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Aquatic Terrestrial Connections

Moderators of Organic Matter Decomposition in Portuguese Streams: A Field Study and Literature Review

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ABSTRACT

1. Organic matter decomposition is a fundamental ecosystem process in streams, which have a large aquatic-terrestrial interface. The sensitivity of organic matter decomposition to changes in environmental conditions promoted by human activities is relatively well understood at large and local scales. In contrast, less is known about the natural variability of organic matter decomposition along natural gradients, especially at intermediate spatial scales. This gap undermines the use of organic matter decomposition as a tool to assess stream functional integrity in biomonitoring programmes. This study aimed at identifying the main moderators of organic matter decomposition under undisturbed conditions to establish natural baseline variation in organic matter decomposition rates in Portuguese streams.
2. A field study was carried out to assess organic matter decomposition rates in 37 least-disturbed streams distributed throughout continental Portugal and Madeira Island. This study addressed gaps in empirical research by using poplar organic matter, including wood, and undertaking the incubation in spring, while previous studies were carried out preferably in autumn/winter and used alder and oak leaf litter. A systematic literature review was also conducted to compile organic matter decomposition rates from least-disturbed streams across continental Portugal and the Azores archipelago.
3. Organic matter type (leaves or wood), shredder access (fine-mesh or coarse-mesh bags), season (autumn/winter or spring/summer), water nitrate concentration, the presence of exotic vegetation, distance to the source, and percentage shade emerged as main moderators of organic matter decomposition in the field study.
4. Organic matter type and identity (genus), shredder access, season, stream type (national Water Framework Directive typologies), hydrological regime (permanent or intermittent), water temperature, conductivity, and soluble reactive phosphorus concentrations were also important moderators of organic matter decomposition for the compilation of the field data and literature review data.
5. There was considerable variation in organic matter decomposition across least-disturbed streams, reflecting differences in organic matter type and identity, shredder access, season, and environmental conditions. Therefore, for organic matter decomposition

to be used as a tool to assess stream functional integrity, it is necessary to standardise the protocol by selecting specific organic matter types and species, mesh types, and incubation seasons to minimise variation caused by these factors. Choosing stream typologies relevant for the decomposition process (e.g., as outlined in the Water Framework Directive), along with identifying reference decomposition ranges for each typology, may facilitate the assessment of deviations in stream functioning from reference conditions.

1 | Introduction

Plant organic matter of terrestrial origin fuels aquatic food webs, being crucial to carbon and nutrient cycling, especially in forest streams, which have a large aquatic-terrestrial interface (Marks 2019). The incorporation of organic matter carbon and nutrients into aquatic food webs is mediated primarily by microbial decomposers (e.g., fungi and bacteria) and invertebrate shredders (Hieber and Gessner 2002). Microbial decomposers assimilate organic matter carbon and nutrients into their biomass and facilitate the feeding activities of shredders by producing extracellular enzymes that soften organic matter and by accumulating microbial biomass that increases the nutritional value of organic matter (Gulis and Suberkropp 2003). Shredders, in turn, feed on coarse organic matter, particularly after it has been conditioned by microbes, and release fine organic particles (including faeces) that serve as food resources for invertebrate collectors (Graça 2001).

The rate at which organic matter decomposes greatly depends on its intrinsic characteristics, with recalcitrant organic matter that is tough, rich in secondary and structural compounds, and poor in nutrients decomposing at a lower rate than labile organic matter. Accordingly, woody substrates generally decompose more slowly than leaf litter (Ferreira, Gulis, and Graça 2006; Ferreira et al. 2021; Spänhoff et al. 2007; Arroita et al. 2012), and decomposition rates of leaf litter across tree species generally correlate negatively with its lignin-to-nitrogen ratio (Ostrofsky 1997; Ramos et al. 2021).

Organic matter decomposition, being fundamentally a biological process, is also sensitive to the environmental conditions that may affect the distribution, abundance, and activity of microbial decomposers and shredders. Important environmental conditions in this context include water temperature (Amani et al. 2019), dissolved nutrient concentration (Ferreira, Castagneyrol, et al. 2015), and pH (Ferreira and Guérold 2017). Generally, organic matter decomposition is faster in streams with moderate nutrient concentration (Woodward et al. 2012), circumneutral pH (Ferreira and Guérold 2017), and at warmer seasons, latitudes, or elevations (Graça, Ferreira, and Coimbra 2001; Boyero et al. 2011; Taylor and Chauvet 2014).

Most studies addressing organic matter decomposition in streams have been carried out at small (local) scales and thus do not consider the variation in decomposition rates that is due to natural environmental variation. Several global studies over the last decade also contributed to our understanding of organic matter decomposition under relatively undisturbed conditions across large scales (e.g., Boyero et al. 2016; Follstad Shah et al. 2017; Tiegs et al. 2019, 2024; Zhang et al. 2019; Yue et al. 2022). However, studies addressing organic matter decomposition under relatively undisturbed conditions at regional or national scales remain scarce (but see Pozo et al. 2011; Feio et al. 2018). This is a significant gap as the drivers of organic matter decomposition can vary depending on the spatial scale under consideration (Tonin et al. 2018). Particularly, studies addressing organic matter decomposition at regional or national spatial scales are needed to assess the potential for organic matter decomposition to be included in official monitoring schemes as an indicator of stream functional condition (Ferreira et al. 2020; Brosed et al. 2022).

Portugal exhibits a high spatial heterogeneity in terms of climate, orography, and lithology along north–south and west–east gradients within its mainland territory, located in the Iberian Peninsula, and between the Iberian mainland and the Atlantic islands (Dinis et al. 2019). Variations in environmental characteristics that reflect this spatial heterogeneity likely affect stream ecosystem processes, such as organic matter decomposition. It is, therefore, important to identify the main moderators of organic matter decomposition under undisturbed conditions to establish natural baseline variation in decomposition rates. For this purpose, a field study was conducted to determine organic matter decomposition rates in least-disturbed streams across mainland Portugal and Madeira Island. In this study, we addressed gaps in empirical research by using poplar (genus *Populus*—a less studied riparian tree genus) leaf litter and wood (a less studied organic substrate), and incubating organic matter in streams during spring (a less studied season). A quantitative systematic literature review of organic matter decomposition studies in least-disturbed Portuguese streams was also performed. The aim was to identify the main moderators of organic matter decomposition, which is essential for considering it as a tool to assess functional stream integrity.

2 | Methods

2.1 | Field Study

The field study consisted of a coordinated distributed experiment (*sensu* Fraser et al. 2013) conducted by 23 researchers from the Marine and Environmental Sciences Centre (MARE), which has a large spatial implantation in Portugal, including Madeira Island. The experiments were conducted in multiple streams, following standard protocols for field and laboratory procedures. Protocols were designed to be straightforward and organic matter samples were prepared centrally and distributed to the research teams, which allowed the participation of researchers without previous experience in organic matter decomposition studies. Data were then compiled in standardised spreadsheets and centrally analysed.

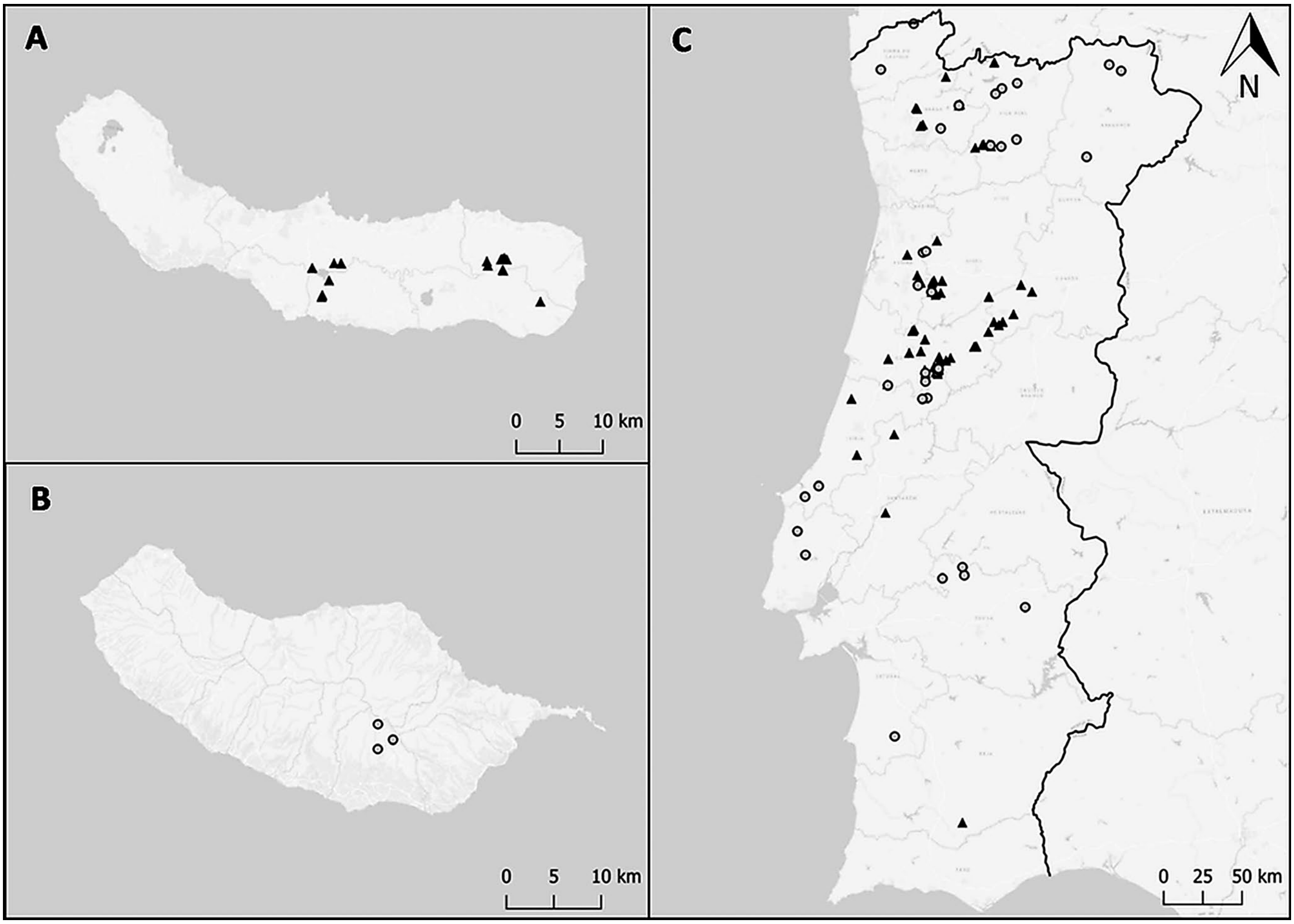

FIGURE 1 | Location of the streams used in the field study (○) and the literature review (▲) in (A) São Miguel Island (Azores archipelago), (B) Madeira Island (Madeira archipelago), and (C) continental Portugal.

2.1.1 | Study Streams

Thirty-seven streams located in mainland Portugal (between 42°4′23.5″N and 37°58′20.0″N; $N=34$) and in Madeira Island (Northeast Atlantic Ocean, Macaronesia region, ~520 km west of Morocco; $N=3$) were used (Figure 1A–C). Stream reaches were generally < 10 m wide and < 60 cm deep, between 0.3 and 79.6 km from the source (determined by GIS), and not greatly disturbed by human activities ("least-disturbed"), except for occasional forestry plantations (Table S1). The geographic distribution of streams resulted in differences in lithology, riparian vegetation, canopy cover, and water characteristics (Table S1).

Information on the methods used for the determination of stream location and characterisation of geomorphology and the surrounding area are shown in Table S1. In particular, shade was estimated visually as the percentage of shading at the zenith, if standing in the middle of the stream reach at noon, and exotic vegetation in the surrounding area was characterised as being absent, present or having an extensive cover (> 30% of the riparian corridor area of the two margins considered together).

Multiple variables were recorded to characterise the stream conditions when deploying leaf litter samples in the streams (see below). These included wet width (mean of three transects over a ~50-m long stream reach), depth and current velocity (mean of six locations where leaf litter samples were deployed), substrate composition and habitat complexity (four categories each) (Table S1). Simultaneously, stream water physical and chemical variables (temperature, pH, conductivity, and dissolved oxygen concentration) were also recorded with field probes (see Table S1 for the equipment references). Additionally, stream water was collected, filtered (fibre-glass filters, 47-mm diameter, 0.7-μm pore size; Whatman GF/F, GE Healthcare UK Ltd., Little Chalfont, UK), stored in two acid-washed plastic bottles (~300 mL each), and frozen for later nutrient analyses. One of the frozen water samples was sent to the Department of Life Sciences of the University of Coimbra for determination of soluble reactive phosphorus (SRP) concentration by spectrophotometry (6715 UV Vis. Spectrophotometer; Jenway, Staffordshire, UK) (APHA 1995). The other frozen water sample was sent to the Centro de Apoio Científico-Tecnolóxico á Investigación (CACTI, University of Vigo, Spain) for determination of nitrate, nitrite, and ammonium concentrations by the colorimetric method (AA3 Bran + Luebbe autoanalyser; SEAL Analytical, Norderstedt, Germany). Dissolved inorganic nitrogen (DIN) concentrations were estimated as the sum of nitrate-nitrogen, nitrite-nitrogen, and ammonium-nitrogen concentrations.

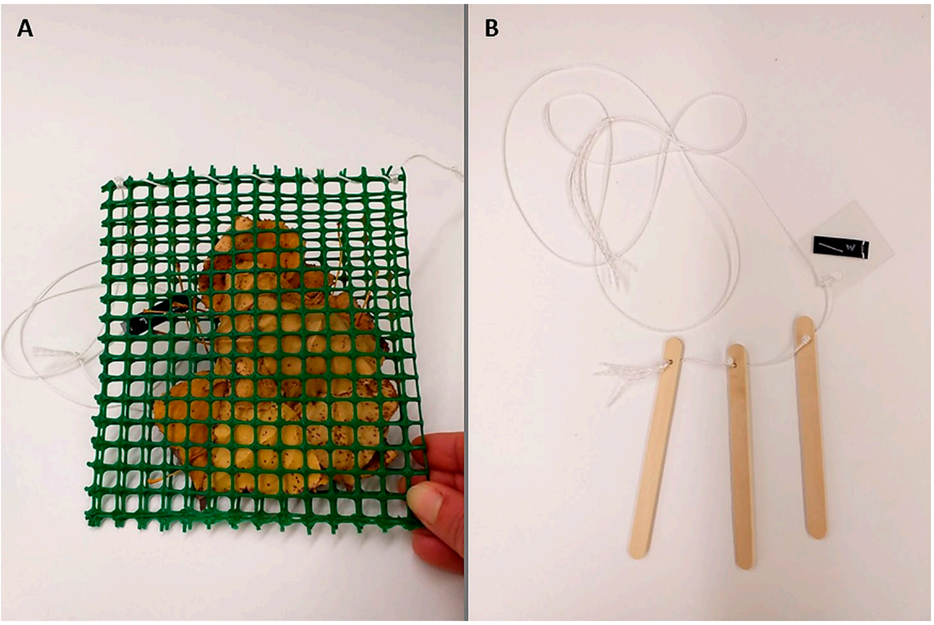

FIGURE 2 | Organic matter samples used in the field study: (A) senescent black poplar (*Populus nigra*) leaf litter enclosed in coarse-mesh bags (10-mm mesh opening) and (B) commercial ice-cream sticks made of Canadian poplar (*Populus* × *canadensis*) wood (3 sticks per sample).

2.1.2 | Organic Matter Decomposition

Poplar (genus *Populus*) leaf litter and wood were used in the field study. Leaf litter contributes the most to the annual organic matter input to streams (up to 66%), with wood (twigs and branches) also making a significant contribution (up to 24%) (values for deciduous riparian forests in the Iberian Peninsula; Abelho and Graça 1996; Molinero and Pozo 2004; Pereira et al. 2024). Additionally, wood often dominates the benthic organic matter standing stock (e.g., 41% in a stream flowing through a deciduous forest in northern Spain), largely due to its slow decomposition rate (Molinero and Pozo 2004). As leaf litter is generally decomposed by the combined efforts of microbial decomposers and invertebrate shredders (Hieber and Gessner 2002; Cornut et al. 2010), and wood decomposition is mostly microbially driven (Ferreira et al. 2021), using both substrates enables the assessment of the performance of both decomposer groups in least-disturbed streams.

Leaves of black poplar (*Populus nigra* L.) were collected immediately after senescence, over two weeks of dry weather in October 2020, from the floor of an even-aged tree stand at the left bank of the Mondego river at the city of Coimbra, central Portugal (40°12′6.1″N, 8°25′44.3″W). Only recently abscised leaves (i.e., shiny yellow leaves) without any sign of damage (e.g., herbivory) were collected. Leaves collected on various dates were mixed, air-dried at room temperature, protected from direct sunlight, and stored in cardboard boxes until used. Batches of air-dry leaves were weighed (2.95–3.05 g) and carefully enclosed in flat (~10 × 15 cm) coarse-mesh bags (10-mm mesh opening) (Figure 2A).

Black poplar is a common riparian tree species in mainland Portugal, but does not occur on Madeira Island. However, black poplar leaves are likely more palatable than those of many tree species in the native laurel forest of Madeira Island, which are often evergreen and have glossy and leathery leaves rich in defensive compounds (Llorent-Martínez et al. 2015, 2017). Black poplar leaves were selected for multiple reasons. They are relatively large and wide, which facilitates collection and allows for reaching the desired mass more easily, allows for using a relatively small number of leaves in mesh bags, and these will not escape the coarse-mesh bags (in contrast with willow, *Salix* spp., leaves that are smaller and narrower). Also, black poplar leaves remain relatively flat after senescence (in contrast with alder, *Alnus glutinosa* (L.) Gaertn., leaves that tend to curl and are very brittle), which allows for the preparation of litter bags without the need to moist the litter before, thus simplifying the process and facilitating storage and postage of litter bags without deterioration of their content. In addition, using black poplar allowed us to expand the knowledge about leaf litter decomposition of a typical riparian species since most published studies used alder leaf litter (very palatable), often in comparison with oak (*Quercus* spp.) leaf litter (recalcitrant) (Table S2).

Commercial wood sticks (i.e., ice-cream sticks, 11.3 cm long × 1.1 cm wide × 0.15 cm thick) of Canadian poplar (*Populus* × *canadensis* Moench) were purchased (Gran Velada S.L., Zaragoza, Spain). Canadian poplar is a natural hybrid of black poplar and necklace poplar (*Populus deltoides* W. Bartram ex Marshall) and was used due to the unavailability of wood sticks made from black poplar. The wood sticks were perforated at one end, at ~1 cm from the edge, and three sticks (total air-dry mass: 3.07–4.28 g) were introduced onto a nylon line, interspaced by knots, to make a sample (Figure 2B).

Ten additional leaf litter and wood stick samples were used to estimate a conversion factor between the initial air-dry mass and initial oven-dry mass, needed to estimate the initial oven-dry mass of the experimental samples. These additional samples were prepared as described above for the experimental samples, oven-dried to constant mass (60°C, ≥72 h), and weighed (±0.1 mg) for determination of initial oven-dry mass. The conversion factor was calculated as the ratio between initial oven-dry mass and initial air-dry mass (0.89 for leaf litter and 0.91 for wood sticks) and was multiplied by the initial air-dry mass of experimental samples to obtain their initial oven-dry mass (leaf litter: 2.61–2.70 g; wood sticks: 2.79–3.89 g). Leaching was not considered in the conversion factor to make results more comparable to those in the literature where most studies do not rule out leaching from the estimation of decomposition rates (Table S2). Also, not accounting for leaching in the conversion factor simplifies the protocol, which is needed if this is to be applied at large scales.

Leaf litter and wood stick samples were all prepared at the Department of Life Sciences of the University of Coimbra, individually placed into zip-lock plastic bags, and either hand-delivered or sent by postal mail to all partners during February 2021. Leaf litter and wood stick samples ($N=6$ each) were deployed in each stream between 19 March and 20 May 2021 (leaf litter: 37 streams, 222 samples total; wood sticks: 33 streams, 198 samples total). The organic matter was deployed in streams during the spring to prevent samples in southern Portugal and Madeira Island from being affected by the floods common during winter. Also, spring is the season when official biomonitoring programmes are implemented under the scope of the Water Framework Directive (INAG 2008a). From the perspective of using organic matter decomposition as a biomonitoring tool of stream functional integrity, it would be advantageous to implement the method in a season less prone to floods to reduce sample loss and in a season that coincides with the time of official biomonitoring programmes to reduce costs. However, more information is needed about the decomposition of organic matter during spring as most studies have addressed this process during autumn/winter.

Before deployment, the plastic bags containing the leaf litter samples were filled with stream water for ~30 min to render the samples moist and less susceptible to fragmentation and floatation when introduced into the streams. Samples were secured with nylon lines to submerged rocks, roots, or iron bars driven into the substrate in areas with relatively calm current velocity (i.e., "runs") distributed over a ~50-m long stream reach. Pools were avoided as samples could become buried under natural accumulating organic matter and sediments, while riffles were avoided as samples would be exposed to turbulent currents that would exacerbate leaf litter mass loss due to physical fragmentation.

Leaf litter and wood stick samples were retrieved after 28 and 77 days, respectively. Each sample was placed in a separate plastic bag and transported refrigerated in ice boxes to the laboratory. Upon arrival, samples were either processed within 24 h or frozen for later processing. Leaf litter and wood stick samples were individually placed into a tray filled with tap water for thorough cleaning and recovery of all leaf litter and wood pieces. The remaining organic matter (leaf litter or wood sticks) was placed into pre-weighed aluminium boxes, oven-dried (60°C, ≥72 h), and weighed for determination of oven-dry mass remaining. Several samples were lost during incubation across streams (10 leaf litter samples and 37 wood stick samples; all wood stick samples were lost from Ribeira de São Simão). Also, after 28 days of incubation, 14 leaf litter coarse-mesh bags were empty due to fast physical/biological breakdown and were not considered in the calculations (all leaf litter coarse-mesh bags were empty at Ribeira de Corona). Therefore, the final sample size varied between 1 and 6 across streams (36 streams for leaf litter and 32 streams for wood sticks).

Organic matter decomposition rates (k) were estimated on a per-day basis for each sample by applying the linearised form of the negative exponential model: Ln (oven-dry mass remaining/initial oven-dry mass) $= -k \times$ time, and samples were averaged per stream.

2.1.3 | Initial Organic Matter Characterisation

Five batches of air-dry leaf litter (~3 g) were milled (Retsch ZM100; Retsch, Haan, Germany) and five groups of wood sticks (3 sticks each) were drilled (Dremel 500; Dremel, Racine, WI, USA) to produce a fine powder (<0.5 mm size), oven-dried (60°C, ≥72 h), and used to determine the initial organic matter chemical characteristics. Lignin concentrations were determined by the Goering and van Soest method (Goering and van Soest 1970), polyphenol concentrations were determined by the Folin–Ciocalteu method (Bärlocher et al. 2020), phosphorus (P) concentrations were determined by the ascorbic acid method after digestion in the autoclave (Bärlocher et al. 2020), and nitrogen (N) and carbon (C) concentrations were determined with a CNH auto-analyser (IRMS Thermo Delta V advantage with a Flash EA, 1112 series; Thermo Fisher Scientific Inc., Waltham, MA, USA). Results are expressed as percentage of oven-dry mass. Nutrient concentrations were used to estimate C:N and C:P molar ratios.

2.2 | Quantitative Systematic Literature Review

The literature review addressed the organic matter decomposition of individual species in least-disturbed Portuguese streams to identify the natural factors contributing to heterogeneity in organic matter decomposition across a broad geographical area from northern to southern Portugal and the Azores Archipelago. Least-disturbed streams refer to streams in the best available condition in each study area, as used for the definition of reference conditions according to the Water Framework Directive (European Commission 2000). Thus, although we only extracted information for streams considered as least-disturbed or reference in the empirical studies (Table S2), the least-disturbed streams included in our analysis may be affected by human activities to some degree.

2.2.1 | Literature Search and Study Selection

A comprehensive literature search was conducted on 4 January 2022 to locate all relevant studies (i.e., those that have reported organic matter decomposition of individual species in least-disturbed Portuguese streams). The literature search was carried out using the Web of Science (WoS) platform (database: WoS Core Collection; editions: Science Citation

Index Expanded (SCI-EXPANDED), Conference Proceedings Citation Index—Science (CPCI-S), and Book Citation Index—Science (BKCI-S)). No lower time limit was applied during the search process. The following combination of keywords and fields (AU: author; TS: topic) was used: AU = ("Canhoto C" OR "Graça MAS" OR "Ferreira V" OR "Abelho M" OR "Pascoal C" OR "Cássio F" OR "Cortes R" OR "Sampaio A" OR "Raposeiro PM") AND TS = ((stream OR river) AND decomposition). The inclusion in the search string of all senior authors who have addressed organic matter decomposition in Portuguese streams ensured that all relevant studies would be identified. This search retrieved 223 studies, 58 of which were retained and 165 were excluded. Excluded studies included those conducted in micro- or mesocosms (83 studies), lacking decomposition data (24), conducted outside Portugal (16), review papers (16), duplicate publications (8), and studies exclusively focusing on disturbed streams (5). Three additional studies were included as they fulfilled the inclusion criteria (i.e., they reported organic matter decomposition of individual species in least-disturbed Portuguese streams), but were not detected in the WoS search (Cortes et al. 1994; Sampaio et al. 2008; Simões et al. 2020). Therefore, 61 studies were selected for inclusion in the literature review (Table S2).

2.2.2 | Data Extraction

Selected studies fulfilled the inclusion criteria, but not all of the reported information regarding the decomposition of organic matter was relevant and was therefore not extracted. Data most often not extracted from selected studies were related to organic matter incubated in disturbed streams (e.g., streams flowing through intensive plantations, affected by nutrient enrichment, metal contamination or experimental manipulation) or to incubation of organic matter as mixtures of several species (Table S2). In particular, when studies reported organic matter decomposition for stream reaches along a disturbed gradient (e.g., increasing nutrient concentrations), data were extracted for the least-disturbed stream reach(es) only (e.g., Pascoal et al. 2001, 2003; Pascoal and Cássio 2004; Pereira et al. 2016, 2017; Castela et al. 2008). Organic matter decomposition data reported in the text or tables were extracted directly, while those reported in figures were extracted with WebPlotDigitizer (https://automeris.io/WebPlotDigitizer/); in some cases, data were requested from authors. Generally, organic matter decomposition was reported as decomposition rate on a per-day basis (k, /d); in studies where it was reported as decomposition rate on a per degree-day basis (k, /dd; Ferreira, Larrañaga, et al. 2015; Ferreira et al. 2021; Pereira et al. 2017, 2021; Simões et al. 2020, 2021), it was multiplied by the average water temperature to convert it into k (/d), and in studies where it was reported as percentage mass loss (Abelho and Graça 1996; Ferreira and Canhoto 2014; Graça and Poquet 2014; Pereira et al. 2016; Ramos et al. 2021) or percentage mass remaining (Balibrea et al. 2020), it was converted into k (/d) by applying the linearised form of the negative exponential model [Ln(1—fraction mass loss) = − k × time (days) and Ln(fraction mass remaining) = − k × time (days), respectively]. Studies generally used leaves that were not pre-leached, and therefore, decomposition rates also account for mass loss due to leaching. The selected studies, therefore, contributed 623 decomposition rate (k, /d) estimates to the database (the database is available on Zenodo, https://doi.org/10.5281/zenodo.17202560).

Additionally, information regarding stream location and environmental conditions was extracted, including hydrological regime (i.e., permanent, intermittent) and water characteristics (e.g., temperature, conductivity, pH, nutrient concentrations) (the database is available on Zenodo). Stream typology, according to the Water Framework Directive (INAG 2008b), was identified for each stream based on location. Study characteristics such as incubation season (i.e., autumn/winter, spring/summer), organic matter type (i.e., leaves, wood) and its identity (genus), and mesh type used (i.e., coarse, fine) were also recorded (the database is available on Zenodo).

2.3 | Data Analysis

2.3.1 | Field Study

Initial organic matter chemistry was compared between leaf litter and wood by t-tests (N = 5 samples). Decomposition rates were compared between leaf litter and wood sticks across streams by paired t-test (excluding streams with missing values, N = 31 streams). The relationship between leaf litter and wood stick decomposition rates was assessed by linear regression (excluding streams with missing values, N = 31 streams). These analyses were performed on Statistica software (version 7, StatSoft Inc., Tulsa, OK, USA). Variability in decomposition rates of leaf litter and wood (considering all stream types together), and of these substrates within each stream type (only stream types with $N > 2$ were used), was assessed with coefficients of variation (CVs).

Streams were grouped by agglomerative hierarchical clustering (AHC) analysis (using Euclidean distance) based on normalised decomposition rates of both leaf litter and wood sticks (excluding streams with missing values, N = 31 streams) to ensure comparability between organic matter types; a cut-off value of 2 (~60% of maximum Euclidean distance) was defined in the AHC to generate at most three relatively homogeneous groups with $N > 1$. Decomposition rate means and standard errors (SE) were computed for each main group. A distance-based linear model [DistLM analysis, with Akaike information criterion corrected (AICc) as the selecting criterion and step-wise procedure] and a distance-based redundancy analysis (dbRDA) were performed to find linear combinations of environmental variables [Log(x + 1) transformed, and normalised] that explained the greatest variation of decomposition patterns (N = 30 streams); highly colinear variables (with correlation $|r| \geq 0.95$) and one stream for which several environmental measures were absent were removed from the analyses. AHC, DistLM and dbRDA were performed using Primer software (version 6, Primer-E Ltd., Plymouth, UK) with the PERMANOVA add-on package.

2.3.2 | Quantitative Systematic Literature Review

Decomposition rates were compared between coarse-mesh and fine-mesh bags across plant taxa by paired t-test (N = 19 taxa). The relationship between decomposition rates in coarse-mesh and fine-mesh bags was assessed by linear regression ($N=19$ taxa). For the portion of the database that allowed for a factorial design ($N=394$ decomposition rates), decomposition rates were compared between the two most common leaf genera (*Alnus* vs. *Quercus*), mesh types (coarse-mesh vs. fine-mesh bags), and seasons (spring/summer vs. autumn/winter) by three-way analysis of variance (ANOVA), followed by Tukey's test. The ANOVA considering multiple decomposition rates per study as independent observations may violate independence assumptions. However, only a few research teams address litter decomposition in Portugal, and litter decomposition methods are highly standardised (Bärlocher et al. 2020), and therefore many different studies share the same methodologies and streams. Thus, for simplicity, we considered that the factorial design explored in the ANOVA (litter genera × mesh type × season) derived from a single large study. The normality assumption was tested with Shapiro–Wilk's test, and the homoscedasticity assumption was tested with Bartlett's test. These analyses were performed using Statistica 7 software (Statsoft). Variability in decomposition rates of *Alnus* and *Quercus* leaf litter, leaf litter enclosed in coarse-mesh and fine-mesh bags, leaf litter incubated during spring/summer and autumn/winter, and leaf litter incubated in distinct stream types was assessed with CVs. To reduce variability, CVs were also calculated for smaller groups, using a hierarchical approach to order factors (only stream types with $N>2$ were used).

2.3.3 | Field Study and Quantitative Systematic Literature Review

The two databases (field study and literature review) were merged, and a factor analysis of mixed data (FAMD) was used to analyse the total number of observations with shared variables ($N=331$ decomposition rates). Also called principal component analysis of mixed data, FAMD is a principal component method dedicated to analysing a dataset containing both quantitative and qualitative variables; therefore, allowing the examination of the proximity between observations by considering both types of variables, exploring the association between all variables (quantitative and qualitative). All variables were considered active in the analysis since all can influence the observations. Correlation coefficients between each quantitative/continuous variable and the first two dimensions of the FAMD were computed. In contrast, a one-way ANOVA was performed for each of the first two dimensions, and an F-test was derived to assess the influence of each qualitative/categorical variable on each dimension. The FAMD was performed on R software (R Core Team 2023) using the Factoextra, FactoMineR, and ggplot2 packages for data analysis and visualisation.

3 | Results

3.1 | Field Study

Leaf litter had higher polyphenols (6.4×), phosphorus (7.1×), and nitrogen (11.2×) concentrations than wood sticks, but lignin and carbon concentrations were lower (by 52% and 6%, respectively) (*t*-test, $p<0.001$) (Table 1). Consequently, leaf litter had lower C/N (11.1×) and C/P (9.6×) molar ratios than wood sticks (*t*-test, $p<0.001$ and 0.036, respectively) (Table 1).

TABLE 1 | Initial chemical characteristics of black poplar (*Populus nigra*) leaf litter and Canadian poplar (*Populus ×canadensis*) wood sticks. Values are means ± SE ($N=5$). Comparisons between organic matter types were done by *t*-test and *p*-values are shown. DM, dry mass.

Characteristics	Leaf litter	Wood sticks	*t*-test
Lignin (% DM)	17.21 ± 0.49	35.55 ± 0.74	< 0.001
Polyphenols (% DM)	2.81 ± 0.22	0.44 ± 0.04	< 0.001
Phosphorus (P, % DM)	0.14 ± 0.02	0.02 ± < 0.01	< 0.001
Nitrogen (N, % DM)	0.56 ± 0.02	0.05 ± < 0.01	< 0.001
Carbon (C, % DM)	41.20 ± 0.28	43.49 ± 0.04	< 0.001
C/N (molar)	87 ± 3	970 ± 45	< 0.001
C/P (molar)	798 ± 73	7655 ± 2824	0.036

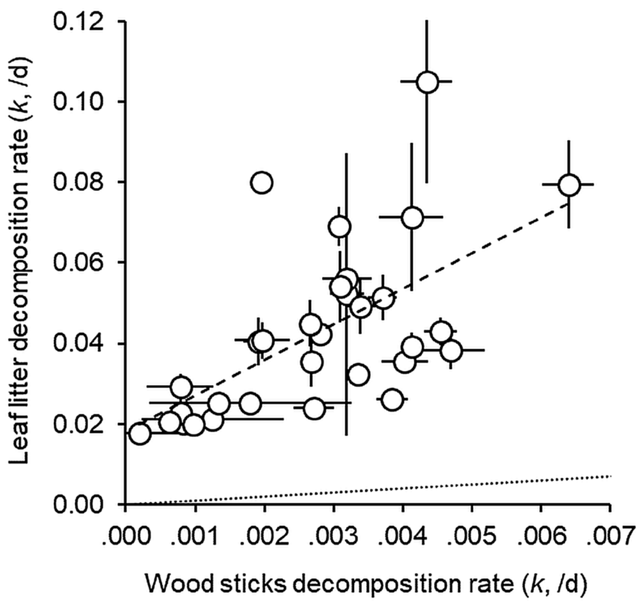

FIGURE 3 | Relationship between the decomposition rates of back poplar leaf litter after 28 days incubation and Canadian poplar wood sticks after 77 days incubation in the streams used in the field study ($N=31$). Values are means per stream, and error bars indicate SE ($N=1$–6 samples). The dotted line indicates the 1:1 line, and the dashed line indicates the best fit of the linear regression model: $Y=8.836 \times x+0.018$, $p<0.001$, $R^2=0.38$.

Leaf litter lost 39%–87% of its initial dry mass after 28 days of incubation across streams, which translated into decomposition rates (*k*, /d) of 0.0179–0.1051 (mean ± SE: 0.0428 ± 0.0035, $N=36$), and wood sticks lost 1%–37% of initial dry mass after 77 days, which translated into decomposition rates (*k*, /d) of 0.0002–0.0064 (mean ± SE: 0.0028 ± 0.0003, $N=32$) (Table S1 and Figure 3). Decomposition rates were 6.8× to 94.1× faster for leaf litter than for wood sticks across streams (paired *t*-test, $p<0.0001$), and there was a positive relationship between both (linear regression, $R^2=0.38$, $p<0.001$, $N=31$; Figure 3). Variation in decomposition rates was high and similar for leaf litter and wood sticks when considering all streams together (CV = 50% and 53%, respectively) and when considering small

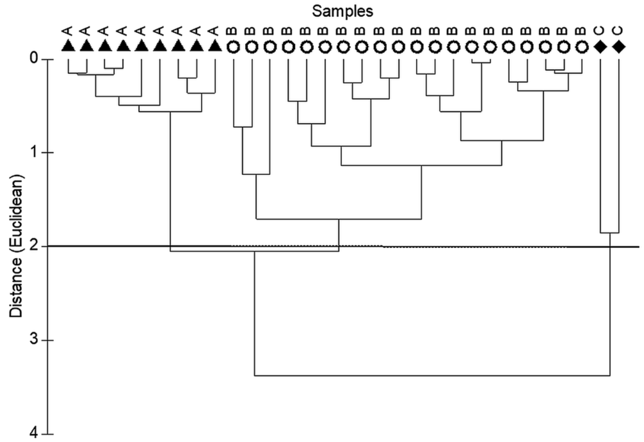

FIGURE 4 | Agglomerative hierarchical clustering (group average) performed on the triangular resemblance matrix (Euclidean distance) of decomposition rates of black poplar leaf litter and Canadian poplar wood sticks (normalised means per streams; *N* = 31) used in the field study. A cutting line (see Methods) was added to the cluster to indicate the three groups of streams considered in further analyses. ▲, slowest decomposition; ○ intermediate decomposition; ◆, fastest decomposition.

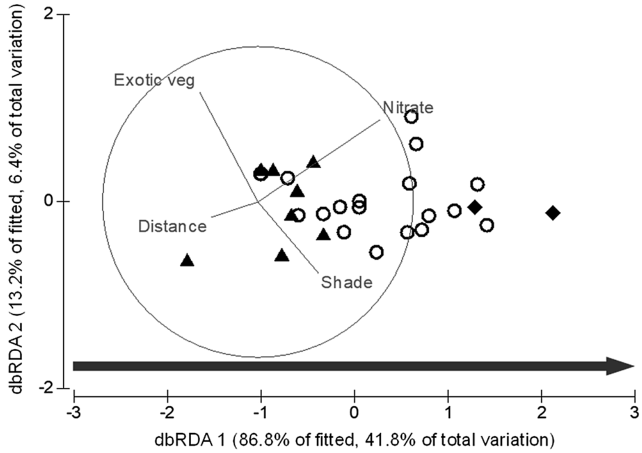

FIGURE 5 | Distance-based redundancy analysis (dbRDA) plots the four variables (exotic vegetation, nitrate, distance to the source, and shade in the channel) that explain more variation in the decomposition rates of black poplar leaf litter and Canadian poplar wood sticks in the field study streams (*N* = 30). Symbols reflect the three groups of streams identified on the cluster analysis: ▲ group A (slowest decomposition), ○ group B (intermediate decomposition), and ◆ group C (fastest decomposition) (Figure 4). The circle corresponds to the correlation circle (varying between 0 at the centre and 1 at the periphery), being independent of the axes' scales. The arrow on axis 1 indicates increasing decomposition rates from left to right.

northern rivers (type N1 <= 100) only (CV = 48% and 55%, respectively). Low to high variation in decomposition rates (CVs = 14%–42%) was found in other stream types, but the sample size was low (3–5 streams) (Table S3).

Decomposition rates (of both leaf litter and wood sticks together) discriminated three main groups of streams (cluster analysis; Figure 4). Group A had the lowest mean decomposition rates (*k*, /d) (leaf litter mean ± SE: 0.0225 ± 0.0012, *N* = 9; wood sticks mean ± SE: 0.0010 ± 0.0002, *N* = 9), followed by group B (leaf litter mean ± SE: 0.0463 ± 0.0033, *N* = 20; wood sticks mean ± SE: 0.0033 ± 0.0002, *N* = 20), and group C (leaf litter mean ± SE: 0.0922 ± 0.0128, *N* = 2; wood sticks mean ± SE: 0.0054 ± 0.0010, *N* = 2) (Figure 4).

The variables selected by the dbRDA constrained axes explained 48.2% of the total variation in decomposition rates in the field study (Figure 5 and Table S4.1). DIN was highly collinear with other physicochemical variables ($|r| > 0.68$) and was thus removed for modelling purposes. The reduced final model included four environmental variables influencing decomposition rates, including nitrate (partial correlation $r = 0.78$), exotic vegetation ($r = -0.38$), distance to the source ($r = -0.30$), and shade ($r = 0.39$) (Figure 5 and Tables S4.2 and S4.3). Shade was the only variable not significantly correlated with the decomposition rates (Table S4.2). Additionally, decomposition rates from left to right of the dbRDA (Figure 5, arrow; Table S4.4) indicate increasing decomposition rates and how they relate to the selected environmental gradients.

3.2 | Quantitative Systematic Literature Review

3.2.1 | Database Description

The database is available on Zenodo, https://doi.org/10.5281/zenodo.17202560. The first study reporting organic matter decomposition in least-disturbed Portuguese streams dates from 1994 (Cortes et al. 1994). Since then, 61 studies have been conducted over 28 years until early 2022 (Table S2). Seven studies provided a single decomposition rate estimate to the database, but most studies contributed multiple estimates (2–96) as they encompassed multiple streams, seasons, leaf species, and mesh types (e.g., Feio et al. 2010; Ferreira and Canhoto 2014; Raposeiro et al. 2014; Ferreira, Raposeiro, et al. 2016; Ramos et al. 2021). The database includes 623 decomposition rate estimates sourced from about 60 streams (lack of precision is related to some ambiguity in reported stream names). These streams were primarily located in the central and northern regions of mainland Portugal, with more recent additions (since 2014) from the Azores Archipelago. This resulted in an uneven distribution of decomposition rate estimates, with 72% in central Portugal, 20% in the Azores, 8% in northern Portugal, and 0.3% in southern Portugal. Leaf litter was the organic substrate most often used (98% of estimates); it derived from 42 native and exotic plants, but native oak (34%) and alder (30%) were the most frequently used taxa. Coarse-mesh bags, which allow microbes and macroinvertebrates to enter, were used more often than fine-mesh bags that exclude macroinvertebrates (56% vs. 44% of estimates). Furthermore, organic matter was more often incubated during autumn/winter than spring/summer (59% vs. 41% of estimates).

3.2.2 | Organic Matter Decomposition in Portuguese Streams

Mean (±SE) organic matter decomposition rates (*k*, /d) in coarse-mesh bags was 0.0306 (±0.0023, *N* = 347) and in

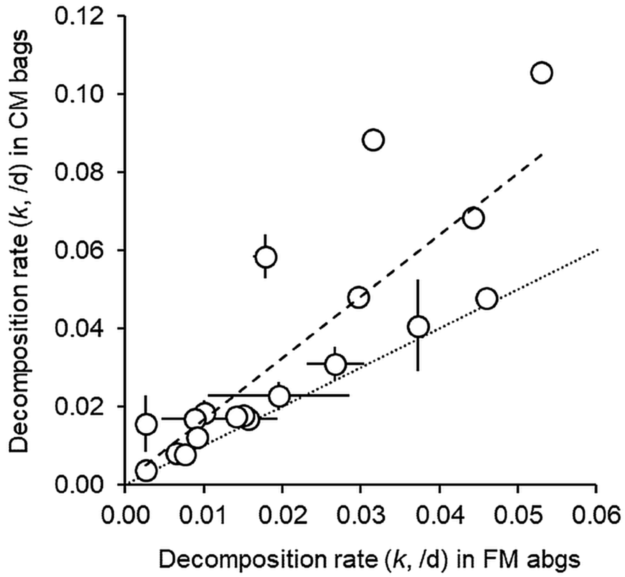

FIGURE 6 | Relationship between the organic matter decomposition rates in coarse-mesh (CM) and fine-mesh (FM) bags for the same plant taxa used in the literature review ($N = 19$). Values are means per plant taxon, and error bars indicate SE. The dotted line indicates the 1:1 line, and the dashed line indicates the best fit of the linear regression model: $Y = 1.5693 \times x + 0.0013$, $p < 0.001$, $R^2 = 0.71$.

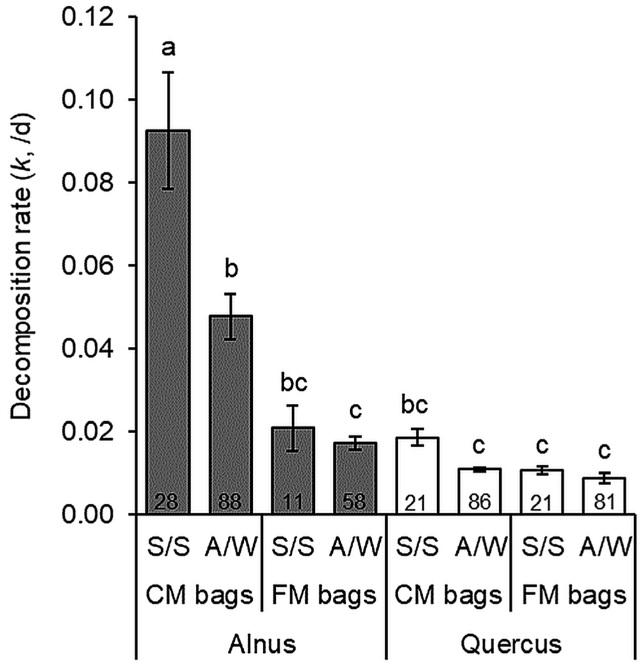

FIGURE 7 | Decomposition rates ($N = 394$) for *Alnus* and *Quercus* leaf litter enclosed in coarse-mesh (CM) and in fine-mesh (FM) bags and incubated in spring/summer (S/S) and in autumn/winter (A/W) in streams from the literature review. Comparisons were made by three-way ANOVA, and different letters indicate significant differences ($p < 0.050$). Values are means, and error bars indicate SE; numbers at the base of columns indicate the sample size.

fine-mesh bags was 0.0131 (±0.0008, $N = 276$) across the database. When considering only organic matter from plant taxa occurring in the environment (whether native or exotic, potentially familiar to stream organisms compared to those used solely for experimental purposes), the mean (±SE) organic matter decomposition rates (k, /d) in coarse-mesh bags was 0.0338 (±0.0025, $N = 314$) and in fine-mesh bags was 0.0135 (±0.0009, $N = 251$). When considering mean values for each plant taxon, decomposition rates were 1.04× to 6.27× faster in coarse-mesh than in fine-mesh bags (paired t-test, $p = 0.004$), and there was a strong positive relationship between them (linear regression, $R^2 = 0.71$, $p < 0.001$, $N = 19$; Figure 6).

Decomposition rates were affected by leaf litter identity (*Alnus* vs. *Quercus*), mesh type (coarse-mesh vs. fine-mesh bags), season (spring/summer vs. autumn/winter), and all the interactions between these factors within the subset of the database that allowed for a complete factorial design (three-way ANOVA, $p \leq 0.038$; Figure 7 and Table S5). Specifically, decomposition rates were faster for *Alnus* than for *Quercus* leaf litter only for coarse-mesh bags, faster in coarse-mesh than in fine-mesh bags only for *Alnus*, and faster in spring/summer than autumn/winter only for *Alnus* in coarse-mesh bags (Tukey's tests, $p \leq 0.006$) (Figure 7). Variation in decomposition rates was very high (CVs: 73%–163%) for both leaf litter taxa, mesh types, and seasons, and for all stream types (Table S6). When considering more homogeneous groups (i.e., specific leaf litter enclosed in a given mesh type and incubated in a given season and stream type), variation in decomposition rates was still very high (CV > 50%) in most cases; low to moderate variation in decomposition rates (CV < 30%) was generally associated with a low sample size (3–12 estimates) (Table S6).

3.3 | Overall Moderators of Organic Matter Decomposition in Portuguese Streams: Combining Field Study Data and Literature Review Data

The field study and literature review databases shared several qualitative (stream type, hydrological regime, substrate type, mesh type, plant genus, and season) and quantitative variables (temperature, conductivity, pH, and DIN and SRP concentrations), which were selected for a joint database. The field study contributed with ~18% ($N = 60$) of the total observations ($N = 331$), expanding the available information regarding the decomposition rate for the genus *Populus*, for woody substrates, for spring and for streams in the central coastal area (L; Portuguese classification) (Table S1). The field study also added information regarding decomposition rates for streams in Madeira Island, and in the northern-southern transition area (N4) and the southern area of mainland Portugal (S3, S1 small), which were not present in the database derived from the literature review (Figure 1A–C and Tables S1 and S3).

The first two dimensions of the FAMD performed on observations from the joint database explained 20.6% of the total variation in decomposition rates (Figure 8A and Table S7.1). Temperature, conductivity, and SRP concentrations were the quantitative variables with the highest contributions to the FAMD first dimension (Figure 8A and Table S7.2). The quantitative/continuous variables significantly correlated with the FAMD first dimension were temperature ($r = 0.77$, $p < 0.001$),

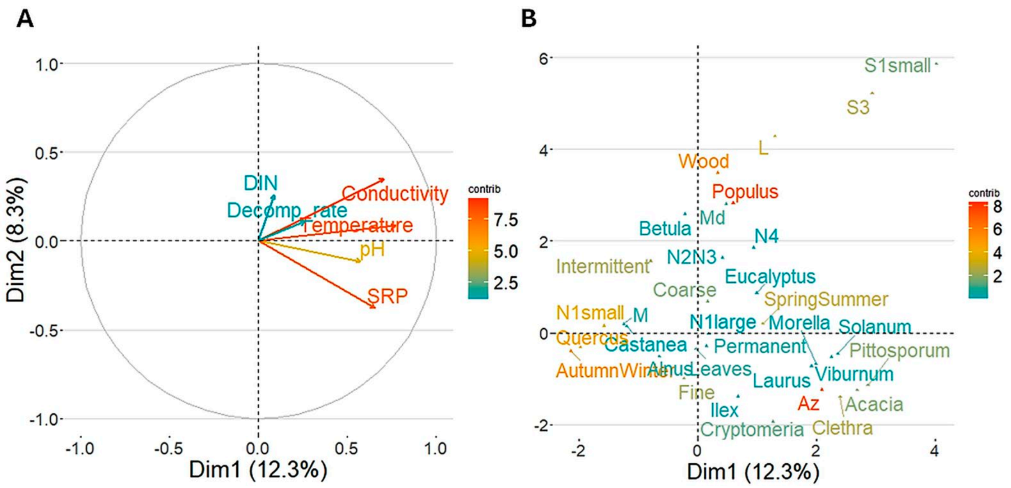

FIGURE 8 | Factor analysis of mixed data (FAMD) on the database joining data from the field study and the literature review projecting (A) square correlation coefficients for quantitative active variables (water characteristics) and (B) square correlation ratios for qualitative active variables. Colours indicate the contribution of variables to the first two principal dimensions, which explain 20.6% of the total variation in decomposition rates. On panel (B), variables and levels are as follows: Stream type (S1small, small southern rivers; S3, rivers in the sedimentary deposits of the Tagus and Sado rivers; N1small, small northern rivers; N1large, medium-large northern rivers; N2, medium-large rivers in the high Douro region; N3, small rivers in the high Douro region; N4, rivers in the northern-southern transition; M, northern mountain rivers; L, centre littoral rivers; Az, Azores; INAG 2008b), hydrological regime (intermittent, permanent), substrate type (leaves, wood), genus (*Acacia*, *Alnus*, *Betula*, *Castanea*, *Clethra*, *Cryptomeria*, *Eucalyptus*, *Ilex*, *Laurus*, *Morella*, *Pittosporum*, *Populus*, *Quercus*, *Solanum*, *Viburnum*), and season (autumn/winter, spring/summer). For the colour code, please refer to the online version of the paper.

conductivity ($r=0.71$, $p<0.001$), SRP ($r=0.66$, $p<0.001$), pH ($r=0.57$, $p<0.001$), and decomposition rate ($r=0.26$, $p<0.001$). Those significantly correlated with the FAMD second dimension were conductivity ($r=0.35$, $p<0.001$), DIN ($r=0.26$, $p<0.002$), SRP ($r=-0.38$, $p<0.001$), pH ($r=-0.11$, $p<0.001$), and decomposition rate ($r=0.11$, $p<0.001$) (Figure 8A and Table S7.3). Therefore, decomposition rates tend to increase along the FAMD first two dimensions, but particularly along the FAMD first dimension, which explains a larger portion of the total variation (Figure 8A). This pattern aligns closely with variations in temperature and conductivity (Figure 8A).

The qualitative/categorical variables with the highest contributions to the two FAMD dimensions (i.e., higher than 2 in at least one dimension; range 0–19.56) were substrate type (wood), genus (*Populus*, *Quercus*, *Clethra*, *Acacia*, *Pittosporum*, and *Cryptomeria*), season (both autumn/winter and spring/summer), stream type (Azores (Az), L, S3, N1 small, and S1 small), hydrological regime (intermittent), and mesh type (coarse and fine) (Figure 8B and Table S7.4). The qualitative/categorical variables significantly associated with the FAMD first dimension were stream type (one-way ANOVA, $R^2=0.77$, $p<0.001$), genus ($R^2=0.74$, $p<0.001$), season ($R^2=0.58$, $p<0.001$), and hydrological regime ($R^2=0.03$, $p<0.001$). Those significantly associated with the FAMD second dimension were genus ($R^2=0.78$, $p<0.001$), stream type ($R^2=0.72$, $p<0.001$), substrate type ($R^2=0.44$, $p<0.001$), mesh type ($R^2=0.25$, $p<0.001$), hydrological regime ($R^2=0.16$, $p<0.001$), and season ($R^2=0.03$, $p<0.001$) (Figure 8B, Table S7.5). Specifically, substrate and mesh types were mainly segregated along the second dimension (Figure 9A,B, respectively). Autumn/winter versus spring/summer were mainly segregated along the first dimension (Figure 9C), with spring/summer associated with higher values of decomposition rates (Figure 8A). Intermittent versus permanent streams were mainly segregated along the second dimension (Figure 9D). Some observations of intermittent streams (top right corner of Figure 9D), belonging to spring/summer (top right corner of Figure 9C) for rivers from the littoral (L) and southern areas of Portugal (S1 small and S3) (top right corner of Figure 9E), and considering *Populus* litter (top right corner of Figure 9F) are associated with higher decomposition rates. Additionally, Azores streams (bottom right corner of Figure 9E), using a great variety of leaf litter genera that are not used in continental streams (bottom right corner of Figure 9E), were segregated along the first and second dimensions and were associated with higher SRP concentrations (Figure 8A).

4 | Discussion

In the field study, we showed the practicality of using organic matter decomposition as a biomonitoring tool by non-experts and identified key moderators of organic matter decomposition in least-disturbed Portuguese streams. By complementing the field study with a literature review compiling studies addressing organic matter decomposition rates in least-disturbed streams across the country, we identified additional moderators of organic matter decomposition at a broader spatial scale. We also found high variation in decomposition rates, even under relatively homogeneous conditions (e.g., specific leaf litter enclosed in a given mesh type and incubated in a given season and stream type), which could stem from small methodological differences among studies.

4.1 | Organic Matter Type and Shredder Access

The faster decomposition rate of poplar leaf litter (up to 94×) compared to wood sticks observed in the field study reflects the

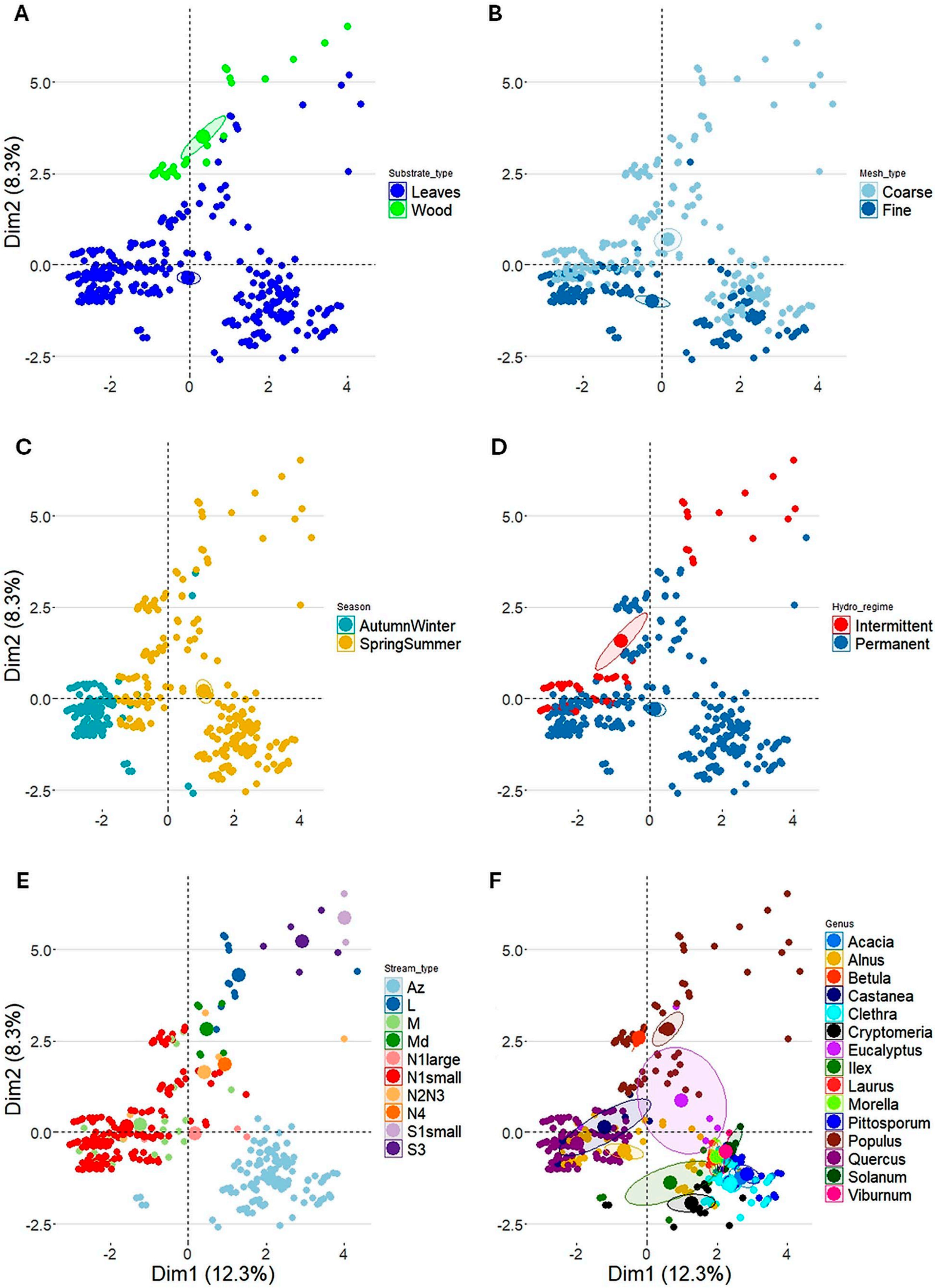

FIGURE 9 | Legend on next page.

FIGURE 9 | Factor analysis of mixed data (FAMD) projecting observations along the first two principal dimensions by grouping streams using the levels of the qualitative variables: (A) substrate type, (B) mesh type, (C) season, (D) hydrological regime, (E) stream type, and (F) genus. A confidence ellipse (0.95) was added around each group and the larger circles are the centroids of each category. On panel (E), acronyms are as follows: S1small, small southern rivers; S3, rivers in the sedimentary deposits of the Tagus and Sado rivers; N1small, small northern rivers; N1large, medium-large northern rivers; N2, medium-large rivers in the high Douro region; N3, small rivers in the high Douro region; N4, rivers in the northern-southern transition; M, northern mountain rivers; L, centre littoral rivers; Az, Azores (INAG 2008b). For the colour code, please refer to the online version of the paper.

higher lability of leaf litter as opposed to wood (Ferreira, Gulis, and Graça 2006; Ferreira et al. 2021; Spänhoff et al. 2007; Gulis et al. 2008; Arroita et al. 2012). This difference can be attributed to the lower carbon-to-nutrient ratios found in leaf litter compared to wood, leading to a reduced stoichiometric imbalance between leaf litter and microbial decomposers. Consequently, microbial decomposers can colonise leaf litter more efficiently than wood (Stelzer et al. 2003; Ferreira, Gulis, and Graça 2006; Gulis et al. 2008; Arroita et al. 2012). Furthermore, microbial colonisation of leaf litter further decreases its carbon-to-nutrient ratios (Gulis and Suberkropp 2003), making it more appealing to shredders (Graça, Cressa, et al. 2001; Graça and Cressa 2010). On the contrary, the high carbon-to-nutrient ratios of wood and its slower colonisation by microbial decomposers make it an unattractive food resource for most shredders, especially at early decomposition stages (Spänhoff et al. 2006).

Despite the faster decomposition of poplar leaf litter compared to wood observed in the field study, both were correlated, and the variation in decomposition rates (coefficient of variation) was high and similar for both substrates, suggesting that the decomposition of leaf litter and wood was similarly sensitive to variations in environmental conditions among streams. However, previous studies have yielded contrasting results. For instance, Simon and Benfield (2001) found that the decomposition of wood veneers did not differ between streams, while that of leaf litter (in coarse-mesh bags) was faster in streams with shredders than in those without, irrespective of all streams having high nutrient concentrations. Gulis et al. (2004) found that the decomposition of wood was more sensitive than that of leaf litter (in fine-mesh bags) to differences in nutrient concentrations among streams, especially for wood substrates with a high surface area-to-volume ratio (veneers). Ferreira et al. (2021) found that the decomposition of wood tongue depressors was more sensitive to differences in nutrient concentrations among streams than leaf litter in coarse-mesh bags. However, this heightened sensitivity was not necessarily observed compared to leaf litter in fine-mesh bags. Collectively, these studies suggest that the range of nutrient concentrations among streams and shredder access to leaf litter interact to determine the relative sensitivity of decomposition rates between substrate types to environmental conditions. Effects of nutrient concentrations, in particular, are more visible along gradients that include low to moderate nutrient concentrations because microbial activity is stimulated with small to moderate nutrient enrichment over oligotrophic backgrounds (Gulis et al. 2006; Woodward et al. 2012); high nutrient concentrations are generally associated with intensive agriculture and are accompanied by detrimental changes in environmental conditions (e.g., oxygen depletion, sedimentation, pesticides) that impair decomposer activity (Woodward et al. 2012). Therefore, it may be difficult to see any influence of nutrient concentration on organic matter decomposition among streams when the variation in nutrient concentrations is low (Simon and Benfield 2001) or when streams with low and high nutrient concentrations derived from intensive agriculture are being compared (Woodward et al. 2012).

Additionally, the decomposition rates of poplar leaf litter observed in the field study (0.0179–0.1051, /d) encompassed a significant portion of the range in decomposition rates found across plant taxa (in coarse-mesh bags) in the literature review (0.0039–0.1056, /d). Poplar leaf litter was collected from a single stand of trees over a short time period with relatively constant weather conditions, and therefore, the variation in leaf litter decomposition rates suggests strong sensitivity to variation in environmental conditions among streams (see below). However, it is worth noting that the number of decomposition rate estimates derived from the field study for poplar leaf litter exceeded the number of estimates derived from the literature review for most leaf litter taxa (except for alder and oak, which were the taxa most often used in previous studies). Therefore, the wide range of decomposition rates for poplar leaf litter in this field study may reflect a higher variation in the conditions to which leaf litter was exposed. Similarly, large ranges in decomposition rates were also found for alder leaf litter incubated in least-disturbed streams in central Portugal (Feio et al. 2010) and worldwide (Boyero et al. 2011) and oak leaf litter incubated in least-disturbed streams in central Portugal (Feio et al. 2010; Ferreira, Castela, et al. 2016). Variation in decomposition rates was also high in the literature review for *Alnus* and *Quercus* leaf litter incubated in specific conditions (i.e., when enclosed in a given mesh type and incubated in a given season and stream type), which may suggest high variability in environmental conditions within each stream type or may be due to methodological differences among studies. Still, it is important to note that poplar leaf litter was incubated in spring, while previous studies preferentially used alder and oak leaf litter incubated in autumn/winter, which limits direct comparisons between the field study and previous studies. Being poplar such a common riparian species, their decomposition in streams should be more closely assessed in future studies.

Decomposition rates were notably higher for leaf litter in coarse-mesh than in fine-mesh bags in the literature review, especially for genera like *Cryptomeria* (6.27×), *Alnus* (3.28×), *Solanum* (2.81×), and *Fraxinus* (2.00×). However, for eight genera, the decomposition rates were higher in coarse-mesh than in fine-mesh bags by less than 25% (1.04–1.25×; *Salix*, *Morella*, *Castanea*, *Pittosporum*, *Eucalyptus*, *Populus*, *Laurus*, and *Viburnum*). Coarse-mesh bags allow shredder access to leaf litter, which can stimulate decomposition rates, especially in streams with high shredder density and when the leaf litter is more labile

(Hieber and Gessner 2002; Gulis et al. 2006). In contrast, leaf litter with physical (e.g., thick or waxy cuticle) and chemical defences (e.g., high concentration of essential oils), such as those from *Eucalyptus* or *Laurus* trees, will not benefit from shredder access as much as leaf litter that is soft and less protected (Gonçalves and Canhoto 2009). Also, leaf litter enclosed in coarse-mesh bags incubated in streams with low shredder density (such as *Morella*, *Pittosporum*, *Laurus*, and *Viburnum* in Azorean streams) will not decompose much faster than those enclosed in fine-mesh bags, with any observed differences between mesh types being most likely due to increased physical abrasion by current and sediments on coarse-mesh bags (Raposeiro et al. 2014; Ferreira, Raposeiro, et al. 2016). Similar decomposition rates between coarse-mesh and fine-mesh bags were also found in three biomes in Brazil (Feio et al. 2018).

The role of macroinvertebrate shredders in leaf litter decomposition was especially evident when examining leaf litter from *Alnus* (palatable) and *Quercus* (recalcitrant), the plant genera with the largest sample size in the literature review database. Decomposition rates showed distinct patterns: (i) they were higher for *Alnus* than for *Quercus* leaf litter only in coarse-mesh bags, where macroinvertebrates can access the litter; (ii) they were higher in coarse-mesh than fine-mesh bags only for *Alnus* leaf litter, which is more palatable and thus attracts more shredders than *Quercus* leaf litter; and (iii) they were higher in spring/summer (when insect shredders are at their late instars, i.e., have higher biomass) than in autumn/winter but only for *Alnus* leaf litter in coarse-mesh bags. Accordingly, the FAMD analysis identified substrate type, mesh type, and plant genus as some of the most relevant categorical variables associated with organic matter decomposition rates when considering both databases together.

Our results align with those of global coordinated distributed experiments (Boyero et al. 2016; Ferreira et al. 2019) and syntheses (Zhang et al. 2019; Yue et al. 2022) that also found overall faster decomposition rates in coarse-mesh than in fine-mesh bags. The differences in decomposition rates between leaf litter and wood in the field study, and the apparent species-specific sensitivity of organic matter decomposition to shredder presence in the literature review, also align with global syntheses that identified correlations between organic matter characteristics (e.g., lignin concentrations, carbon-to-nitrogen ratio) and decomposition rates (Zhang et al. 2019) or the ratios between decomposition rates in coarse-mesh and fine-mesh bags (i.e., shredder effect on decomposition; Yue et al. 2022). However, looking at organic matter identity, we have identified taxa that may be less sensitive to shredder presence (i.e., showing small differences in decomposition rates between coarse-mesh and fine-mesh bags) and should not be considered in biomonitoring if human activities are expected to affect stream invertebrates.

4.2 | Stream Environmental Conditions

Organic matter decomposition rates were grouped into three clusters, which likely reflect environmental differences across streams and merit targeted follow-up. Indeed, environmental variables explained about half of the variation in organic matter decomposition across streams, which is noteworthy considering the large heterogeneity across streams and relatively small sample size (30 streams used in the analyses). Only four of the environmental variables assessed seem to be responsible for the identified variation in organic matter decomposition across streams (nitrate concentration in water, percentage shade, presence of exotic vegetation, and distance to the source), three of which can be modified by human activities that affect water chemistry and riparian vegetation. Although streams were minimally disturbed by human activities, differences in geology and land uses in the catchment could account for differences in water chemistry. Previous studies have shown that small to moderate increases in dissolved nitrogen concentrations can stimulate the activities of microbial decomposers and organic matter decomposition in streams (Ferreira, Gulis, and Graça 2006; Gulis et al. 2006; Rosemond et al. 2015). Percentage shade, distance to source, and presence of exotic vegetation (e.g., eucalyptus plantations) are all likely correlated with shredder density. A higher percentage of shade occurs in streams with more dense riparian vegetation, which is more common in small streams (close to the source) at the upper sections of hydrographic basins. These small forest streams are also those likely to have higher shredder density compared with larger downstream sections, in accordance with the River Continuum Concept (Vannote et al. 1980). In contrast, streams flowing through eucalyptus (*Eucalyptus globulus*) plantations are known to have lower shredder density and biomass compared with streams flowing through native vegetation in the Iberian Peninsula (Larrañaga et al. 2009). Shredder density (and biomass and abundance in mesh bags) is generally positively correlated with organic matter decomposition (Piscart et al. 2009; Encalada et al. 2010).

Organic matter decomposition is a fundamental ecosystem process in streams (as those used in this study), but it can also be relevant in large rivers where microbial activity is significant and small oligochaetes (mostly tubificids) can contribute to organic matter decomposition with their mining activities (Chauvet et al. 1993; Baldy et al. 1995, 2002). Therefore, organic matter decomposition can also be a potentially useful biomonitoring tool in large rivers (Feio et al. 2021). The establishment of reference conditions in large rivers is, however, more challenging as they are more frequently altered by human activities and have higher flows that can displace litter bags and finer sediments that deposit over them, preventing access to decomposers.

For the overall database (field study and literature review together), decomposition rates were positively correlated with temperature, conductivity, and SRP concentrations. Positive correlations between decomposition rates and temperature or SRP concentrations have been reported before (Taylor and Chauvet 2014; Rosemond et al. 2002; Gulis et al. 2006). Microbial and shredder activity are generally stimulated by increases in water temperature (González and Graça 2003; Ferreira and Chauvet 2011) and in phosphorus concentrations (Rosemond et al. 2002; Gulis et al. 2006). This effect is especially pronounced where microbial activity is limited by low temperature and low nutrient availability. Temperature and conductivity are higher in stream types located in the southern region of the country (e.g., L, S3 and S1 small), leading to the segregation of some stream types along the first dimension of FAMD, which correlates with the variable stream type. Temperature and nutrient concentrations have also been identified as important variables controlling organic matter decomposition rates at the global scale (Boyero et al. 2016; Zhang et al. 2019; Tiegs et al. 2024). Consequently, temperature and nutrient concentrations seem to be important moderators of organic matter decomposition across scales (local to global). Conductivity was also identified as an important moderator of organic matter decomposition in Spanish streams (Pozo et al. 2011).

It is important to note that water temperature can naturally vary among streams located at different elevations or ecoregions and greatly contribute to variation in organic matter decomposition rates (Taylor and Chauvet 2014). The direct effects (e.g., due to changes in biological activities) of natural differences in water temperature among streams on organic matter decomposition could be discarded by expressing decomposition rates on a per degree-day basis (instead of on a per-day basis as done here). However, this would require the deployment of temperature dataloggers in the streams for the duration of the organic matter incubation, which would add an extra level of difficulty to the protocol and make it more expensive, which is not appealing in the scope of large-scale biomonitoring programmes. Additionally, expressing decomposition rates on a per degree-day basis would not rule out the indirect effects of natural differences in water temperature among streams (e.g., due to higher shredder density in colder streams) on organic matter decomposition. Therefore, streams of unknown ecological status must be compared with similar "least-disturbed" streams (i.e., at similar elevation, ecoregion) when using organic matter decomposition as a biomonitoring tool. The same reasoning applies to natural differences in water chemistry among streams (e.g., arising from differences in geology), which can greatly affect organic matter decomposition. Again, streams should be compared within the same geological context.

Season and stream hydrological regimes were also correlated with FAMD's first dimension. Generally, organic matter decomposition is faster in warmer (spring/summer) than colder seasons (autumn/winter) (Graça, Ferreira, and Coimbra 2001; Ferreira and Canhoto 2015; Mora-Gómez et al. 2015). This increase in decomposition rates during warmer seasons is due to higher water temperature, which stimulates the activities of microbial decomposers and invertebrate shredders, and to the ontogenetic stage in which insect shredders are (i.e., late larval instars with large body sizes) (González and Graça 2003; Ferreira and Chauvet 2011).

The hydrological regime is also an important variable controlling aquatic communities and processes. Recent meta-analyses have shown lower macroinvertebrate diversity and density, as well as lower decomposition rates, in intermittent than in perennial streams (Soria et al. 2017; Ferreira et al. 2023; Yang et al. 2023). In this study, however, hydrological regime had a very small contribution to the variation in organic matter decomposition across streams (R^2: 0.03–0.16), which could be explained by most studies being carried out during autumn/winter when even intermittent streams have flowing water. In flowing water conditions, other variables such as water chemistry, organic matter type and species, and mesh type may be more relevant.

Although stream type is associated with distinct environmental conditions and biotic communities affecting organic matter decomposition (see above), this study revealed a strong correlation between stream type and plant genus. For instance, decomposition rates derived from Azorean streams involved plant genera not previously studied in mainland Portugal streams (*Clethra*, *Cryptomeria*, *Ilex*, *Laurus*, *Morella*, *Pittosporum*, *Solanum*, and *Viburnum*). Also, decomposition rates derived for rivers in the littoral (L) and southern areas of Portugal (S3 and S1 small streams), which were mainly intermittent, pertained to *Populus* organic matter exclusively contributed by the field study.

4.3 | Methodological Considerations

The protocol used in the field study was designed to be as simple as possible to allow the participation of researchers without previous experience with the method, and simultaneously assess the feasibility of the method to be implemented by non-experts using organic matter decomposition in biomonitoring programmes. The simplifications introduced into the protocol also considered time constraints associated with large-scale biomonitoring programmes that often imply long field campaigns and limited time for sample processing. Therefore, freezing organic matter samples after retrieval from the streams for later processing (as done here, and in other large studies; Gulis et al. 2006; Hladyz et al. 2011; Colas et al. 2017) allows for better time management compared with the common practice in ecological studies of processing the organic matter samples fresh.

Also, organic matter samples were processed for the determination of oven-dry mass remaining only, while determination of ash-free dry mass is often preferred to rule out inorganic sedimentation on the organic matter, which "artificially" increases mass remaining and leads to the underestimation of decomposition rates. However, not all participants had access to a muffle furnace, and igniting organic matter samples in large-scale biomonitoring programmes also complicates the approach and makes it less appealing. Nevertheless, organic matter samples were thoroughly cleaned with abundant water on a tray. Still, in streams with high rates of inorganic sedimentation, as those in calcareous lithology, igniting oven-dry organic matter for determination of the ash fraction may be needed to avoid underestimating decomposition rates.

The type of organic matter used is also a factor that can affect the practicability of the protocol in terms of time and budget (Arroita et al. 2012). In the field study, we used two organic matter types with ecological relevance (i.e., leaf litter and wood; Molinero and Pozo 2004) and found that both could potentially be useful bioindicators of stream functioning, with one being preferred over the other depending on specific conditions. For instance, if streams naturally differ in hydromorphology and leaf litter mass loss could be greatly due to physical fragmentation, wood sticks could be preferable to detect changes in ecosystem functioning driven by environmental changes caused by human activities. On the contrary, if streams naturally have low shredder density or if environmental changes are expected to affect shredder communities, then leaf litter could be used.

The use of leaf litter is, however, time-consuming, especially if a large number of leaf litter bags need to be prepared and later processed (Arroita et al. 2012). Previous large-scale biomonitoring studies have used a combination of alder leaf litter enclosed in coarse-mesh and fine-mesh bags, which allows capturing the effects of environmental changes that affect both microbial decomposers and invertebrate shredders (Colas et al. 2017; Brosed et al. 2022). These studies, however, used strategies to deal with the large number of leaf litter samples which included freezing the leaf litter bags after collection for later processing (Colas et al. 2017) and distributing the samples in the streams over three winters (Brosed et al. 2022). Ideally, senescent leaf litter should be collected at a single location to ensure homogeneity of the material (as done here); intraspecific differences in leaf litter of different origins may affect decomposition rates and compromise its use to detect differences in environmental conditions among streams (Ferreira et al. 2020). However, using leaf litter collected at a single location may not be feasible if large amounts are needed, would require transportation to the regions where it needs to be deployed in large-scale biomonitoring programmes, and may carry risks of disseminating pathogens associated with leaf litter across regions (sterilising leaf litter would add another level of complexity to the protocol). For these reasons, commercial organic matter may be an appealing alternative (Ferreira et al. 2021).

Commercial wood sticks (e.g., tongue depressors, ice-cream sticks, as used here), in particular, are easy to obtain and process (Arroita et al. 2012). However, as wood decomposes more slowly than leaf litter, longer incubation periods are needed, which exposes wood samples to being lost to vandalism and floods (e.g., 3.7× more wood sticks than leaf litter samples were lost in our field study). Wood stick decomposition is also mostly carried out by microbial decomposers; therefore, it is less sensitive to environmental changes that affect invertebrate communities (Ferreira et al. 2021). Other standardised substrates that have also been tested in previous studies include cotton strips and tea bags, which are easy to obtain and require an incubation time similar to that of leaf litter, although decomposition is microbially driven (Ferreira et al. 2021). Therefore, the type of organic matter to use needs careful consideration and depends on the number of streams to be biomonitored, time and budget constraints, and whether invertebrates are likely to be affected by environmental changes or not (Ferreira et al. 2020).

5 | Conclusions

This study illustrates the large-scale nation-wide application of the organic matter decomposition methodology in a scenario similar to that of a biomonitoring programme (although with fewer streams), in which a common protocol is applied at multiple sites by practitioners that do not necessarily have previous experience on the topic. A similar exercise was done in France where water agency technicians deployed and sampled alder leaf litter enclosed in coarse-mesh and fine-mesh bags at 82 stream sites across the mainland territory to assess the effects of hydromorphological and water chemical alterations on leaf litter decomposition (Colas et al. 2017). These studies show that the methodology is simple enough to be implemented at large scale by non-experts.

This study also revealed a considerable variation in organic matter decomposition across least-disturbed Portuguese streams, reflecting differences in substrate type and identity, shredder access, season, and environmental conditions. Addressing this variation in organic matter decomposition is essential for it to be used as an indicator of stream functional integrity (Ferreira et al. 2020). Standardising the protocol, including selecting specific organic matter types and species, mesh types, and incubation season, can help minimise variation caused by these factors (Ferreira et al. 2020). However, addressing variation due to natural heterogeneity in environmental conditions is challenging. Nonetheless, establishing stream typologies based on environmental characteristics that are not much affected by human activities (e.g., geology, climate), along with identifying reference decomposition ranges for each typology, may facilitate the assessment of deviations in stream functioning from reference conditions. However, to move towards the proposal of a definitive protocol for using organic matter decomposition as a biomonitoring tool, collaboration between researchers and environmental agencies is needed for testing alternative protocols (e.g., different organic matter types and species, mesh types, incubation seasons) at the large scale (preferably using the stream network already in place within the scope of official biomonitoring programmes). This collaboration has been hindered by the need for a huge initial investment to ensure enough time and human resources.

Author Contributions

Conceptualization: V.F., M.J.F.; Developing methods; V.F.; Data analysis, data interpretation: V.F., S.R.Q.S.; Preparation of figures and tables: V.F., S.R.Q.S., A.R.C.; Conducting the research, writing: All authors.

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Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The data that support the findings of this study are openly available in Zenodo at https://zenodo.org/, reference number https://doi.org/10.5281/zenodo.17202560.

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Supporting Information

Additional supporting information can be found online in the Supporting Information section. **Table S1:** Location and characterisation of the 37 streams used in the field experiment during spring 2021 and decomposition rates of black poplar leaf litter and Canadian poplar wood sticks. The methods used are shown at the bottom of the table. **Table S2:** Source, full reference and information on extracted and non-extracted data from the studies selected for inclusion in the literature review ($N=61$). **Table S3:** Sample size (N), mean, standard deviation (SD), and coefficient of variation (CV) of decomposition rates of poplar leaf litter and wood incubated in streams of distinct typologies (only typologies with $N>2$ are shown) in the field study. **Table S4:** Output tables of the dbRDA analysis. **Table S5:** Three-way analysis of variance (ANOVA) to assess the effects of leaf genus (*Alnus* vs. *Quercus*), mesh type (coarse-mesh vs. fine-mesh bags), and season (spring/summer vs. autumn/winter) on leaf litter decomposition rates. **Table S6:** Sample size (N), mean, standard deviation (SD), and coefficient of variation (CV) of decomposition rates of *Alnus* and *Quercus* leaf litter enclosed in coarse-mesh and fine-mesh bags and incubated during Spring/Summer (S/S) and Autumn/Winter (A/W) in streams of distinct typologies (only typologies with $N>2$ are shown) in the literature review. **Table S7:** Output tables of the FAMD analysis.