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Organic-matter decomposition as a bioassessment tool of stream functioning: A comparison of eight decomposition-based indicators exposed to different environmental changes☆

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ABSTRACT

Organic-matter decomposition has long been proposed as a tool to assess stream functional integrity, but this indicator largely depends on organic-matter selection. We assessed eight decomposition-based indicators along two well-known environmental gradients, a nutrient-enrichment gradient (0.2–1.4 mg DIN/L) in central Portugal and an acidification gradient (pH: 4.69–7.33) in north-eastern France to identify the most effective organic-matter indicator for assessing stream functional integrity. Functional indicators included natural leaf litter (alder and oak) in 10-mm and 0.5-mm mesh bags, commercial tea (Lipton green and rooibos teas in 0.25-mm mesh bags), wood sticks (wood tongue depressors) and cotton strips. Biotic indices based on benthic macroinvertebrates ($IPtI_N$ for Portugal and IBGN for France) were calculated to compare the effectiveness of structural and functional indicators in detecting stream impairment and to assess the relationship between both types of indicators. The effectiveness of organic-matter decomposition rates as a functional indicator depended on the stressor considered and the substrate used. Decomposition rates generally identified nutrient enrichment and acidification in the most acidic streams. Decomposition rates of alder and oak leaves in coarse-mesh bags, green and rooibos teas and wood sticks were positively related with pH. Only decomposition rates of rooibos tea and wood sticks were related with DIN concentration; decomposition rates along the nutrient-enrichment gradient were confounded by differences in shredder abundance and temperature among streams. Stream structural integrity was good to excellent across streams; the $IPtI_N$ index was unrelated to DIN concentration, while the IBGN index was positively related with pH. The relationships between decomposition rates and biotic indices were loose in most cases, and only decomposition rates of alder leaves in coarse-mesh bags and green tea were positively related with the IBGN. Commercial substrates may be a good alternative to leaf litter to assess stream functional integrity, especially in the case of nutrient enrichment.

1. Introduction

Streams and rivers provide numerous services to human populations (Palmer & Richardson, 2009; Harrison et al., 2010). This fact justifies the legislation that mandates the bioassessment of these ecosystems so that streams and rivers with impaired ecological integrity are identified and recovery measures can be undertaken (e.g., US Clean Water Act; EU Water Framework Directive). However, although ecosystem ecological integrity is described by both structure and function, stream bioassessment is generally based on structural indicators such as the composition of aquatic communities, while functional indicators based on ecological processes are rarely considered (Ferreira et al., 2020). Structure and function are, however, not always linked, and so considering only structural indicators may not allow the full evaluation of stream ecological integrity (McKie & Malmqvist, 2009; Young & Collier, 2009; Niyogi et al., 2013; Feckler & Bundschuh, 2020).

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As for structural indicators, a good functional indicator should comply to the following criteria: (i) be *a priori* predictive, (ii) be sensitive to human-induced impacts, (iii) be able to respond to multiple human impacts, (iv) be easy to interpret (e.g., monotonic response), (v) be applicable over large-scales, (vi) be prone to repeatability, and (vii) be cheap and easy to use (Bonada et al., 2006; Ferreira et al., 2020). Among the multiple ecosystem processes that could potentially be used as tools to assess stream functional integrity, organic-matter decomposition is one of the most promising as it complies with the above mentioned criteria (Gessner & Chauvet, 2002; Young et al., 2008; Imberger et al., 2010; Ferreira & Guérold, 2017; Ferreira et al., 2020). The proposal of organic-matter decomposition as a bioassessment tool of stream functional integrity is based on a strong theoretical background gathered from over 50 years of organic-matter decomposition experiments (Webster & Benfield, 1986; Abelho, 2001; Tank et al., 2010). The decomposition of plant organic matter derived from the riparian vegetation is a fundamental stream ecosystem process, which allows the incorporation of plant carbon and nutrients into aquatic foodwebs (Wallace et al., 1997; Marks, 2019), and is mostly a biological process carried out by microbial decomposers and invertebrate detritivores (Hieber & Gessner, 2002; Cornut et al., 2010). The rate at which organic matter decomposes depends on three main and non-exclusive general factors: environmental conditions, organic-matter characteristics, and the presence, diversity, and activity of microbial and invertebrate consumers.

Considering environmental conditions (e.g., water chemistry), organic-matter decomposition rates are generally stimulated by moderate nutrient enrichment (Woodward et al., 2012; Ferreira et al., 2015; Rosemond et al., 2015) and inhibited by acidification (Ferreira & Guérold, 2017). Therefore, organic-matter decomposition can discriminate between reference and impaired conditions, and potentially between different types of impairment, and it is *a priori* predictive (Gessner & Chauvet, 2002; Young et al., 2008; Ferreira et al., 2020).

Organic-matter characteristics (i.e., physical and chemical traits) also strongly modulate its decomposition, with high quality organic matter (i.e., soft, with high concentrations of nutrients and low concentrations of structural and secondary compounds) decomposing faster than recalcitrant organic matter (Gessner & Chauvet, 1994; Arroita et al., 2012; Ferreira et al., 2016b). Therefore, the sensitivity of organic-matter decomposition to its characteristics should be taken into account when selecting the species or type of organic-matter (e.g., leaves, wood) to use as a bioassessment tool. However, there is also wide intraspecific variability in organic-matter characteristics (LeRoy et al., 2007; Graça & Poquet, 2014), which can be a problem in large scale bioassessment if it is not possible to get organic matter from a common source. This challenge can be overcome by using commercial organic substrates that are highly standardized and can be acquired in large quantities, such as wood sticks (e.g., ice cream sticks, coffee mixers, tongue depressors; Aristi et al., 2012; Niyogi et al., 2013) or cotton strips (Imberger et al., 2010; Tiegs et al., 2013).

The composition of consumer communities plays a major role on organic-matter decomposition too, with decomposition rates being stimulated by increases in the abundance and biomass of shredders (i.e., leaf-eating macroinvertebrates) in general or in that of highly efficient shredder taxa (Dangles et al., 2004; Hagen et al., 2006; Piscart et al., 2009; Iñiguez-Armijos et al., 2016). Shredder abundance and biomass can, in turn, be affected by environmental changes. A straightforward example is the decrease in abundance and biomass of highly efficient acid sensitive shredder taxa (e.g., gammaridae) with acidification (Dangles et al., 2004). Macroinvertebrate shredder access to organic matter can be controlled in decomposition assays by the use of bags with different mesh sizes, such as fine mesh ($\leq$0.5 mm opening) and coarse mesh (typically 5 or 10 mm opening) that prevents and allows macroinvertebrate access to the organic matter, respectively.

In this study, we aimed to determine which are the most effective indicators in detecting impaired ecosystem functioning under conditions of contrasting environmental change i.e., those that most clearly reveal differences between impacted and reference sites and whose response magnitude matches the magnitude of the environmental change. We assessed eight decomposition-based indicators (including leaf litter and commercial substrates) incubated in streams along a nutrient-enrichment gradient in central Portugal and an acidification gradient in north-eastern France (five streams each), knowing that moderate nutrient enrichment generally stimulates and acidification generally inhibits organic-matter decomposition. As organic-matter decomposition strongly depends on the substrate intrinsic characteristics, selected substrates ranged from low (alder leaf litter) to high recalcitrance (wood sticks), and included standardized substrates (green and rooibos tea, wood sticks, cotton strips) and substrates with higher intraspecific variability (alder and oak leaf litter). Coarse- and fine-mesh bags were used to incubate leaf litter as shredder access to leaves (allowed in the former bag type, but not in the latter) is a key factor controlling leaf-litter decomposition. We also compared the performance of organic-matter decomposition with structural measures based on the benthic macroinvertebrate communities to assess redundancy or complementarity among functional and structural indicators, and to assess the relationship between both types of indicators.

2. Materials and methods

2.1. Study streams

2.1.1. Streams along a nutrient-enrichment gradient in central Portugal

Five streams differing in nutrient concentrations (from low nutrient concentrations to moderate nutrient enrichment) were selected in Caramulo mountain, central Portugal (Gulis et al., 2006; Table 1). Streams flow through native deciduous forests dominated by *Castanea sativa* L. and *Quercus* spp., abandoned agricultural fields and fields where extensive agriculture is carried while allowing for *Alnus glutinosa* (L.) Gaertn. and *Salix* spp. trees in the riparian area. Selected streams are representative of least-impacted, oligotrophic streams (Malhapão) to moderately nutrient-enriched streams in central Portugal (Gulis et al., 2006; Ferreira et al., 2016a). Streams are small (2nd – 4th order; 1.5–5.0 m wide), and underlined by siliceous bedrock. During the study, stream water was circumneutral, cool and had low conductivity (Table 1).

2.1.2. Streams along an acidification gradient in north-eastern France

Five streams differing in pH (from circumneutral to acidic, with Al concentration increasing in the same direction) were selected in the Vosges mountains, north-eastern France (Dangles et al., 2004; Table 2). Streams are small (1st – 2nd order; 0.5–2.0 m wide) and flow through mixed deciduous-coniferous forests (mainly *A. glutinosa*, *Fagus sylvatica* L., and *Picea abies* (L.) H. Karst.). The study area, underlined by sandstone bedrock, is highly sensitive to acidification, and decades of atmospheric deposition resulted in surface water acidification (Probst et al., 1990). Nevertheless, local differences in bedrock mineral composition can lead to variations in stream water buffering capacity, resulting in large pH differences among nearby streams (Dangles et al., 2004).

2.1.3. Stream water characteristics

Water temperature was recorded hourly with data loggers and hourly values were averaged to produce daily means. Stream water pH and electrical conductivity were recorded with field or laboratory meters. Water was filtered through glass fiber filters (47-mm diameter, 0.7-µm pore size; Whatman GF/F, GE Healthcare UK Limited, Little Chalfont, UK) into acid washed plastic bottles and transported cold to the laboratory for chemical characterization. Water was analyzed for nitrate, nitrite, ammonia, soluble reactive phosphorus (SRP), and total aluminum (after acidification with HNO_3) concentrations, and for acid-neutralizing capacity (ANC). Methods used for stream water

Table 1
Water physical and chemical characteristics (mean ± SE or min – max) of the five streams in central Portugal during organic-matter incubation (March 15 – May 22, 2017; n = 1–4, except for temperature where n = 68). Streams are ranked by increasing DIN concentration, from reference to most nutrient-enriched. DIN, dissolved inorganic nitrogen (NO_3–N + NO_2–N; NH_4 was below detection limit); SRP, soluble reactive phosphorus; Cond, electrical conductivity; Temp, mean daily temperature.

Streams	DIN (mg/L)			SRP (μg/L)			pH			Cond (μS/cm)			Temp (°C)		
Malhapão	0.21	±	0.03	19	±	5	7.14			17			12.73	±	0.17
Tojosa	0.72	±	0.25	23	±	1	7.01	–	7.33	15	–	34	12.69	±	0.22
Múceres	0.74	±	0.12	30	±	3	6.85	–	7.45	52	–	54	13.21	±	0.18
Caramulo1	1.21	±	0.04	22	±	3	7.08	–	7.17	56	–	64	11.59	±	0.16
Caramulo2	1.42	±	0.18	36	±	4	7.11	–	7.16	68	–	71	12.07	±	0.12

Table 2
Water physical and chemical characteristics (mean ± SE) of the five streams in north-eastern France during organic-matter incubation (February 28 – April 7, 2017; n = 4, except for temperature where n = 39). Streams are ranked by decreasing pH, from reference to most acidic. ANC, acid neutralizing capacity; Cond, electrical conductivity; Temp, mean daily temperature.

Streams	pH			ANC (μeq/L)			Al (μg/L)			NO_3 (mg/L)			Cond (μS/cm)			Temp (°C)		
La Maix	7.33	±	0.14	447.64	±	80.38	102	±	67	2.09	±	0.20	67	±	9	7.78	±	0.13
Chevrosgoutte	6.68	±	0.08	95.37	±	20.98	119	±	32	1.53	±	0.21	42	±	2	8.37	±	0.07
Repafosse	5.45	±	0.21	5.18	±	4.57	136	±	47	1.39	±	0.15	29	±	1	7.83	±	0.12
Grand Bras	4.74	±	0.11	−15.41	±	5.48	379	±	90	1.87	±	0.33	22	±	1	6.54	±	0.11
Gentil Sapin	4.69	±	0.15	−20.02	±	8.44	368	±	113	2.04	±	0.43	22	±	1	6.61	±	0.15

characterization are described in Table S1.

2.2. Organic matter, experimental design and sample processing

Organic matter included leaf litter of native tree species: alder (*A. glutinosa*) leaves were collected from a riparian stand of even-aged trees and oak (*Quercus robur* L.) leaves were collected from a native mixed deciduous forest, in autumn 2016, central Portugal. Leaves were air-dried at room temperature in the dark and stored in card boxes until used. Portions of air-dry leaves (~2.50 g) were sprayed with distilled water to render them soft and less susceptible to break due to handling, and placed into coarse-mesh (10-mm mesh opening) and fine-mesh (0.5-mm mesh opening) bags (~12 × 15 cm), which were then closed in tetrahedral shape. Coarse-mesh bags were used to allow access of shredders, including large cased caddisflies, to the leaf litter, but they would also allow for physical fragmentation due to current velocity and sediments. However, as the experiment was carried out in spring, when water levels are low, we expected physical fragmentation to play a minor role on leaf-litter decomposition (Ferreira et al., 2006). Commercial substrates were also used as these are more standardized and easy to get than leaf litter. Green (*Camellia sinensis* (L.) Kuntze; EAN 8722700055525) and rooibos (*Aspalathus linearis* (Burm.f.) R. Dahlgren; EAN 8722700188438) teas provided in tetrahedral-shaped woven nylon bags (~5-cm side and ~0.25-mm mesh opening; Lipton, Unilever) were enclosed individually (air-dry mass: ~1.81 and 1.98 g, respectively) into tetrahedral coarse-mesh bags. *Betula* sp. wood sticks (untreated wood tongue depressors; 15.0 × 1.7 cm, 1-mm thick; Goodwood Medical Care Ltd., Dalian District, China) were enclosed in groups of three (air-dry mass: ~4.90 g) into tetrahedral coarse-mesh bags. Cotton fabric ('Empa', Swissatest Product no. 222; Empa, St. Gallen, Switzerland) was cut into 14 × 5 cm strips and tied in groups of two inside tetrahedral coarse-mesh bags with a cable binder. Each substrate had a single source and subsamples were distributed by both the Portuguese and French teams to ensure uniform initial substrate characteristics. Details on substrate source, characteristics and selection criteria are given in the Supplementary Material.

Nine samples of each of the eight decomposition-based indicators (alder and oak leaf litter in coarse- and fine-mesh bags, green and rooibos teas, wood tongue depressors, and cotton strips) were deployed in the five streams in north-eastern France on February 28, 2017 and in the five streams in central Portugal on March 15, 2017 (9 samples × 8 decomposition-based indicators × 5 streams × 2 regions = 720 samples total). On the same day (day 0), extra sets of five samples prepared as described above for each decomposition-based indicator were taken to the field, submersed in one stream in each region for ~ 10 min and returned to the laboratory. These extra samples were used to estimate the initial ash-free dry mass (AFDM), taking into account losses due to handling, as described below for the experimental samples. A conversion factor between organic-matter initial air-dry mass and initial AFDM was calculated to allow the estimation of initial AFDM for experimental samples. In the case of cotton strips, initial tensile strength was determined (see below).

Samples (n = 3) were retrieved from the streams on three dates: after 9, 22, and 38 days for streams in France and after 9, 16 and 68 days for streams in Portugal (the large time interval between the second and third sampling dates in Portugal was due to logistical constraints). However, due to fast decomposition rates in some streams over the nutrient-enrichment gradient, no mass remaining was recovered for alder and oak in coarse-mesh bags and for cotton strips on the last sampling date (day 68). After retrieval, samples were stored individually in zip-lock plastic bags and returned to the laboratory in a cooler. In the laboratory, the organic-matter remaining in each bag (including tea bags) was extracted and rinsed with distilled water on top of a 0.5-mm sieve to remove sediments and retain organic-matter fragments. Organic-matter mass remaining (except for cotton strips), keeping the entire sample together (e.g., the three wood sticks were kept together), was transferred into pre-weighed aluminum pans, oven-dried (70 °C for 48 h), weighed (±0.1 mg), ignited (550 °C for 4 h), and reweighed to allow estimation of AFDM. Cotton strips were oven-dried (70 °C for 48 h) and then analyzed using a tensiometer (Ametek CS225, Ametek Inc., Largo, USA) to determine tensile strength as a surrogate for decomposition; the two measures taken per sample (one per cotton strip) were averaged to produce a single mean value per sample. Results were expressed as percentage of initial AFDM or tensile strength: remaining value/initial value × 100.

2.3. Benthic macroinvertebrates and biotic indices

In Portugal, benthic macroinvertebrate communities were sampled on March 24, 2017 (day 9 of the organic-matter decomposition experiment) following the Portuguese official protocol that complies with the Water Framework Directive (INAG, 2008). Benthic macroinvertebrate composition was used to compute the $IPtI_N$ (North Invertebrate Portuguese Index) multimetric index (Feio et al., 2019). Since shredders play a crucial role in leaf-litter decomposition, shredder families were also identified (Tachet et al., 2002). In France, benthic macroinvertebrate communities were sampled on March 22, 2017 (day 22 of the organic-matter decomposition experiment) following the French official protocol that complies with the Water Framework Directive (AFNOR NF T90-350; AFNOR, 2004). Taxa were then classified in pre-defined faunistic groups corresponding to their tolerance toward pollution, and the IBGN (Global Normalized Biological Index) index was calculated. Details on benthic macroinvertebrate sampling and index calculation are given in the Supplementary Material.

2.4. Data analysis

Organic-matter decomposition rates (k) for streams in Portugal were estimated assuming an exponential decay by fitting a linear model to the fraction of AFDM (or tensile strength) remaining (ln-transformed) over time (only dates with available data were considered; Fig. S1). For streams in France, very slow biological organic-matter decomposition in the two most acidic streams precluded a good fit of the negative exponential model to the data and thus decomposition rates (k) were estimated by fitting a linear model with free intercept to the fraction of AFDM remaining (or tensile strength) over time. To take into account differences in water temperature among streams, organic-matter decomposition rates were expressed in a per degree-day basis (k,/dd) by replacing time in the models above with the cumulative daily mean temperature by the sampling date.

The magnitude of the nutrient enrichment or acidification effect on organic-matter decomposition rates (i.e., the effect size) was assessed by the standardized mean difference Hedges' g, with Malhapão being the reference stream in the nutrient-enrichment gradient owing to its lower nutrient concentrations and La Maix being the reference stream in the acidification gradient owing to its higher pH (Borenstein et al., 2009; formulas are given in the Supplementary Material).

Pearson correlations were performed among organic-matter indicator decomposition rates. The relationships between organic-matter decomposition rates in streams in central Portugal and dissolved inorganic nitrogen (DIN) concentrations, $IPtI_N$ index scores, and number of shredders were assessed by linear regression or quadratic models. The relationships between organic-matter decomposition rates in streams in north-eastern France and pH, IBGN index scores, and number of shredders were assessed by linear regression models. Linear regressions were also used to assess the relationships between $IPtI_N$ index scores and DIN concentrations and between IBGN index scores and pH.

Analyses were performed on STATISTICA 6 (models; StatSoft, Inc., Tulsa, OK, USA) and OpenMEE (effect sizes; Wallace et al., 2017).

3. Results

3.1. Organic-matter decomposition along a nutrient-enrichment gradient

Organic-matter decomposition in streams along the nutrient-enrichment gradient in central Portugal followed a negative exponential model (except for wood sticks in Malhapão, which only lost 3.7% of initial mass over the 68 days incubation) (Fig. S1; Table S2). Decomposition was fast for alder and oak leaves in coarse-mesh bags and for cotton strips, especially in Múceres and Caramulo2, the two streams with the highest number of shredders (Fig. S1; Table 3). Decomposition rates (k,/dd; exponential model) across streams were in the order (presented as the range in mean values across streams): alder leaves in coarse-mesh (0.0037–0.0181) > cotton strips (0.0023–0.0154) > alder leaves in fine-mesh (0.0011–0.0042) ~ oak leaves in coarse-mesh (0.0010–0.0037) ~ green tea (0.0019–0.0026) > oak leaves in fine-mesh (0.0007–0.0020) > rooibos tea (0.0002–0.0011) > wood sticks (0.0001–0.0009) (Table S2). No significant correlations were found among decomposition rates for any pair of decomposition-based indicators (Table S3).

Organic-matter decomposition rates were generally stimulated in nutrient-enriched streams compared with Malhapão, the stream with the lowest nutrient concentrations, although the magnitude of the effect size did not always increase with nutrient concentration (Fig. 1). Surprisingly, the decomposition of oak leaves on coarse mesh bags was not significantly affected (or it was even inhibited) in nutrient-enriched streams (Fig. 1). Alder leaves in fine-mesh bags, rooibos tea, wood sticks and cotton strips were the substrates that most consistently detected impairment (Fig. 1).

Decomposition rates of rooibos tea showed a significant hump-shaped relationship and those of wood sticks showed a marginally significant positive linear relationship with DIN concentrations (Fig. 2; Table S4). There was no significant relationship between organic-matter decomposition rates and DIN concentrations for any of the other indicators (Table S4).

Table 3
Biotic indices based on benthic macroinvertebrates, total number of individuals, and number and percentage of shredders in benthic samples from the five streams in central Portugal (March 24, 2017) and in north-eastern France (March 22, 2017). Biotic indices: $IPtI_N$ for central Portugal ($IPtI_N \geq 0.87$ indicates excellent ecological condition), IBGN for north-eastern France ($13 \leq$ IBGN ≤ 16 indicates good and IBGN ≥ 17 indicates excellent ecological condition). Streams are ranked by increasing DIN concentration (from reference to most nutrient-enriched) or decreasing pH (from reference to most acidic) for central Portugal and north-eastern France, respectively.

Region	Stream	Biotic index	No. individuals	No. shredders	% shredders
Central Portugal	Malhapão	0.91	1185	33	3
	Tojosa	1.11	725	39	5
	Múceres	1.07	3320	146	4
	Caramulo1	0.99	1810	16	1
	Caramulo2	1.13	2289	390	17
North-eastern France	La Maix	17	834	477	57
	Chevrosgoutte	17	733	526	72
	Repafosse	15	1444	825	57
	Grand Bras	13	311	221	71
	Gentil Sapin	15	617	275	45

3.2. Organic-matter decomposition along an acidification gradient

Organic-matter incubated in streams along the acidification gradient in north-eastern France lost mass following distinct dynamics among indicators and streams: until the first sampling date (day 9), mass loss was accentuated for alder leaves in both mesh types and rooibos and green teas (10–18% initial mass lost for the first three indicators, 52–62% for green tea), while decomposition was very slow for oak leaves in both mesh types, wood sticks and cotton strips (<8% initial mass lost) (Fig. S2). After the first sampling date, organic-matter decomposition (a) generally stabilized in the two most acidic streams (Gentil Sapin and Grand Bras; note the non-significant slopes on Table S5), (b) proceeded at very low rates for oak leaves in fine-mesh bags, green and rooibos teas and wood sticks, (c) proceeded at a rate similar to the initial rate for alder leaves in both mesh types and oak leaves in coarse-mesh bags, or (d) proceeded at a rate faster than the initial rate for cotton strips (Fig. S2). These distinct dynamics translated into organic-matter decomposition rates (k,/dd; linear model) across streams in the order (presented as the range in mean values across streams): cotton strips (0.0009–0.0033) > alder leaves in coarse-mesh (0.0005–0.0034) > oak leaves in coarse-mesh (<0.0001–0.0016) ~ green tea (0.0007–0.0010) > alder leaves in fine-mesh bags (0.0003–0.0011) > rooibos tea (0.0001–0.0008) > oak leaves in fine-mesh bags (<0.0001–0.0005) > wood sticks (<0.0001–0.0004) (Table S5). There were significant positive correlations between decomposition rates for most pairs of decomposition-based indicators, except when considering wood sticks, which were correlated only with alder leaves in coarse-mesh bags and green tea, and when considering cotton strips, which were correlated only with alder leaves in fine-mesh bags and oak leaves in coarse-mesh bags (Table S3).

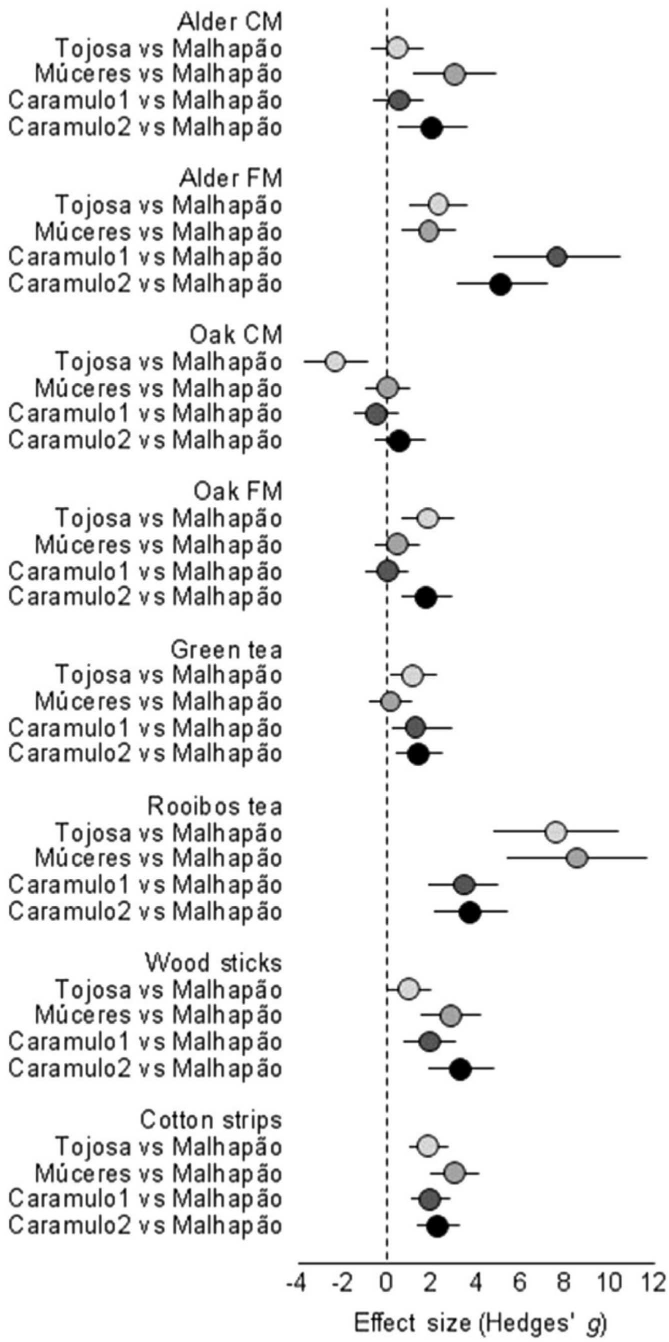

Fig. 1. Effect of nutrient enrichment on organic-matter decomposition given by the standardized mean difference Hedges' g (±95%CI) for each impacted–reference stream pair (with Malhapão considered as the reference for its lower nutrient concentrations). Impacted streams are ranked by increasing DIN concentration (increasing darkness). The dashed vertical line on 0 indicates no effect of nutrient enrichment (i.e., $k_{\text{impacted}} = k_{\text{reference}}$), while effect sizes >0 indicate a stimulation and effects sizes <0 indicate an inhibition of organic-matter decomposition in impacted streams; effects are significant if the 95%CI does not include 0.

Decomposition rates of green tea were not significantly affected by acidification, while decomposition rates of wood sticks were strongly inhibited in all acidic streams (Fig. 3). For the other six indicators, decomposition rates were inhibited only at the two most acidic streams (Gentil Sapin and Grand Bras) compared with La Maix (pH = 7.33) (Fig. 3).

Decomposition rates of alder leaves in coarse-mesh bags and green and rooibos teas showed a significant positive linear relationship with pH, while for oak leaves in coarse-mesh bags and wood sticks the relationship was marginally significant (Fig. 4; Table S6). There was no significant relationship between organic-matter decomposition rates and pH for alder and oak leaves in fine-mesh bags and cotton strips (Table S6).

3.3. Benthic macroinvertebrates and biotic indices

The $IPtI_N$ scores were above the threshold for excellent ecological condition (≥0.87) in all streams along the nutrient-enrichment gradient (Table 3) and were unrelated with DIN concentration (linear regression, $p = 0.294$; Table S4). Shredders were found in all streams (16–390 individuals/sample), with abundance being highest in Múceres and Caramulo2 (146 and 390 individuals/sample, respectively) (Table 3). Shredder contribution to total macroinvertebrate abundance, however, varied between 1% and 17%, with the minimum and maximum values being found in the most nutrient-enriched streams (Caramulo1 and Caramulo2, respectively) (Table 3). Shredder assemblages were dominated by caddisflies (mostly Calamoceratidae, Limnephilidae and Sericostomatidae) in Múceres (64%) and Caramulo2 (92%), and by stoneflies (mostly Leuctridae) in Tojosa (59%), Malhapão (61%) and Caramulo1 (31%) (Table S7).

The IBGN scores indicated good (13–16) to excellent (≥17) ecological conditions in streams along the acidification gradient (Table 3), although they were positively related with pH (linear regression, $p = 0.047$; Table S6). Shredders were present in high numbers in all streams (221–825 individuals/sample) and had a large contribution to total invertebrate abundance (45–72%) (Table 3). Shredder assemblages were dominated by stoneflies (Leuctridae and Nemouridae) in the acidic streams (Gentil Sapin, Grand Bras, Repafosse and Chevrosgoutte; 63–85%) and by amphipods (Gammaridae) in the circumneutral stream (La Maix; 88%) (Table S8).

3.4. Relationships between functional and structural indicators

Organic-matter decomposition rates along the nutrient-enrichment gradient were unrelated with the $IPtI_N$ scores (Table S4). Decomposition rates of wood sticks showed a significant and positive relationship (linear regression, $p = 0.023$) and decomposition rates of alder and oak leaves in coarse-mesh bags showed a marginal significant and positive relationship ($p = 0.064$ and 0.084, respectively) with the number of shredders (Table S4). For streams along the acidification gradient, decomposition rates of green tea showed a significant and positive relationship (linear regression, $p = 0.040$) and decomposition rates of alder leaves in coarse-mesh bags showed a marginal significant and positive relationship ($p = 0.061$) with the IBGN scores (Table S6). No relationship was found between organic-matter decomposition rates and number of shredders (Table S6).

4. Discussion

The performance of organic-matter decomposition as a functional indicator differed between types of environmental change and among decomposition-based indicators (Table S9). Along the nutrient-enrichment gradient, only two indicators (out of eight) responded to increases in nitrogen concentration (rooibos tea and wood sticks; based on correlations), although four (alder leaves in fine mesh bags, rooibos tea, wood sticks and cotton strips) had their decomposition significantly stimulated in all nutrient-enriched streams compared with reference (based on effect sizes). Along the acidification gradient, five indicators (out of eight) responded to decreases in pH (based on correlations), but only for one (wood sticks) was decomposition significantly inhibited in all acidic streams compared with reference (based on effect sizes).

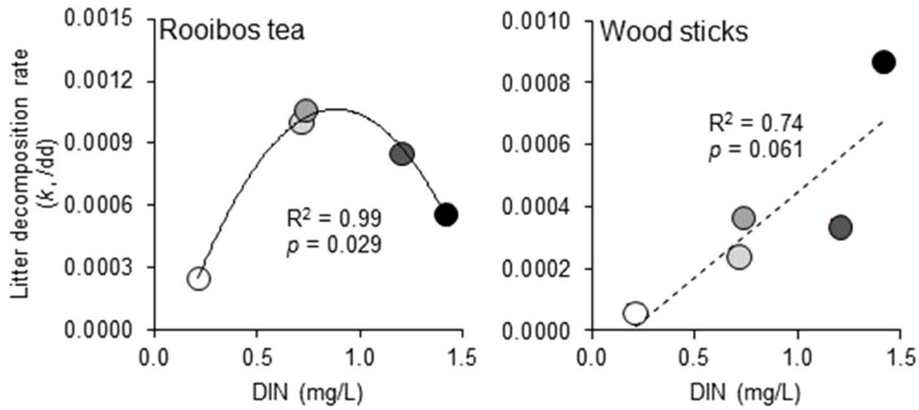

Fig. 2. Relationships between decomposition rates (on a per degree-day basis; exponential decay model) of rooibos tea and wood sticks and DIN concentrations in streams in central Portugal. Solid line indicates significant relationship; Dashed line indicates marginally significant relationship (see Table S4). Symbol color indicates degree of nutrient-enrichment: reference stream (white) to most nutrient-enriched stream (black).

4.1. Organic-matter decomposition along a nutrient-enrichment gradient

Moderate nutrient enrichment of oligotrophic streams generally stimulates organic-matter decomposition (Woodward et al., 2012; Ferreira et al., 2015; Rosemond et al., 2015). Microbial decomposers can take nutrients directly from the water column and, thus, increases in the availability of dissolved inorganic nutrients often promote increases in microbial activities, fungal biomass and aquatic hyphomycete species richness (Gulis & Suberkropp, 2003; Ferreira et al., 2006; Gulis et al., 2006). Enhanced microbial colonization of organic matter leads to increases in substrate nutritional quality (e.g., from fungal biomass accumulation and nutrient immobilization) and softness (from microbial enzymatic activities), which facilitates invertebrate colonization with concomitant increases in invertebrate abundance, biomass and taxa richness (Gulis et al., 2006; Greenwood et al., 2007; Manning et al., 2015). Indeed, in this study, decomposition rates of alder leaves in fine-mesh bags, rooibos tea, wood sticks and cotton strips were significantly stimulated in all nutrient-enriched streams compared with the reference stream (i.e., discriminated between stream types). The stimulation of decomposition rates with nutrient enrichment has been shown to depend on the background nutrient concentrations and on the magnitude of the nutrient enrichment, being generally (i.e., in the absence of limiting or confounding factors) high for oligotrophic streams than undergo large increases in nutrient concentrations (Ferreira et al., 2015). These two aspects (i.e., reference conditions and magnitude of the nutrient enrichment) affect the output of effect sizes. However, the reference stream is representative of reference streams in central Portugal (Ferreira et al., 2016a), and the streams considered along the nutrient gradient are representative of streams flowing through small-scale agricultural fields (Gulis et al., 2006). Only decomposition rates of rooibos tea and wood sticks were related with nitrogen concentration in water (i.e., identified the magnitude of the impact). Decomposition rates of rooibos tea discriminated nutrient-enriched streams from the reference stream, but they showed a hump-shaped relationship with nitrogen concentration in water, which may complicate interpretation as low decomposition rates are found at both low and high nitrogen concentrations (Woodward et al., 2012). Low decomposition rates at low nitrogen concentrations likely resulted from nutrient limitation of microbial activity: microbial decomposers can retrieve nutrients from the substrate and the water column, relying on water nutrients especially when the substrate is nutrient poor (as in the case of rooibos tea) (Gulis & Suberkropp, 2003). Low decomposition rates at high nitrogen concentrations may have resulted from inhibition of decomposer activity by changes in environmental variables concomitant with increases in nutrient concentrations (e.g., increase in pesticide concentration or fine sediment loads from agricultural activities) (Woodward et al., 2012; Pereira et al., 2016), as high nutrient enrichment *per se* would likely not inhibit decomposer activity (we recall that ammonia was below detection limit), as shown in multiple laboratory experiments using nitrogen concentrations higher than those found in our study (e.g., Ferreira & Chauvet, 2011; Jabiol et al., 2019). Wood sticks were the only indicator for which decomposition rates were significantly stimulated in all nutrient-enriched streams compared with the reference stream, and for which decomposition rates were positively related with nitrogen concentrations in water, making it a potentially good indicator of nutrient effects on stream functioning as it not only discriminates nutrient-enriched from reference stream but it may also indicate the magnitude of the nutrient enrichment.

The effects of nutrient enrichment on the decomposition of other indicators may have been confounded by changes in other variables. In fact, indicators exposed to invertebrates and on which they are known to feed (i.e., alder and oak leaves in coarse-mesh bags) decomposed very fast, especially in the two streams with the highest number of shredders (Múceres and Caramulo2). Even cotton strips, which are regarded as not appealing to shredders (Tiegs et al., 2013), decomposed very fast and had obvious signs of having been shredded. When streams differ in shredder abundance or biomass, this generally overcomes differences in water characteristics in controlling organic-matter decomposition (Hagen et al., 2006; Piscart et al., 2009; Iñiguez-Armijos et al., 2016). The effects of shredder abundance or biomass on organic-matter decomposition may be exacerbated if samples are incubated at a time of low organic-matter standing stock in the benthos, as in spring in this study, since shredders may concentrate on the samples and artificially increase the shredder density there against background levels (Frainer et al., 2014). Also, leaf litter in coarse-mesh bags was exposed to abrasion by current velocity and sediments, although physical fragmentation likely played a small role on organic-matter matter decomposition in this study as the experiment was carried out in spring and water levels were low (Ferreira et al., 2006). The interplay of these different factors on organic-matter decomposition can explain the lack of correlation between decomposition rates of different indicators. Therefore, leaf litter in coarse-mesh bags and cotton strips should not be used as indicators of nutrient enrichment in streams where invertebrate abundance may confound the effects of nutrient enrichment. In these streams, slow decomposing organic matter, where invertebrates do not feed preferentially (e.g. wood), or organic matter protected from shredders and physical abrasion (e.g. leaf litter in fine-mesh bags and tea), may be preferable.

Also, streams differed in water temperature, and although expressing organic-matter decomposition in terms of degree-days corrects for the direct effects of increases in temperature (i.e., stimulated metabolic activities at higher temperature), it does not take into account indirect

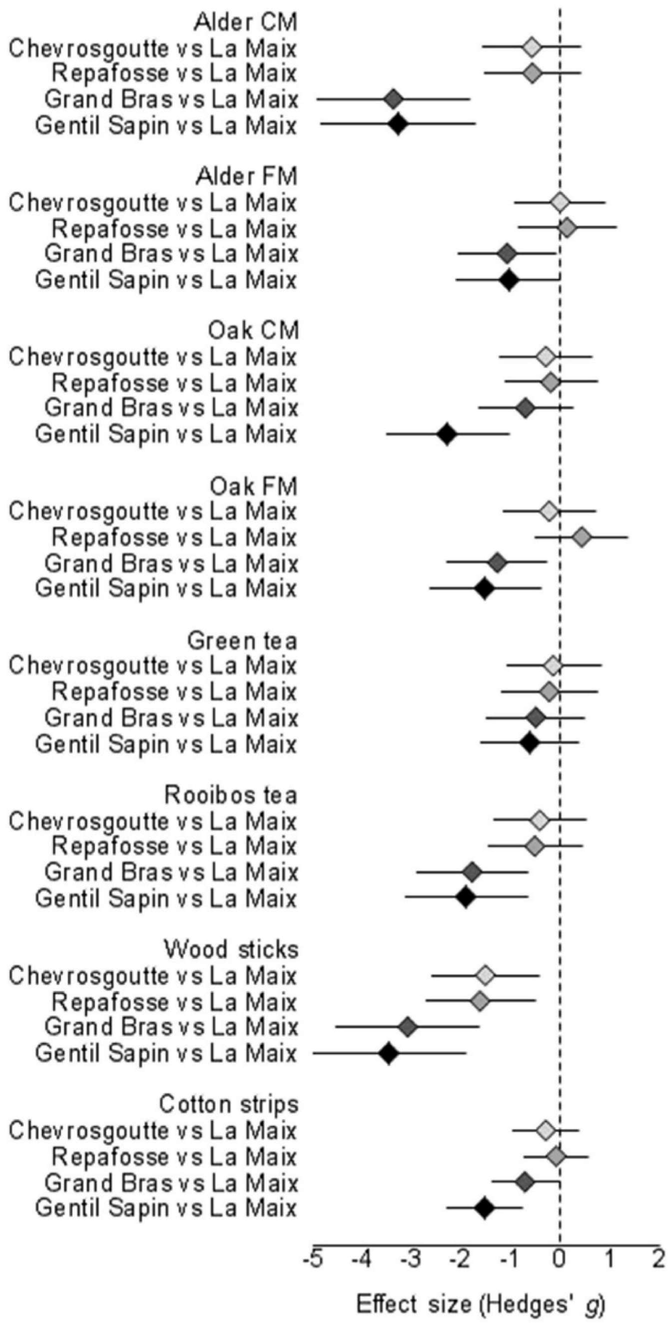

Fig. 3. Effect of acidification on organic-matter decomposition given by the standardized mean difference Hedges' g ($\pm$95%CI) for each impacted–reference stream pair (with La Maix considered as the reference for its higher pH). Impacted streams are ranked by decreasing pH (increasing darkness). The dashed vertical line on 0 indicates no effect of acidification (i.e., $k_{impacted} = k_{reference}$), while effect sizes >0 indicate a stimulation and effects sizes <0 indicate an inhibition of organic-matter decomposition in impacted streams; effects are significant if the 95%CI does not include 0.

effects such as the possibility for the presence of more efficient taxa or instars in the warmer streams. In this study, the warmer water in the three streams with lower nitrogen concentrations (Malhapão, Tojosa and Múceres, cumulative thermal mean by day 68: 853–898 °C) may have stimulated organic-matter decomposition to levels observed in the two streams with highest nitrogen concentration but cooler water (Caramulo1 and Caramulo2, cumulative thermal mean by day 68: 777 and 821 °C, respectively), contributing to the weak relationships between decomposition rates and nitrogen concentrations.

4.2. Organic-matter decomposition along an acidification gradient

Acidification, often accompanied by increases in Al concentration, generally has a negative effect on organic-matter decomposition (Ferreira & Guérold, 2017). This is a consequence from the well documented decreases in microbial activities, fungal biomass and aquatic hyphomycete species richness, and in the abundance and biomass of efficient acid-sensitive shredder taxa (e.g., Gammaridae) in acidic streams (Dangles et al., 2004; Simon et al., 2009; Cornut et al., 2012). In fact, organic-matter decomposition was generally inhibited in the two most acidic streams (Gentil Sapin and Grand Bras, pH = 4.69 and 4.74, respectively) compared with the reference stream (La Maix, pH = 7.33). Also, decomposition rates of alder and oak leaves in coarse-mesh bags, green and rooibos teas and wood sticks were negatively related with water pH. However, the decomposition of most indicators was not significantly affected by the slight-moderate acidification observed in Chevrosgoutte and Repafosse (pH = 6.68 and 5.45, respectively). It is possible that microbial activity is stimulated under slightly acidic conditions as this might favor the activity of some enzymes (e.g., pectinases; Chamier & Dixon, 1982). Still, the strong correlation between decomposition rates of most indicators suggests that they are sensitive to the same moderators. The decomposition of wood sticks, despite being very slow, was both significantly inhibited in all acidic streams compared with the reference stream (i.e., discriminated between stream types) and significantly related with water pH (i.e, identified the magnitude of the impact), suggesting its potential as an indicator of acidification effects on stream functioning (Table S9).

4.3. Organic-matter decomposition along contrasting gradients and incubation time

The difference in the direction of the response of organic-matter decomposition between the studied gradients is attributed to the subsidy nature of nutrient enrichment in oligotrophic streams and the stressor nature of acidification in circumneutral streams, as discussed above. The difference in the magnitude of response of organic-matter decomposition between gradients may be due to differences in the magnitude of the gradients themselves. Differences in the magnitude of the response may have also resulted from differences in the incubation duration. Organic-matter incubation lasted for up to 38 days along the acidification gradient, which was enough to allow fast decomposing indicators (alder leaves in coarse-mesh bags, green tea and cotton strips) to overpass 50% mass remaining in the circumneutral streams and have a good separation of streams, but was not enough for the other indicators to go over 40% mass loss. On the other hand, organic-matter incubation lasted for up to 68 days along the nutrient-enrichment gradient, which was too long for the fast decomposing indicators (alder and oak leaves in coarse-mesh bags and cotton strips) that lost most of their mass before the last sampling date, while it allowed the recalcitrant indicators or those in fine-mesh bags to reach or even overpass 50% mass loss in some streams. It emerges from this that incubation duration is an essential factor to consider when using organic-matter decomposition as a functional indicator (Bergfur, 2007; Bergfur et al., 2007).

4.4. Benthic macroinvertebrates and biotic indices

Despite differences in macroinvertebrate communities among streams (total abundance: up to 5 × ; shredder abundance: up to 24 × ; shredder percentage: up to 19 ×), the $IPtI_N$ index classified all streams along the nutrient-enrichment gradient as having excellent ecological condition, and was unrelated with nitrogen concentration or organic-matter decomposition rates. This lack of agreement between functional and structural indicators in impaired streams has been observed in other studies (McKie & Malmqvist, 2009; Young & Collier, 2009; Niyogi et al., 2013) and suggests that both components of ecosystem ecological integrity need to be considered for a complete evaluation of the

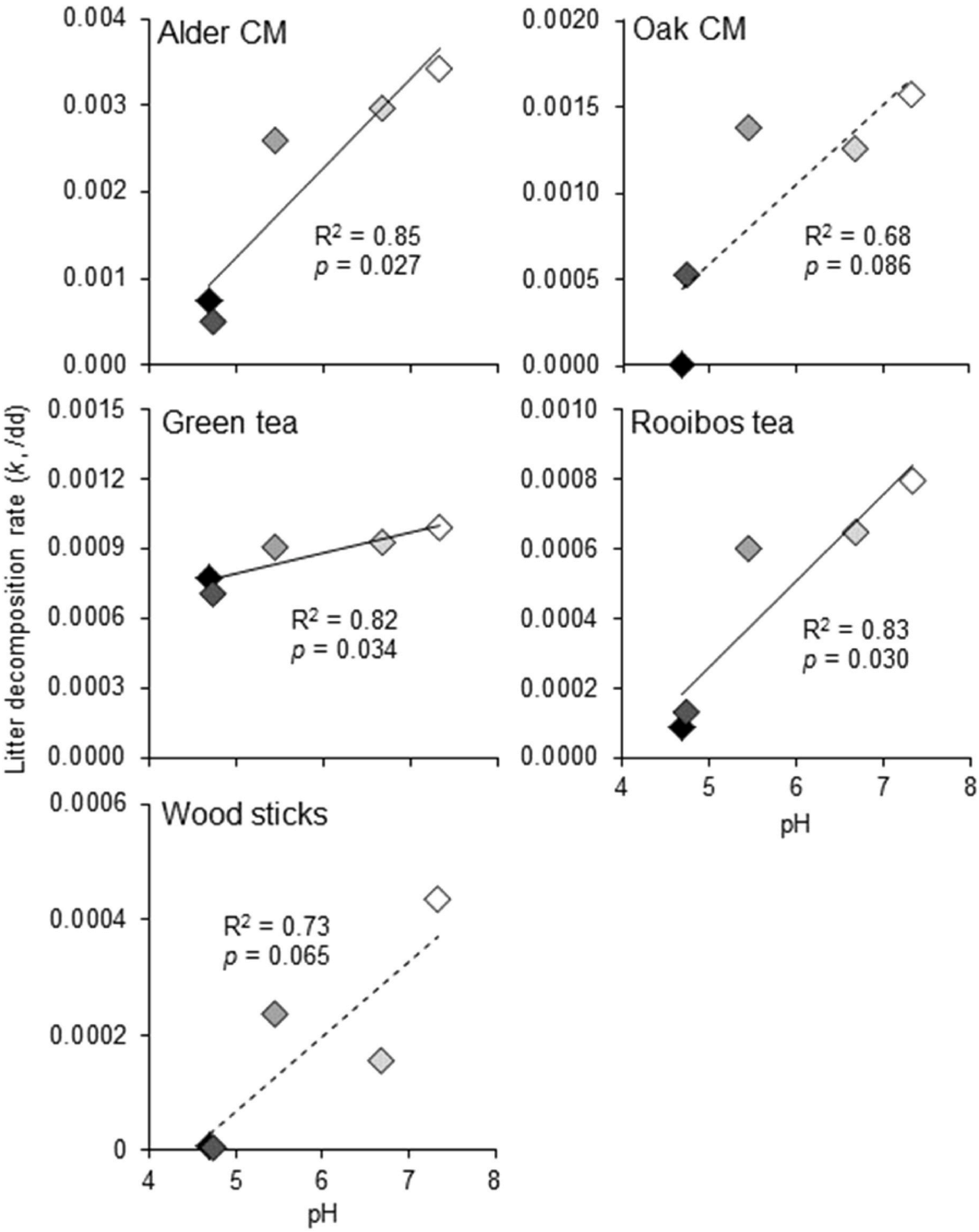

Fig. 4. Relationships between decomposition rates (on a per degree-day basis; linear decay model) of alder and oak leaf litter in coarse-mesh bags, green and rooibos teas, and wood sticks and pH in streams in north-eastern France. Solid line indicates significant relationship; Dashed line indicates marginally significant relationship (see Table S6). Symbol color indicates degree of acidification: reference stream (white) to most acidic stream (black).

ecological condition of nutrient-enriched streams.

The IBGN biotic index classified all streams along the acidification gradient as having good to excellent ecological condition, despite the disappearance of acid-sensitive Gammaridae from acidic streams. Decomposition rates of alder leaves in coarse-mesh bags and green tea were positively related with the IBGN index scores suggesting some agreement between structural and functional indicators. However, neither the IBGN biotic index nor the decomposition rates of alder leaves in coarse-mesh bags and green tea were able to identify impairment (except for decomposition rates of alder leaves in the two most acidic streams).

4.5. Decomposition-based indicators of stream impairment

From a practical perspective, the organic-matter decomposition tool is easy to implement since it does not require advanced technical skills (e.g., taxonomic knowledge), uses simple material and equipment (e.g., mesh bags, balance, oven), is low time consuming (especially if commercial substrates are used and there is no need for leaf collection or bag preparation), and it can be easily standardized across large scales (e.g., by the use of commercial substrates; Aristi et al., 2012; Tiegs et al., 2019).

Four out of eight decomposition-based indicators had their decomposition stimulated in all nutrient-enriched streams compared with reference, and seven out of eight decomposition-based indicators had their decomposition inhibited in the two most acidic streams compared with reference, suggesting that organic-matter decomposition is potentially sensitive to human-induced impacts and can also respond to multiple types of human impacts (i.e., nutrient enrichment, acidification) (Table S9). However, the effects of nutrient enrichment on organic-matter decomposition were confounded by macroinvertebrate abundance and potentially also by water temperature for some substrates (e.g., leaf litter in coarse-mesh bags and cotton strips), suggesting that detection of nutrient-enrichment effects on stream functioning may be complicated by changes in other environmental variables. Also, most decomposition-based indicators detected acidification effects only in the two most acidic streams suggesting that at slight impact levels, effects may not be detected with most indicators (Table 3). Still, decomposition of wood sticks discriminated impacted and reference streams in both gradients and was positively related with nitrogen concentration and pH, indicating that it has potential to be used as a functional indicator of human impacts. Applying wood sticks to other stressor types could help to confirm this tendency.

Our results are in line with those of a recent systematic review addressing the effectiveness of organic-matter decomposition as a bioassessment tool of stream functional integrity under environmental change (Ferreira et al., 2020). This review found that organic-matter decomposition was sensitive to stream water acidification, multiple stressors and restoration practices in 100% of studies addressing these environmental changes, nutrient enrichment (82%), mining (75%), forestry (71%), and overall agriculture/pasture effects (63%). Organic-matter decomposition has therefore the potential to be used to assess effects of a large range of environmental changes.

5. Conclusion

Despite the recurrent calls for the inclusion of functional indicators in stream bioassessment, their incorporation into routine bioassessment programs is limited (Ferreira et al., 2020). Although organic-matter decomposition has been proposed as a strong functional indicator candidate, a common bottle neck to its use is its dependence on natural organic matter (i.e. leaf litter), which requires large efforts to be collected and may be difficult to standardize. Here, we show that there are commercial substrates that are very promising as functional indicators (Table S9), which could promote their use in stream bioassessment. Understanding how different substrates respond to environmental changes and defining the aims of bioassessment (e.g., detect impact or identify the magnitude of impact) will be important when choosing the most adequate substrate for a given situation. Those interested in using decomposition-based indicators for bioassessment can also conduct initial tests with multiple substrates to examine which indicators are most sensitive to their particular stressor/system.

Credit author statement

Verónica Ferreira: Conceptualization, Methodology, Formal analysis, Investigation, Resources, Writing - Original Draft, Writing - Review & Editing **João Silva:** Investigation, Writing - Review & Editing **Julien Cornut:** Conceptualization, Methodology, Investigation, Writing - Review & Editing **Olímpia Sobral:** Investigation, Writing - Review & Editing **Quentin Bachelet:** Investigation, Writing - Review & Editing **Jonathan Bouquerel:** Investigation, Writing - Review & Editing **Michael Danger:** Methodology, Investigation, Resources, Writing - Review & Editing.

Declaration of competing interest

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

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envpol.2021.118111.

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