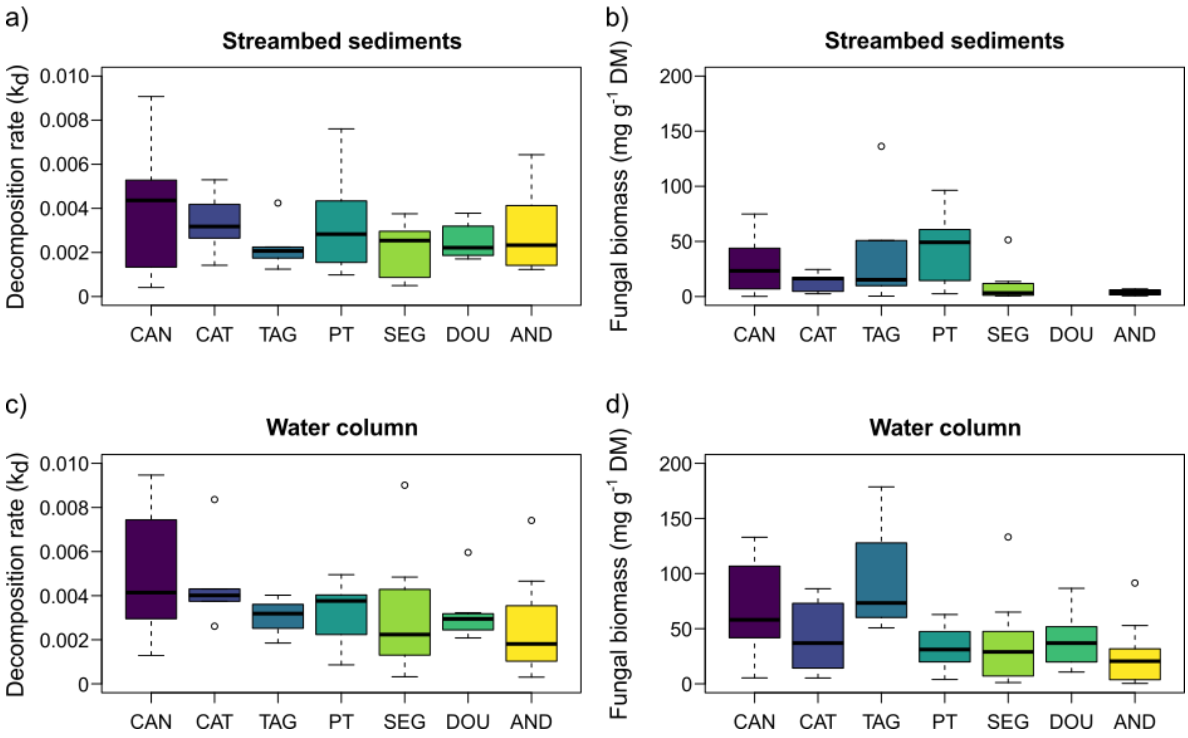

**Figure 4.** Boxplots of: (a) decomposition rate ($k_d$) in the water column, (b) decomposition rate ($k_d$) in the streambed sediments, (c) fungal biomass in the water column, and (d) fungal biomass in the streambed sediments. The colours of the bars correspond to the regions (as shown in Figure 1). Region abbreviations are as follows: CAN, Cantabrian Mountain range; CAT, Catalonia; TAG, Mid Tagus; PT, North Portugal; SEG, Segura; DOU, South Douro; and AND, Andalusia.

mean fungal biomass (94.0 ± 19.2 mg FB g DM$^{-1}$ and 66.9 ± 9.9 mg FB g DM$^{-1}$, respectively), while the Segura (34.5 ± 8.0 mg FB g DM$^{-1}$), northern Portugal (32.9 ± 4.3 mg FB g DM$^{-1}$), and Andalusia (23.8 ± 5.6 mg FB g DM$^{-1}$) regions exhibited the lowest values. In contrast, the northern Portugal region exhibited the highest mean fungal biomass in streambed sediments (44.3 ± 7.1 mg FB g DM$^{-1}$), followed by the Tagus region (42.5 ± 18.6 mg FB g DM$^{-1}$). The Segura and Andalusia regions showed the lowest mean fungal biomass in streambed sediments (9.7 ± 3.9 mg FB g DM$^{-1}$ and 3.8 ± 0.7 mg FB g DM$^{-1}$, respectively). On average, fungal biomass in the water column compartment (47.6 ± 9.6 mg g$^{-1}$) was double that in streambed sediments (23.6 ± 6. 6 mg FB g DM$^{-1}$).

Our models generally supported additive stressor effects on ecosystem functions and the sediment: water column ratios, with only one interactive term supported in the model of streambed sediment fungal biomass (Figure 5; Tables S5 & S6). Water column decomposition was positively associated with DOC (R$^2$ = 7.0%) and SRP (R$^2$ = 7.0%) but decreased with drying duration (R$^2$ = 9.7%) and water temperature (R$^2$=4.3%). In contrast, streambed sediment decomposition was primarily driven by the positive effects of DOC enrichment (R$^2$ = 22.9%). The sediment: water column ratio for the decomposition rate was positively related to DOC (R$^2$ = 5.7%), drying duration (R$^2$ =1.7) but negatively related to SRP (R$^2$ = 6.9%).

The fungal biomass in the water column compartment was positively associated with DOC (R$^2$ = 8.5%) and DN (R$^2$ = 1.4%), while it had a negative relationship with water temperature (R$^2$ = 13.0%), riparian canopy openness (R$^2$ = 7.6%), and drying duration (R$^2$ = 3.3%). Fungal biomass in the sediments was positively associated with DOC (R$^2$ = 9.5%), DO deficit (R$^2$ = 4.9%), and discharge (R$^2$ = 9.6%), but was negatively affected by water temperature (R$^2$ = 5.3%) and DN (1.7%). An antagonistic interaction between drying duration and DN enrichment was statistically supported, explaining 5.3% of the variance. The sediment: water column ratio for fungal biomass was positively related to drying duration ($R^2$ = 6.3%), water temperature ($R^2$ =1.6%), and discharge ($R^2$ = 1.2%), but showed a negative correlation with DN ($R^2$ = 3.7%) (Figure 5).

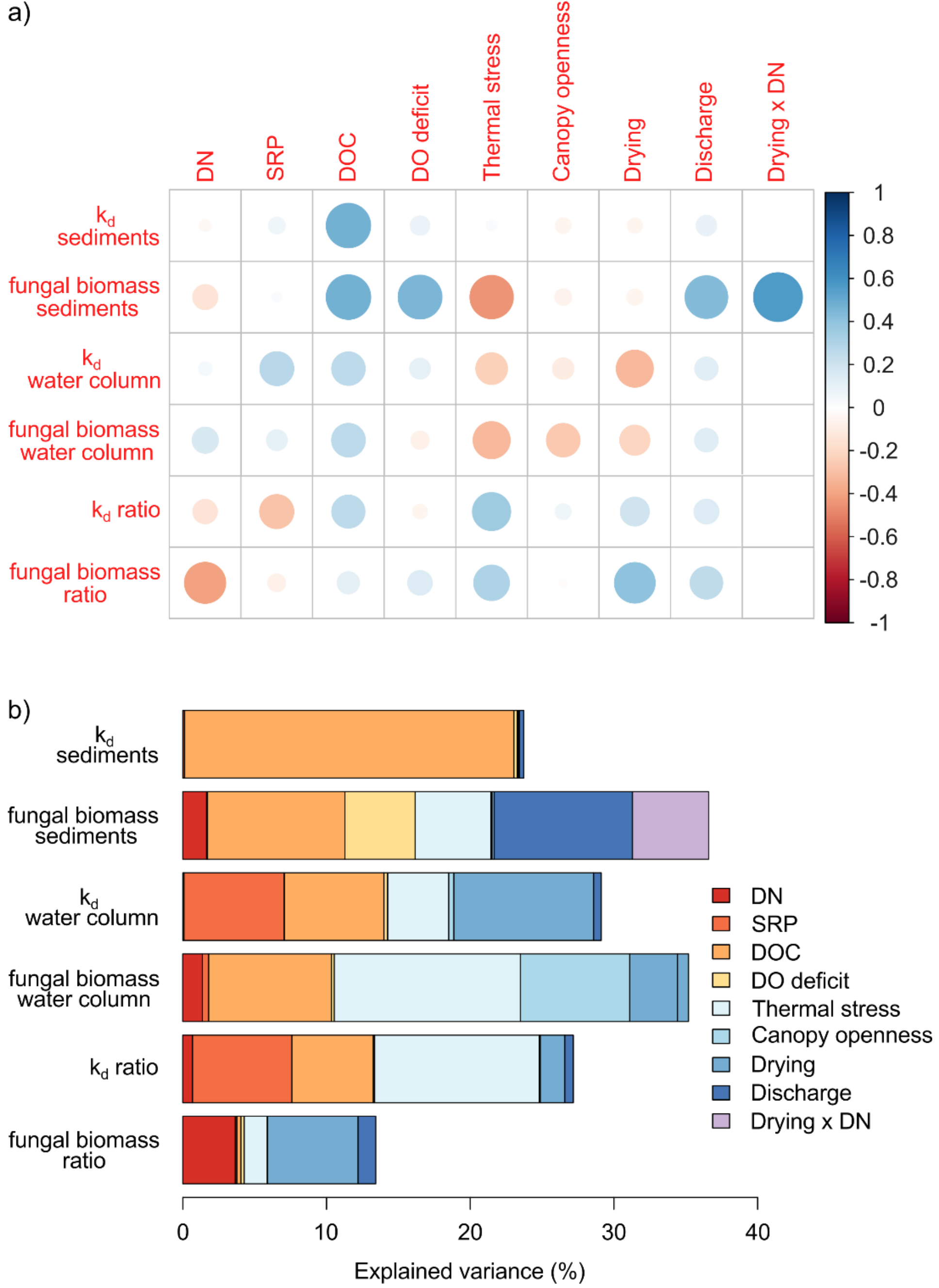

**Figure 5**. (a) Averaged and standardised model coefficients for the decomposition rate ($k_d$) and fungal biomass in the water column and streambed sediment. Red tones represent negative predictor effects, while blue tones indicate positive effects. (b) Mean explained variance of stressors and hydrology for the decomposition rate ($k_d$) and fungal biomass in both stream compartments. For simplicity, the importance of random factors is not shown here (see Table S7 for further details).

## 4. Discussion

Our results demonstrate that fungal communities and their functions are shaped by multiple abiotic drivers, offering valuable insights into the effects of global change on stream ecosystems. Specifically, while DOC positively influenced biodiversity and ecological functions, water temperature, riparian canopy openness, and drying duration were associated with lower biodiversity and functionality. These findings highlight the critical role of climatic stressors in regulating stream microbial biodiversity and function, suggesting that climate change may significantly reduce fungal biodiversity, decomposition activity, and trophic transfer. Additionally, we found that stream sediments can sustain OM decomposition and fungal biomass accrual, even under stressors, such as thermal and drying stress, providing an “insurance capacity” against human impacts.

## 4.1 Multiple stressor effects on fungal biodiversity and functions

Our study identified DOC, water temperature, riparian canopy openness, and drying duration as key drivers of fungal biodiversity and functions. Organic matter enrichment, represented here by DOC, positively influenced fungal diversity and functions, likely due to its ability to enhance heterotrophic microbial activity, including that of aquatic fungi (Danger et al., 2016; Kuehn et al., 2014). Furthermore, in our study, higher DOC levels were associated with a higher occurrence of wood saprotrophs, suggesting that dissolved carbon may originate from the decomposition of deadwood, potentially from deciduous tree species (Błońska et al., 2019).

Conversely, increasing water temperature was associated with reduced fungal biodiversity and functions, except in sediment decomposition. Within our thermal stress gradient (10.7–28.3°C), this negative response may be related to the fact that aquatic fungal communities exhibit their highest species richness in temperate streams (Duarte et al., 2016), within an optimal temperature range of 15 – 25°C (Graça et al., 2023; K. Sridhar & Bärlocher, 1993; Suberkropp, 1984). Additionally, species richness typically follows a unimodal relationship with temperature, peaking at around 10°C (Duarte et al., 2017; Gonçalves et al., 2013). Studies reporting positive temperature effects have generally focused on gradients ranging from below to near-optimal conditions (Fernandes et al., 2009; Ferreira, Chauvet, et al., 2015), with such patterns being more consistent during colder months (Ferreira & Canhoto, 2014, 2015). In contrast, our study was conducted during summer, when the mean water temperature of most streams exceeded this optimal range, reaching the upper end of the thermal gradient (10.7–28.3°C). This was particularly evident in the Andalusian (mean: 21.9ºC, range: 17.8-28.3ºC), Catalan (19.7ºC, 17.8-21.3ºC) and Segura (17.5ºC, 13.3-21.8ºC) basins, which showed the lowest biodiversity values and generally reduced functional metrics. These findings align with previous studies that reported reductions in functional richness across broader water temperature gradients (4-22ºC) (Fenoy et al., 2021).

Consistent with previous observational and manipulative research, drying stress – particularly prolonged drying – negatively affected fungal functional diversity and functions in water column compartments (Arias-Real et al., 2020, 2023; Viza et al., 2022). Flowing water is essential for the sporulation and dispersal of aquatic hyphomycetes (Bärlocher, 1992b). Although microbial decomposers, including aquatic hyphomycetes, often recover their activity quickly following rewetting events (Bruder et al., 2011; Foulquier et al., 2015; Niyogi et al., 2020; Pohlon et al., 2013), our results suggest that prolonged drying during summer consistently inhibits their biological activity across regions.

Our results also indicated that riparian degradation, represented by riparian canopy openness, was generally associated with reduced fungal biodiversity and functions. Streams with reduced riparian forest diversity and cover are expected to experience declines in the quality and quantity of OM inputs (Kominoski et al., 2011), as well as more severe summer conditions with increased water temperatures and radiation inputs (Garner et al., 2017). This reduction in OM availability may lower fungal biodiversity and activity through bottom-up effects, as reduced resource availability, which simplifies the range of niches available for these organisms (Fernandes et al., 2013; Laitung & Chauvet, 2005; Tonin et al., 2018).

The effects of light availability further illustrate the complexity of riparian degradation. Field studies have shown varied outcomes, with some reporting no significant impact (Elosegi et al., 2018) and others highlighting negative effects, potentially due to increased solar radiation inhibiting fungal activity under open canopies (Albariño et al., 2008; Tonin et al., 2018). In addition, the absence of canopy cover reduces OM availability and background biomass of litter-associated communities (Oester et al., 2025). Experimental mesocosm studies have also yielded mixed findings. Some suggest a positive priming effect, where increased light enhances autotrophic production and labile OM availability, thereby stimulating fungal growth and accelerating OM decomposition (Danger et al., 2013; Lagrue et al., 2011; Rier et al., 2007). Conversely, other mesocosm studies report negative priming, where labile carbon from algal exudates suppresses the decomposition of recalcitrant carbon (Ashberry et al., 2021; Halvorson et al., 2019).

Surprisingly, and in contrast to previous research (Gulis et al., 2006; Menéndez et al., 2011; Pascoal et al., 2005; Pereira et al., 2016), nutrient enrichment was not identified as a dominant driver of fungal functions, possibly reflecting low nutrient limitation in Iberian rivers (Bruder et al., 2016). Our nutrient enrichment gradients were skewed towards higher concentrations, largely due to arid climates and pervasive agricultural activities. As a result, we may not have captured scenarios where moderate increases in nutrient concentrations stimulate fungal activity and diversity. Furthermore, our study was conducted in summer, when temperatures are higher compared to other seasons, and at elevated temperatures, fungal decomposer activity appears to be achieved at much lower nutrient levels (Fernandes et al., 2014). In regions with high agricultural activity, additional stressors, such as sedimentation and pesticides, may reduce decomposer activity despite higher nutrient concentrations (Pascoal et al., 2005; Pereira et al., 2016; Woodward et al., 2012). A continental-scale study, including colder regions and oligotrophic streams, found a non-linear relationship between nutrient levels and OM decomposition, with macroinvertebrate-mediated decomposition rates peaking at intermediate nitrogen and phosphorus concentrations (Woodward et al., 2012). Field experiments often report weaker effects of nutrient enrichment compared to laboratory studies, with variability influenced by substrate type (e.g., wood vs. leaf litter) and climate (Bruder et al., 2016; Ferreira, Castagneyrol, et al., 2015). Our study underscores the importance of considering a broad range of geographic conditions and multiple stressors for a more comprehensive assessment of nutrient effects, which may be less pronounced than previously suggested (Duarte et al., 2017; Woodward et al., 2012).

Nonetheless, nutrient enrichment generally had weak positive effects on taxonomic and functional diversity metrics and fungal functions in the water column, with nitrogen showing minimal impact (Ferreira & Graça, 2016; Lecerf & Chauvet, 2008). Enhanced decomposition rates under nutrient-enriched conditions were linked to increased fungal activity (Gulis et al., 2006; Menéndez et al., 2011; Pascoal et al., 2005), as fungi can absorb nutrients directly from the water column (Sridhar & Bärlocher, 2000; Suberkropp & Chauvet, 1995). In contrast, nutrient enrichment negatively affected fungal decomposition and biomass accrual in streambed sediments, likely due to reduced oxygen availability caused by summer flow reductions and fine sediment deposition from agricultural activities (Bruder et al., 2016). Although this mechanism was not directly measured, it warrants further investigation. Additionally, nitrogen was correlated with litter saprotrophs, while SRP was associated with wood saprotrophs, indicating substrate-specific nutrient limitations (Danger et al., 2016).

Our study also supports the idea that microbial activity can be maintained in sediments even in the presence of multiple stressors. Previous studies in intermittent streams hypothesised that sediment moisture acts as a refuge for aquatic fungi during drying events, preserving microbial activity at levels comparable to those under flowing conditions (Arias-Real et al., 2020; Ghate & Sridhar, 2015; Gionchetta et al., 2024; Schreckinger et al., 2021). Riparian shading and flash storms further enhance this "insurance capacity" by maintaining sediment water content and detrital resources (Gionchetta, Oliva, et al., 2019; Herbst, 1980). Additionally, our data suggest that streambed sediments can buffer extreme temperatures and protect microbial activity during extreme climatic events, while slight to moderate organic matter enrichment can stimulate the development of heterotrophic microbial biomass, supporting higher activity levels in sediments than in the column water (Gionchetta, Oliva, et al., 2019). However, microbial communities may respond differently according to their taxonomic group, with fungi being the least affected by hydrological alterations (Gionchetta, Romaní, et al., 2019).

Discharge positively influenced fungal functional diversity and functions, with a notable correlation to basin area (Figure S1). Sites with higher average discharge tend to have fewer periods or areas of dry conditions, thereby maintaining aquatic functions (Bruder et al., 2011). Higher discharge also buffers against temperature extremes, particularly in summer, by increasing the thermal capacity of the water column through a greater volume of water (Caissie, 2006). Additionally, higher flow velocities, associated with increased discharge, enhance fluxes of dissolved nutrients and oxygen, thereby stimulating fungal diversity and activity, such as sporulation and decomposition rates (Abril et al., 2016; Bruder et al., 2016; Ferreira & Graça, 2006).

## 4.2 Implications for stream management and biomonitoring in a global change context

Despite the prevalence of antagonistic interactive effects in experimental studies (Gutiérrez-Cánovas et al., 2022; Jackson et al., 2016; Velasco et al., 2018), our findings reinforce a growing body of literature suggesting that the combined effects of multiple stressors in real-world ecosystems tend to be additive rather than interactive (Birk et al., 2020; Gutiérrez-Cánovas et al., 2022; Lourenço et al., 2023). Notably, our analysis did not support stressor interactions, even though we captured long stress gradients (Feld et al., 2016; Segurado et al., 2022), representing a wider spectrum of stress conditions than those typically explored in experimental studies (Graça et al., 2023; Jackson et al., 2016).

Two reasons may explain these contrasting patterns between observational and manipulative studies. First, manipulative studies are typically short-term and may not account for acclimation, adaptation, recovery, or resilience mechanisms that occur over longer periods (Collins et al., 2020; Orr et al., 2020). Second, while manipulative studies often apply stressors simultaneously, stressor dynamics in natural ecosystems tend to occur sequentially or discretely, enabling organisms to resist and recover more effectively (e.g. heatwaves, Jackson et al., 2021). Fortunately, from a management perspective, non-interactive effects are among the simplest to address, as stressors can be mitigated independently or in combination without unintended side effects (Brown et al., 2013; Côté et al., 2016; Spears et al., 2021).

Organisms commonly used in biomonitoring programmes, such as diatoms, macrophytes, and invertebrates, are particularly vulnerable to nutrient and organic pollution (Birk et al., 2020; Hering et al., 2006). For this reason, most management actions focus on reducing nutrient pollution and improving water quality. However, our study demonstrates that stressors expected to intensify with climate change—such as warming, drying, and riparian degradation—are more critical for fungal biodiversity and functions (Barros et al., 2024; Bruder et al., 2016; Fenoy et al., 2021; Mora-Gómez et al., 2016). For example, climate change is expected to negatively affect fungal biodiversity and functions through increased water temperature (Tassone et al., 2023), prolonged drying (Messager et al., 2021), and riparian canopy loss (Pace et al., 2021, 2022). Nonetheless, as organic matter enrichment was positively associated with fungal biodiversity and function, climate change impacts may be partially offset by anticipated increases in DOC under conditions of low flow and hydrological fragmentation (Granados et al., 2022). Collectively, these findings suggest that mitigating warming through riparian restoration and maintaining natural flow conditions are among the most effective strategies for preserving fungal biodiversity and functions in a global change context.

While previous studies have found that taxonomic and trait metrics respond similarly to multiple stressors (Lourenço et al., 2023), our models indicate that functional trait diversity and composition were much more explanatory than taxon richness. This may partly reflect the large geographic scale of our study, where differences in taxonomic biogeography may blurry ecological responses. However, as traits are shared across species with contrasting spatial distributions, functional diversity and composition seems to be more robust tools for capturing the impacts of global change on streams at large scales, aiding to capture broad biodiversity response to environmental change.

Our study also offers insights into the advantages and limitations of using ecosystem functions as biomonitoring indicators for multiple stressor effects. Although there have been recent calls to incorporate ecosystem functioning into biomonitoring, few studies have evaluated their response to multiple stressors in natural streams (Brauns et al., 2022; but see Pereira et al., 2016; Smeti et al., 2019). While previous studies have suggested that aquatic fungi and their activities, such as OM decomposition, can serve as bioassessment tools for stream functioning—responding to hydromorphological alterations, nutrient availability, and pollution levels (Barros et al., 2024; Colas et al., 2017; Ferreira et al., 2021) —our study surprisingly found that decomposition and fungal biomass did not reflect changes in nutrient enrichment, one of the main focuses of stream biomonitoring. Other studies have shown complex responses of OM decomposition to nutrients (Woodward et al., 2012), ranging from strong (Arroita et al., 2012) to weak effects (Brauns et al., 2022; Tiegs et al., 2024), suggesting that these indicators may be less sensitive than invertebrates or diatoms for detecting nutrient pollution. However, our functional measurements effectively captured hydroclimatic stressors such as warming, drying, and riparian degradation, highlighting their potential to complement traditional biomonitoring methods. Despite the cost-effectiveness of using wooden sticks to estimate decomposition (Arroita et al., 2012), ergosterol analysis and result interpretation require more sophisticated and expensive methodologies and expertise.

In conclusion, our study demonstrates that fungal biodiversity and functions are shaped by multiple stressors, which are likely to intensify under climate change. While organic matter enrichment positively influenced fungal biodiversity and functions, thermal and drying stresses, as well as riparian degradation, had negative effects. Importantly, our findings highlighted the critical role of streambed sediments in buffering the impacts of thermal and drying stresses, providing streams with resilience against global change. The predominance of additive (non-interactive) stressor effects suggests that these stressors can be managed independently, simplifying the implementation of targeted mitigation measures. Together, these results underscore the urgent need for action to preserve the conditions that support stream microbial biodiversity and their associated functions, thereby safeguarding the essential services provided by freshwater ecosystems in a global change context.

## Author contributions

**Conceptualisation:** A.V., E.F., C.G.C., **Developing methods**: E.F., A.B., R.C., M.M. (fungal spore identification), A.V., R.A.R., I.M.-S., I.M. (decomposition, fungal biomass and fungal traits), A.L. (DOC and nutrient analyses), C.G.C. (coordination, study design and fieldwork protocol). **Conducting the research**: A.V., E.F., M.A., M.Á., R.A.R., C.A., J.B., A.B., R.C., Í.D., I.F., A.J.G.M., A.L., I.M.-S., M.M., I.M., C.P., M.T.M., C.G.C. **Data analysis:** C.G.-C. **Preparation of figures and tables:** A.V., C.G.C.; A.V. and C.G.-C. led the writing, and E.F., M.A., M.Á., R.A.R., C.A., J.B., A.B., R.C., Í.D., I.F., A.J.G.M., A.L., I.M.-S., M.M., I.M., C.P. and M.T.M. revised and edited the text, providing critical comments.

## Acknowledgments

R.A.R. held a postdoctoral grant, “Juan de la Cierva” (JDC2022-048728-I), funded by the Spanish Ministry of Science and Innovation and the Next Generation EU. C.G.C. is supported by a Ramón y Cajal contract (RYC2022-038189-I), funded by the Spanish Ministry of Science, Innovation and Universities (MICIU) through the Spanish State Research Agency (AEI) (MICIU/AEI/10.13039/501100011033) and by the European Social Fund Plus (ESF+). This research was carried out with the support of a Junior Leader Fellowship awarded to C.G.C. (LCF/BQ/PR22/11920005), funded by the 'la Caixa' Foundation (ID 100010434), and the “Generación de Conocimiento 2022” project (TrophicStress, PID2022-141732NA-I00), funded by MICIU/AEI/10.13039/501100011033 and the EU FEDER programme. M.T.M. is supported by a predoctoral grant, “Formación de Profesorado Universitario” (FPU22/04290), funded by MICIU, as well as by the BiodivERsA and Water JPI 2020-2021 COFUND Call for Transnational Research Proposals through the ResPond project (BiodivRestore-614), funded by AEI and the European Union NextGenerationEU/PRTR (PCI2022-132973/AEI/10.13039/501100011033). J.B. and M.Á.-C. received funding from the project “*Los efectos del calentamiento en el funcionamiento fluvial: Una respuesta mediada a través de la biomasa*” (BIORESP; PID2023-150641OB-I00), funded by MICIU/AEI/10.13039/501100011033 and by FEDER, EU. A.L.'s work was supported by the INHOT (CNS2022-135690) and FLUPRINT (EUR2023-143456) projects, funded by MCIN/AEI/10.13039/501100011033 and the European Union NextGenerationEU/PRTR. R.C. and A.B. received funding from the Biodiversa+ project MOSTFUN (SNSF: 31BD30_216821). C.A. is supported by a predoctoral grant, “Formación de Personal Investigador” (PRE2020-094732), associated with the ClimaRiskinPond project (PID2019-104580GA-I00), funded by MCIN/AEI/10.13039/501100011033 and by the European Social Fund (ESF) under the motto “Investing in your future.” E.F. was supported by a postdoctoral grant (POSTDOC-21-00225), funded by the Junta de Andalucía. The work of I.M.-S., M.M. and I.M. received funding from the project PID2020-115708RB-C21, funded by MCIN/AEI/10.13039/501100011033.

Additionally, I.M.-S. was supported by a Basque Government predoctoral grant (2023, J0D0Z-T5967-AJZ8).

## Data Availability Statement

Data are available from the authors upon reasonable request.

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## Figures and Tables captions

**Figure 1.** Locations of the study sites within the Iberian Peninsula and their context within Europe (red square).

**Figure 2.** Boxplots showing the variation in (a) taxonomic richness, (b) functional richness (FRic), and (c) functional dispersion (FDis) across the study regions. Region abbreviations: CAN – Cantabrian Mountain Range, CAT – Catalonia, TAG – Mid Tagus, PT – North Portugal, SEG – Segura, DOU – South Douro, and AND – Andalusia.

**Figure 3.** (a) Averaged and standardised model coefficients for the different fungal biodiversity facets, including taxonomic richness (Ric), functional richness (FRic), functional dispersion (FDis), and centroids of the first and second trait axes (Axis 1 and Axis 2). Red tones represent negative predictor effects, while blue tones indicate positive effects. (b) Mean explained variance of stressors and hydrology for the different fungal biodiversity facets, including taxonomic richness (Ric), functional richness (FRic), functional dispersion (FDis), and centroids of the first and second trait axes (Axis 1 and Axis 2). The random factor importance is not shown for simplicity (see further details in Table S7).

**Figure 4.** Boxplots of: (a) decomposition rate ($k_d$) in the water column, (b) decomposition rate ($k_d$) in the streambed sediments, (c) fungal biomass in the water column, and (d) fungal biomass in the streambed

sediments. The colours of the bars correspond to the regions (as shown in Figure 1). Region abbreviations are as follows: CAN, Cantabrian Mountain range; CAT, Catalonia; TAG, Mid Tagus; PT, North Portugal; SEG, Segura; DOU, South Douro; and AND, Andalusia.

**Figure 5**. (a) Averaged and standardised model coefficients for the decomposition rate ($k_d$) and fungal biomass in the water column and streambed sediment. Red tones represent negative predictor effects, while blue tones indicate positive effects. (b) Mean explained variance of stressors and hydrology for the decomposition rate ($k_d$) and fungal biomass in both stream compartments. For simplicity, the importance of random factors is not shown here (see Table S7 for further details).

**Table 1**. Fungal traits, their corresponding categories, and the references used for their definition. The table is adapted from Arias-Real et al. (2023) with additional references included.

**Supplementary Material captions**

**Figure S1.** Correlation between response variables.

**Table S1.** Mean values and standard errors (mean ± SE) for the environmental characteristics of the study regions. Region abbreviations are as follows: CAN – Cantabrian Mountain Range, CAT – Catalonia, TAG – Mid Tagus, PT – North Portugal, SEG – Segura, DOU – South Douro, and AND – Andalusia.

**Table S2.** Mean values and standard errors (mean ± SE) for the stressor variables in the study regions. Region abbreviations are as follows: CAN – Cantabrian Mountain Range, CAT – Catalonia, TAG – Mid Tagus, PT – North Portugal, SEG – Segura, DOU – South Douro, and AND – Andalusia.

**Table S3.** Total number of species occurrences per region during the study period.

**Table S4.** Means and standard error (mean±SE) of the taxonomic and functional metrics by region. Abbreviations are as follows: CAN, Cantabrian Mountain range; CAT, Catalonia; TAG, Mid Tagus; PT, North Portugal; SEG, Segura; DOU, South Douro; AND, Andalusia; Ric, taxonomic richness; FRic, Functional richness; FDis, functional dispersion; cent_ax1, centroids of the first trait axis; and, cent_ax2, centroids of the second trait axis.

**Table S5.** Correlations between functional axes and fungal trait categories.

**Table S6.** Summary of averaged coefficients for biodiversity and functional models.

**Table S7.** Explained variance ($R^2$) for biodiversity and functional models.

**Table S8.** Means and standard error (mean±SE) of the decomposition rates and fungal biomass accrual at each study site in both stream compartments (water column and streambed sediments). Abbreviations are as follows: CAN, Cantabrian mountain range; CAT, Catalonia; TAG, Mid Tagus; PT, North Portugal; SEG, Segura; DOU, South Douro; AND, Andalusia; BioM_sediment, fungal biomass in sediments; BioM_water-column, fungal biomass in the water column; kd_sediment, decomposition rates in sediments; kd_water-column, decomposition rates in the water column; ratio_k, ratio of decomposition rates; and ratio_bioM, ratio of fungal biomass.

**Appendix 1**. Details on missing data imputation for stressors.

**Appendix 2.** Details on performance of the models exploring functional and biodiversity responses to multiple stressors.