Aquatic Sciences (2022) 84:4
https://doi.org/10.1007/s00027-021-00834-3

Aquatic Sciences

RESEARCH ARTICLE

Microbial colonization and decomposition of commercial tea and native alder leaf litter in temperate streams

Verónica Ferreira[1] · João Silva[1] · Julien Cornut[1] · Manuel A. S. Graça[1]

Received: 30 June 2021 / Accepted: 24 October 2021

Abstract

Leaf litter decomposition in streams is a fundamental ecosystem process that allows for the cycling of nutrients. The rate at which leaf litter decomposes is greatly controlled by its intrinsic characteristics. However, intraspecific variation in leaf litter characteristics poses a major challenge for large-scale studies aiming at identifying the environmental moderators of leaf litter decomposition. Thus, several standardized organic substrates have been proposed as surrogates for leaf litter. Tea bags were proposed as a standardized alternative to leaf litter for studies in soil and their use in aquatic ecosystems has been growing in recent years. It is therefore necessary to evaluate how tea is colonized and decomposed by aquatic microbial decomposers to assess its usefulness as a surrogate for leaf litter in litter decomposition studies. Here we compared the microbial colonization (based on the reproductive activity of aquatic hyphomycetes) and decomposition of green and rooibos teas and native alder leaf litter in two streams differing in environmental conditions. Colonization of green tea was lower than that of alder leaf litter, but their decomposition rates were similar. In contrast, colonization of rooibos tea was similar to that of alder leaf litter, but it decomposed 3–4 × slower. Results were consistent in both streams. Despite differences in magnitude, dynamics of microbial colonization and decomposition of tea were similar to those of alder leaf litter and were sensitive to substrate characteristics. Tea may be used as a surrogate for leaf litter in studies addressing microbial-driven leaf litter decomposition in streams.

Keywords Aquatic hyphomycetes · Green tea · Microbial decomposers · Rooibos tea · Water nutrients

Introduction

Up to 90% of the primary production in forests and shrub lands escapes herbivory and enters the detrital pathway (Cebrian 1999), mostly as leaf litter (Molinero and Pozo 2004). The decomposition of leaf litter in terrestrial and aquatic ecosystems is a key ecosystem process in the global carbon (C) cycle (Gessner et al. 2010; Marks 2019). A major moderator of leaf litter decomposition is its quality, i.e., its physical and chemical characteristics, with leaf litter with higher concentrations of structural (e.g., lignin) and secondary compounds (e.g., tannins) and higher lignin:nitrogen and C:nitrogen ratios generally decomposing slower than less recalcitrant leaf litter (Gessner and Chauvet 1994; Ostrofsky 1997; Ramos et al. 2021). Leaf litter characteristics vary among plant species (Ostrofsky 1997; Jabiol et al. 2019; Ramos et al. 2021), but there is also substantial intraspecific variation (Lecerf and Chauvet 2008; LeRoy et al. 2012; Graça and Poquet 2014).

Intraspecific variation in leaf litter characteristics is especially problematic for large-scale studies aiming at understanding the environmental moderators of litter decomposition or for large-scale bioassessment programs aiming at using litter decomposition as a functional indicator of ecosystem health (Ferreira et al. 2020). Conspecific leaf litter collected locally at multiple sites may substantially differ in its physical and chemical characteristics as a result from differences in plant growing conditions (e.g., soil, climate) and in plant genotype (Lecerf and Chauvet 2008; LeRoy et al. 2012; Graça and Poquet 2014). Intraspecific variation in litter characteristics often translate into differences in litter decomposition rates (Lecerf and Chauvet 2008; LeRoy et al. 2012; Graça and Poquet 2014), which are partially mediated by distinct microbial decomposer colonization of

✉ Verónica Ferreira
veronica@ci.uc.pt

[1] MARE – Marine and Environmental Sciences Centre, Department of Life Sciences, University of Coimbra, Coimbra, Portugal

Published online: 23 November 2021

and activity on litter of contrasting characteristics (Biasi et al. 2017). Experimental effects of intraspecific variation can be mitigated using leaf litter from a common source. However, it may be difficult, time-consuming and costly to collect enough leaf litter from a single location and to send it to all the sites where it needs to be deployed, especially for large-scale projects with limited budget (Ferreira et al. 2019). When this is possible, researchers still need to face the restrictions to the importation of biological material, bureaucracy and charges imposed by several countries (e.g., BICON 2021; MPI 2021), which my limit their inclusion in large-scale studies using a common natural substrate. Finally, sending leaf litter around is not free of risks as it may contribute to disseminate litter-associated airborne pathogens to new locations (e.g., some *Phytophthora* species; Webber and Rose 2008; Sanfuentes et al. 2016).

Standardized organic substrates, such as commercial tea, have been suggested as an alternative to natural leaf litter (Keuskamp et al. 2013). Tea bags have been used in large-scale studies aiming at assessing the drivers of litter decomposition in the soil, while controlling for intraspecific variation in litter quality (Djukic et al. 2018; Petraglia et al. 2019; Fanin et al. 2020). Although tea bags were first proposed as a method to address litter decomposition in terrestrial systems (Keuskamp et al. 2013), they have recently gain track in aquatic ecosystems, especially in wetlands (Lalimi et al. 2018; Mueller et al. 2018; Marley et al. 2019; Trevathan-Tachett et al. 2021), but also in lakes (Seelen et al. 2019) and streams (Peralta-Maraver et al. 2019; Ferreira et al. 2021). Despite that the tea used in tea bags has undergone a series of modifications during processing for commercialization (e.g., grinding, oxidation), the decomposition of green and rooibos teas in aquatic ecosystems seems to respond to environmental conditions predictably, suggesting that they can be used as a surrogate for the decomposition of natural leaf litter (Peralta-Maraver et al. 2019; Seelen et al. 2019; Trevathan-Tackett et al. 2021; Ferreira et al. 2021). Green and rooibos teas also seem to be considerably colonized by prokaryota, protozoa and eumetazoa invertebrates in wetlands and streams (Peralta-Maraver et al. 2019; Trevathan-Tackett et al. 2021), and to support substantial microbial activity under laboratory conditions simulating wetland environment (Trevathan-Tackett et al. 2020).

As tea bags become more popular as an alternative to natural leaf litter (i.e., they are standardized substrates that are provided ready-to-use), their use to assess litter decomposition in streams will probably increase, making it necessary to assess tea colonization and decomposition by aquatic decomposers under natural stream conditions in comparison with natural leaf litter. In streams, leaf litter is colonized and decomposed first by microbial decomposers, mostly by aquatic hyphomycetes, which mediate the incorporation of plant carbon into the aquatic foodweb (Hieber and Gessner 2002; Cornut et al. 2010). As aquatic hyphomycetes species differ in their enzymatic capabilities (Zemek et al. 1985) and nutrient stoichiometry (Brosed et al. 2017), their community composition and activity are responsive to litter characteristics and environmental conditions (Gulis and Suberkropp 2003; Ferreira et al. 2016; Pereira et al. 2021). For instance, low quality litter may select for decomposer species with high litter mining capabilities or with lower nutrient needs, while nutrient enrichment or warming over low background levels stimulates microbial activity, in the absence of limiting or confounding factors (Gulis and Suberkropp 2003; Ferreira et al. 2006b; Martínez et al. 2014).

Here we incubated green and rooibos teas, and native alder leaf litter, in two streams in central Portugal to assess how the microbial colonization (based on the reproductive activity of aquatic hyphomycetes) and decomposition dynamics of teas compare to those of natural leaf litter under different environmental conditions, but we did not aim at applying the Tea Bag Index (TBI; Keuskamp et al. 2013). Tea bags may be especially advantageous in the scope of large-scale studies, bioassessment programs or citizen science initiatives, but the TBI (Keuskamp et al. 2013) introduces a complexity to the litter decomposition protocol that is not favorable in many of these scenarios (especially in bioassessment and when involving citizen scientists, where simplicity is needed). For instance, it should not be mandatory that both tea types are used together (as in the TBI), but that, depending on the study goal, green tea (labile) or rooibos tea (recalcitrant) could be used in isolation. In addition, recent studies found that the TBI may not be suitable for aquatic systems without adaptations (Seelen et al. 2019; Mori 2021; Mori et al. 2021). We expected microbial colonization and decomposition to be driven by substrate characteristics (e.g., C:nitrogen molar ratio), with aquatic hyphomycete sporulation rates and litter decomposition rates being higher for green tea, and lower for rooibos tea, than for alder leaf litter (Lecerf and Chauvet 2008; Keuskamp et al. 2013). We also expected microbial colonization and decomposition to be stimulated by higher water nutrient concentrations, and this stimulation to be inversely correlated with substrate C:nitrogen molar ratio (i.e., highest stimulation for rooibos tea) (Gulis and Suberkropp 2003). Differences in substrate quality and in environmental conditions were also expected to affect aquatic hyphomycete community composition (Gulis and Suberkropp 2003; Ferreira et al. 2016; Pereira et al. 2021).

Methods

Study streams

The experiment was carried out in two small streams (2–5 m wide) differing in water characteristics located in Caramulo mountain, central Portugal (Table 1). Streams are underlined

by granitic bedrock and flow through abandoned agricultural fields or fields with small agricultural activities. Alder (*Alnus glutinosa* (L.) Gaertn.) and willow (*Salix atrocinerea* Brot.) trees dominate the riparian area at the study site in Múceres stream, and chestnut (*Castanea sativa* Mill.) and elm (*Ulmus glabra* Huds.) dominate the riparian area at the study site in Caramulo stream. During the study, stream water was circumneutral and had low conductivity (Table 1). Water temperature was significantly higher at Múceres than at Caramulo stream (13.2 °C ± 1.5 °C vs. 12.1 °C ± 1.0 °C, mean ± SE; *t* test, $p < 0.001$; Table 1). Soluble reactive phosphorus (SRP) and dissolved inorganic nitrogen (DIN) were higher (20% and 92%, respectively) at Caramulo than at Múceres stream, but significant differences existed for DIN only (*t* test, $p = 0.314$ and $p = 0.034$, respectively; Table 1).

Water temperature was recorded hourly with data loggers (Hobo Pendant UA-001-08, Onset Computer Corp. MA, USA) and hourly values were averaged to produce daily means. When visiting streams, pH and electrical conductivity were recorded with field meters (WTW, Weilheim, Germany). On the same occasions, stream 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 nutrient analysis. Water was analyzed for nitrate, nitrite and ammonia concentrations (ion chromatography; Dionex DX-120, Sunnyvale, CA, USA; ammonia was below detection limit, < 20 µg/L) and for SRP concentrations (ascorbic acid method; APHA 1995).

Commercial tea and native alder leaf litter

Commercial 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 × 5 cm, 0.25-mm mesh opening; Lipton, Unilever) were used. Tea bags are highly standardized, ready-to-use commercial substrates (Keuskamp et al. 2013). Green and rooibos teas have contrasting characteristics, with the green tea having higher concentration of water soluble compounds and of nitrogen (N), lower concentration of lignocellulose, and lower C:N ratio than the rooibos tea (C:N ratio of 12 vs. 43, respectively; Keuskamp et al. 2013), which results in faster decomposition of the former than of the latter tea (Keuskamp et al. 2013; Djukic et al. 2018; Seelen et al. 2019). Initial tea dry mass enclosed in tea bags was estimated as the difference between the mass of the tea bag (1.92 ± 0.01 g for green tea and 2.11 ± 0.01 g for rooibos tea; mean ± SE) and the mass of the empty tea bag (0.12 ± < 0.01 g, $n = 6$). To avoid loss of tea bags, these were enclosed individually into larger tetrahedral bags with coarser mesh size (~ 12 × 15 cm, 10-mm mesh opening), which should not affect microbial colonization of tea litter.

Alder (*A. glutinosa*) leaves recently abscised and without signs of damage (e.g., herbivory) were collected from a stand of even-aged trees located in the riparian area of the Mondego river (Coimbra, central Portugal), in autumn 2016. Alder is a dominant riparian tree species in Europe and its leaf litter is among the most often used in litter decomposition studies (Lecerf and Chauvet 2008; Woodward et al. 2012). Alder leaves are soft, rich in N and poor in defensive compounds (C:N of ratio 16–26; Lecerf and Chauvet 2008; Graça and Poquet 2014; Pereira et al. 2021), and therefore their colonization by aquatic decomposers and their decomposition is generally fast (Gessner and Chauvet 1994; Ferreira et al. 2012; Pereira et al. 2016, 2021). Leaves were air-dried at room temperature, in the dark, and stored in card boxes until used. Air-dry leaves were weighed (2.51 ± 0.01 g, mean ± SE), sprayed with distilled water to make them malleable and less prone to break during handling, and enclosed into tetrahedral fine-mesh bags (~ 12 × 15 cm, 0.25-mm mesh opening).

Table 1 Streams location and water physical and chemical characteristics (mean ± SD or min–max; $n = 1$–4, except for temperature where $n = 68$) during organic-matter incubation (15 March–22 May 2017)

Water variables	Múceres stream	Caramulo stream
Latitude (N)	40° 32′ 01″	40° 34′ 4"
Longitude (W)	8° 9′ 15″	8° 9′ 3″
Elevation (m a.s.l.)	210	430
Temperature (ºC)	13.2 ± 1.5	12.1 ± 1.0
Conductivity (µS/cm)	52–54	68–71
pH	6.85–7.45	7.11–7.16
SRP (µg/L)	30 ± 3	36 ± 4
DIN (µg/L)	738 ± 174	1424 ± 310

DIN dissolved inorganic nitrogen (NO_3-N + NO_2-N; NH_4 was below detection limit); *SRP* soluble reactive phosphorus

Organic substrates decomposition

Twelve samples of each substrate (green and rooibos teas and alder leaf litter) were deployed in the two streams on 15 March 2017 (12 samples × 3 substrates × 2 streams = 72 samples total). On the same day (day 0), extra sets of five samples prepared as described above for each substrate were taken to the field, submersed in one stream 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 substrate initial air-dry mass and initial AFDM was calculated to allow the estimation of initial AFDM for experimental samples.

Samples were recovered from the streams after 9, 16, 54 and 68 days (the large gap between the 2nd and 3rd sampling dates was due to logistical constraints), stored individually in zip-lock plastic bags, and returned to the laboratory in a cooler box. In the laboratory, alder samples were open and rinsed with distilled water on top of a 0.25-mm sieve to retain small litter fragments. Five litter discs (12-mm diameter) were extracted from alder samples (on days 9 and 16), or the entire sample was used (on days 54 and 68), to induce conidial production (see below). The remaining alder leaf litter after discs extraction, or the entire sample after incubation for conidial production, was oven-dried at 70 °C for 48 h and weighed (± 0.1 mg) for determination of dry mass (DM). Leaf litter was then ignited at 550 °C for 4 h and weighed for determination of ash mass. AFDM was estimated as the difference between DM and ash mass, and results were expressed as percentage of initial AFDM (considering the discs extracted; see below). Tea bags were rinsed with abundant distilled water to clean them as much as possible from the fine sediments that had penetrated inside, and the entire sample was used to induce conidial production (see below). Tea DM and AFDM were determined after incubation for conidial production as described for alder leaf litter.

Organic substrates colonization by microbial decomposers

Colonization of decomposing substrates by microbial decomposers was assessed from conidial production by aquatic hyphomycetes. Aquatic hyphomycetes are regarded as the main microbial decomposers of organic substrates in streams (Gulis and Suberkropp 2003; Pascoal and Cássio 2004), and conidial production (i.e., reproductive activity) is often very sensitive to substrate characteristics and environmental conditions (Gessner and Chauvet 1994; Gulis and Suberkropp 2003). Conidial production also allows assessing aquatic hyphomycete species richness and community composition.

Aquatic hyphomycetes conidial production was induced in the laboratory by incubating substrates in 250-mL Erlenmeyer flasks filled with 75 mL of distilled water. Flasks were displayed in an orbital shaker, in a temperature controlled room set at 15 °C and with a 14 h light: 10 h dark regime. Incubations ran for 48 h at 100 rotations per minute. Conidial suspensions were saved into plastic bottles, preserved with 2 mL of 37% formalin, and stored in the dark until processed. Substrates were saved for determination of AFDM as described above.

When preparing filters for conidia identification and counting, conidial suspensions were gently stirred and aliquots were passed through cellulose membrane filters (25-mm diameter, 5-µm pore size; Sartorius Stedim Biotech GmbH, Goettingen, Germany), under gentle vacuum if needed. Filters were stained using 0.05% trypan blue in 60% lactic acid, mounted on a glass slide, and scanned using a microscope at 200× magnification (Leica, DM1000, Wetzlar, Germany) for conidia identification and counting (Gulis et al. 2020). Sporulation rates by aquatic hyphomycetes were expressed as the number of conidia released per mg of substrate AFDM per day, and species richness was expressed as the number of species per sample.

Data analysis

Water temperature, DIN and SRP concentrations were compared between streams by Student's *t* test. Litter decomposition rates (*k*, /d) were estimated assuming an exponential decay by using the linearized form of the negative exponential model: $\ln(AFDM_{remaining}/AFDM_{initial}) = -k \times t$, where $AFDM_{remaining}$ is the mass remaining (g) at time t (days) and $AFDM_{initial}$ is the initial mass (g), with the intersect fixed at $\ln(1) = 0$. To account for differences in water temperature between streams, litter decomposition rates were also estimated on a degree-day basis (*k*, /dd), by replacing time in the model above with the cumulative daily mean water temperature (ºC) by the sampling date. Decomposition (fraction of AFDM remaining, ln-transformed) was compared among substrates and streams by analysis of covariance (ANCOVA; substrate and stream as categorical factors and degree-days as the continuous variable), followed by Fisher's test for post hoc comparisons. Aquatic hyphomycete sporulation rates (log(x)-transformed) and species richness (log(x + 1)-transformed) were compared among substrates and streams by repeated-measures analysis of variance (ANOVA), followed by Tukey's HSD test for post hoc comparisons. Also, sporulation rates at the peak were compared among substrates and streams by two-way ANOVA, followed by Tukey's honest significant difference (HSD) test for post hoc comparisons. Data normality (Shapiro–Wilk's test) and homoscedasticity (Bartlett's test) were assessed, and data were transformed when needed to achieve analysis assumptions. Analyses were done with Statistica 6 (StatSoft, Inc., Tulsa, OK, USA).

Aquatic hyphomycete community composition was compared among organic substrates and streams by analysis of similarity (ANOSIM), based on a Bray–Curtis similarity matrix of species-specific sporulation rates (log(x + 1)-transformed). The species that most contributed to the dissimilarity among substrates and between streams were identified with two-way similarity percentage (SIMPER) analysis performed on the matrix of species-specific sporulation rates (log(x + 1)-transformed). Multivariate analyses were done with Primer 6 (version 6.1.6; Primer-E Ltd, Plymouth, UK).

Results

Organic substrates decomposition

Substrates lost mass exponentially over time, and after 68 days incubation mass remaining ranged between 39 and 64% of initial mass for rooibos tea in Múceres and in Caramulo stream, respectively, between 21 and 13% for green tea, and between 5 and 14% for alder leaf litter (Fig. 1). Decomposition rates (k, /dd) were significantly affected by substrate and stream, with a marginal significant interaction between both factors (Tables 2 and 3). In both streams, decomposition was significantly slower for rooibos tea than for alder leaf litter and green tea (Tukey's test, $p \leq 0.001$), with no significant difference between the latter two substrates ($p = 0.089$ and $p = 0.999$ in Múceres and in Caramulo stream, respectively) (Table 2). Alder leaf litter decomposed significantly faster in Múceres than in Caramulo stream (Tukey's test, $p = 0.010$), while no significant differences between streams were found for green and rooibos teas ($p = 0.998$ and $p = 0.726$, respectively) (Table 2).

Table 2 Decomposition rates (k, $n = 12$) of alder leaf litter and green and rooibos teas incubated in two streams in central Portugal over 68 days (15 March–22 May 2017), standard error (SE) and coefficient of determination of the regression (R^2) ($p < 0.001$ in all cases)

Stream	Substrates	k (/dd)	SE	R^2
Múceres stream	Alder leaf litter	0.0035[a]	0.00016	0.94
	Green tea	0.0021[ab]	0.00021	0.35
	Rooibos tea	0.0010[c]	0.00004	0.96
Caramulo stream	Alder leaf litter	0.0024[b]	0.00012	0.93
	Green tea	0.0026[b]	0.00011	0.94
	Rooibos tea	0.0006[c]	0.00004	0.87

Treatments with the same letter do not significantly differ (ANCOVA followed by Fisher's test, $p > 0.050$)

Organic substrates colonization by microbial decomposers

Colonization of alder leaf litter by aquatic hyphomycetes was fast, and sporulation rates attained their maximum values by day 16 in both streams (1217 and 482 conidia/mg AFDM/d, in Múceres and Caramulo stream, respectively) (Fig. 2). Sporulation rates attained a peak on green tea in Caramulo stream by day 54 (581 conidia/mg AFDM/d), while for green tea in Múceres stream and rooibos tea in both streams there are no marked peak (Fig. 2); but it is possible that the large gap between the 2nd and 3rd sampling dates precluded the detection of peaks. Sporulation rates were significantly affected by substrate, but not by stream (Table 3). The interaction between time, substrate and stream was also significant (Table 3). In Múceres stream, sporulation rates were significantly lower for green tea than for alder leaf litter (Tukey's test, $p = 0.026$), with no significant difference between any of these substrates and rooibos tea ($p = 0.995$ and $p = 0.051$, repectively) (Fig. 2a). In Caramulo stream, sporulation rates did not significantly differ among substrates (Tukey's test, $p \geq 0.343$) (Fig. 2a).

Peak sporulation rates were significantly affected by substrate and the interaction between substrate and stream (two-way ANOVA, $p = 0.003$ in both cases). Peak values were significantly lower for green and rooibos teas than for alder leaf litter in Múceres stream (Tukey's test, $p = 0.003$ in both cases), while no significant differences were found among substrates in Caramulo stream ($p \geq 0.717$). Peak values on alder leaf litter were significantly higher in Múceres than in Caramulo stream (Tukey's test, $p = 0.015$), while no significant differences were found between streams for green and rooibos teas ($p \geq 0.387$).

Aquatic hyphomycete species richness per sample was already high after 9 days incubation (11–13 species), and attained maximum values of 18 species in alder leaf litter in Caramulo stream by day 16 (Fig. 2). It generally increased until day 16, remained stable until day 54, and

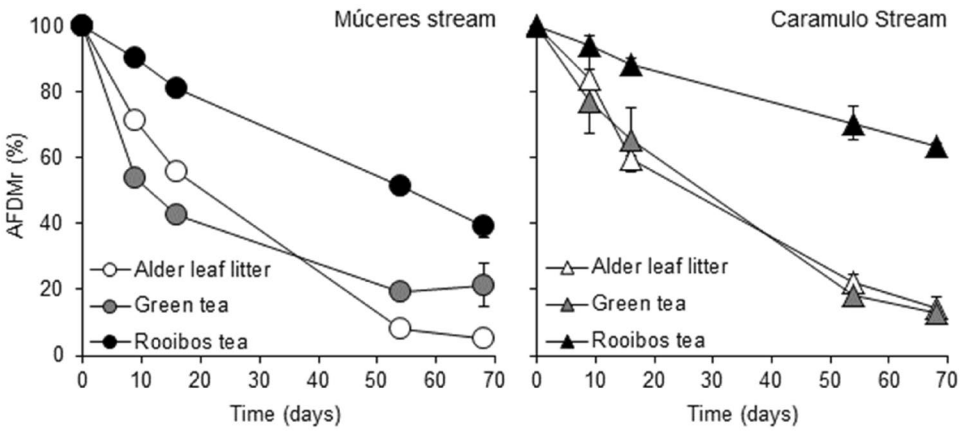

Fig. 1 Ash-free dry mass remaining (AFDMr) of alder leaf litter and green and rooibos teas (mean ± SE, $n = 3$) incubated in two streams in central Portugal over 68 days

Table 3 Summary table for ANCOVA performed on substrate mass remaining and repeated-measures ANOVAs performed on aquatic hyphomycete sporulation rates and species richness associated with alder leaf litter and green and rooibos teas (Substrate) incubated in two streams in central Portugal (Stream) over 68 days (Time)

Source of variation	SS	df	MS	*F*	*p*
ANCOVA on organic substrate mass remaining[1]					
Intercept	0.224	1	0.224	1.321	0.255
ThermalSum	26.937	1	26.937	158.775	< 0.001
Substrate	13.035	2	6.517	38.416	< 0.001
Stream	0.964	1	0.964	5.680	0.020
Substrate × Stream	0.847	2	0.424	2.498	0.090
Error	11.028	65	0.170		
Repeated-measures ANOVA on aquatic hyphomycete sporulation rates[2]					
Intercept	271.521	1	271.521	3026.468	< 0.001
Substrate	1.925	2	0.963	10.729	0.003
Stream	0.001	1	0.001	0.013	0.913
Substrate × Stream	0.481	2	0.240	2.680	0.113
Error	0.987	11	0.090		
Time	5.419	3	1.806	23.705	< 0.001
Time × Substrate	3.795	6	0.632	8.299	< 0.001
Time × Stream	3.778	3	1.259	16.527	< 0.001
Time × Substrate × Stream	1.197	6	0.200	2.619	0.035
Error	2.515	33	0.076		
Repeated-measures ANOVA on aquatic hyphomycete species richness[3]					
Intercept	82.854	1	82.854	23,998.573	< 0.001
Substrate	0.232	2	0.116	33.622	< 0.001
Stream	0.014	1	0.014	3.989	0.071
Substrate × Stream	0.010	2	0.005	1.399	0.288
Error	0.038	11	0.003		
Time	0.123	3	0.041	7.664	0.001
Time × Substrate	0.018	6	0.003	0.577	0.745
Time × Stream	0.039	3	0.013	2.414	0.084
Time × Substrate × Stream	0.049	6	0.008	1.524	0.201
Error	0.176	33	0.005		

[1] ln(fraction AFDMr)

[2] log(x)-transformed

[3] log(x + 1)-transformed

then decreased (Fig. 2). Species richness per sample was significantly affected by substrate, but not by stream or the interaction between factors (Table 3). Species richness was significantly lower for green tea than for alder leaf litter and rooibos tea in both streams (Tukey's test, $p < 0.001$), with no significant difference between the latter two substrates ($p = 0.746$) (Fig. 2).

Overall, 30 aquatic hyphomycete species were identified in this study, with species richness per treatment varying between 21 for green tea in Caramulo stream and 26 for alder leaf litter in Múceres stream and rooibos tea in both streams (Table 4). Aquatic hyphomycete community composition was significantly affected by substrate (ANOSIM, $R = 0.198$, $p = 0.001$), being significantly different among the three substrates ($p \leq 0.011$) (Table 4). Communities also significantly differed between streams (ANOSIM, $R = 0.067$, $p = 0.046$) (Table 4).

Dissimilarity in aquatic hyphomycete community composition among substrates and between streams was moderate to high (55–63%; Table 5). The aquatic hyphomycete species most contributing (at least 7%) to the dissimilarity between green tea and alder leaf litter were *Articullospora tetracladia*, *Flagellospora curvula*, *Clavatospora longibrachiata*, and *Alatospora acuminata*, between rooibos tea and alder leaf litter were *Hydrocina chaetocladia*, *Triscelophorus acuminatus*, *F. curvula*, and *A. tetracladia*, and between green and rooibos tea were *C. longibrachiata*, *T. acuminatus*, and *H. chaetocladia* (Tables 4 and 5). The aquatic hyphomycete species most contributing (at least 7%) to the dissimilarity between streams were *F. curvula*, *C. longibrachiata*, and *H. chaetocladia* (Tables 4 and 5).

Discussion

Here we provide novel information on the microbial colonization and decomposition of tea in comparison with native leaf litter, under different environmental conditions, to assess if dynamics on tea are representative of those on natural leaf litter. Despite differences in magnitude, microbial colonization and decomposition rates of green and rooibos teas followed similar patterns to those of alder leaf litter, with the differences among substrates being attributed to differences in their quality. The consistency of results between the two streams suggests that the relative differences between teas and alder leaf litter are robust to changes in environmental conditions.

Decomposition rates of rooibos tea were lower than those of alder leaf litter, as expected given the more recalcitrant nature of the rooibos tea (e.g., higher C:N ratio), while decomposition rates of green tea were similar to those of alder leaf litter, which was not expected based on the lower C:N ratio of green tea (Lecerf and Chauvet 2008; Keuskamp et al. 2013). However, green tea has compounds with known antimicrobial activity (Hamilton-Miller 1995), which might have limited microbial colonization and activity to some extent. The observed higher decomposition rates of green than of rooibos tea has been widely documented and serves as the basis for the TBI (Keuskamp et al. 2013). In addition, the discussion comparing rooibos tea and alder leaf litter is also valid for the comparison between rooibos and green tea.

Interestingly, the differences in litter decomposition between teas and alder leaf litter did not match the

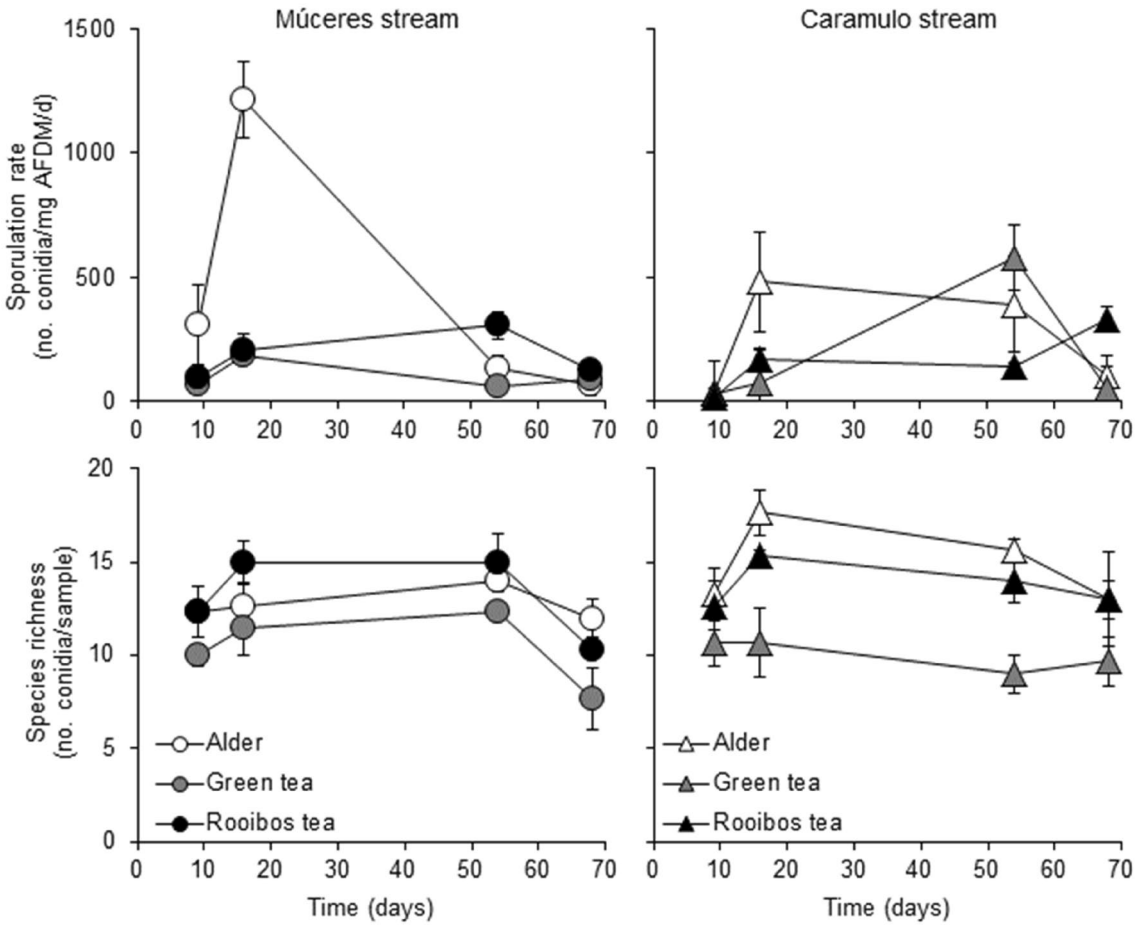

Fig. 2 Aquatic hyphomycete sporulation rates (**a**) and species richness (**b**) associated with alder leaf litter, green and rooibos teas (mean ± SE, $n = 3$) incubated in two streams in central Portugal over 68 days

differences in aquatic hyphomycete sporulation rates (microbial activity) and in species richness. Rooibos tea had aquatic hyphomycete sporulation rates and species richness similar to those of alder leaf litter, despite its recalcitrant nature. It is not uncommon for recalcitrant litter to show similar, or even higher, aquatic hyphomycete species richness than more labile litter; this is often observed for the comparison between alder (labile) and oak (*Quercus robur* L., recalcitrant) leaf litter, but also in other cases (Gulis and Suberkropp 2003; Ferreira et al. 2006a, b, 2016; Canhoto and Graça 1996; Pereira et al. 2021). Higher aquatic hyphomycete species richness in recalcitrant than in labile litter may have two non-exclusive explanations: (i) recalcitrant leaf litter may provide a more stable substrate (i.e., substrate integrity lasts longer), which allows more aquatic hyphomycete species to establish when compared with labile litter that loses structural integrity faster (e.g., previous studies have found an increase in aquatic hyphomycetes species richness with leaf area; Bärlocher and Schweizer 1983; Duarte et al. 2017), and (ii) there may be a faster turnover in decomposer species in more recalcitrant substrates, where changes in quality are likely stronger over the incubation period than those observed for labile litter (Canhoto and Graça 1996). Despite the similar aquatic hyphomycete species richness and identity between rooibos tea and alder leaf litter, community composition (e.g., dominant species based on their contribution to conidial production) differed between substrates, which may explain the differences in decomposition rates. Nutrient stoichiometry differs among aquatic hyphomycete species (Brosed et al. 2017), which partially explains differences in species performance on a given substrate (i.e., species likely perform best where the elemental imbalance between their biomass and the substrate is lowest; Suberkropp and Arsuffi 1984). Also, species differ in their enzymatic capabilities, as reported in multiple studies (Chamier 1985; Zemek et al. 1985; Abdel-Raheem and Ali 2004). However, enzymatic activities are sensitive to experimental conditions and we could not find in a single study where all the dominant species in rooibos tea and alder leaf litter were assessed, making comparisons

Table 4 Relative contribution (average %) of individual aquatic hyphomycete species to total conidial production associated with alder leaf litter and green and rooibos teas incubated in two streams in central Portugal over 68 days (15 March–22 May 2017)

Aquatic hyphomycete taxa	Múceres stream			Caramulo stream		
	Alder leaf litter	Green tea	Rooibos tea	Alder leaf litter	Green tea	Rooibos tea
Alatospora acuminata Inglod	11.21	1.63	4.74	10.76	2.96	3.32
Alatospora pulchella Marvanová	2.87	0.63	0.84	5.92	0.28	1.03
Anguillospora crassa Inglod	1.87	3.54	8.62	4.67	0.50	3.62
Anguillospora filiformis Greath	0.73	0.74	1.15	1.04	0.04	0.76
Anguillospora longissima Ranzoni			0.11	0.12		
Aquanectria submersa (H.J. Huds.) L. Lombard & Crous[1]	0.18			0.07	0.11	0.19
Articulospora tetracladia Inglod	6.33	4.78	4.83	33.35	8.88	9.70
Clavariopsis aquatica De Wild	8.69	6.86	5.23	7.02	4.36	5.19
Clavatospora longibrachiata (Ingold) Sv. Nilsson ex Marvanová & Sv. Nilsson	4.96	33.75	4.65	0.78	20.13	0.98
Culicidospora aquatica R.H. Petersen	0.04					0.04
Dimorphospora foliicola Tubaki	4.08	4.07	1.16	1.03	0.25	1.75
Flagellospora curvula Inglod	40.33	24.18	22.37	4.46	0.35	2.73
Goniopila monticola (Dyko) Marvanová & Descals/*Margaritospora aquatica* Inglod			0.05			0.08
Heliscella stellata (Ingold & V.J. Cox) Marvanová	0.64	3.54	0.07	0.81	11.41	1.17
Hydrocina chaetocladia Scheuer[2]	4.67	6.15	20.65	5.05	14.13	27.18
Lemonniera aquatica De Wild	0.22	0.04	0.54	1.11	0.40	0.33
Lemonniera terrestris Tubaki		0.50	0.07	0.04		
Lunulospora curvula Inglod	1.21	0.26	0.75	1.94	0.63	0.90
Neonectria lugdunensis (Sacc. & Therry) L. Lombard & Crous[3]	0.56	5.19	1.38	4.77	28.04	9.92
Stenocladiella neglecta (Marvanová & Descals) Marvanová & Descals	2.13	0.21	1.19	1.56	1.64	0.82
Tetrachaetum elegans Inglod	3.53	0.80	4.58	11.98	3.50	15.39
Tetracladium marchalianum De Wild	0.39	1.09	1.28	1.13	0.61	1.19
Tripospermum camelopardus Ingold, Dann & P.J. McDougall	0.18					
Tricellula aquatica J. Webster	0.22	0.43	0.03			0.03
Tricladium angulatum Inglod	1.01	0.05	0.14	0.63		
Tricladium attenuatum S.H. Iqbal	2.00	0.43	0.25	0.12	0.04	0.08
Tricladium splendens Inglod	0.22	0.09	0.28	0.23	0.99	0.35
Tricladium curvisporum Descals	0.18					0.06
Triscelophorus acuminatus Nawawi	1.31	1.04	14.89	1.13	0.60	12.78
Triscelophorus monosporus Inglod			0.11	0.16		0.34
Non identified	0.26		0.05	0.12	0.16	0.05
Taxa richness (no. species/treatment)	26	24	26	25	21	26

Total taxa richness per substrate and stream is also shown

[1]Syn. *Heliscus submsersus* H.J. Huds.

[2]Syn. *Tricladium chaetocladium* Inglod

[3]Syn. *Heliscus lugdunensis* Sacc. & Therry

of potential decomposing efficiency among the relevant species difficult. Still, community sporulation was similar between rooibos tea and alder leaf litter (but peak values were lower in the former), which may have resulted from a tradeoff between investment in reproduction and in growth, since litter decomposition was lower for rooibos tea than for alder leaf litter. Aquatic hyphomycete reproductive activity is generally sensitive to substrate quality (Gessner and Chauvet 1994; Ramos et al. 2021), but similar sporulation rates have been found between litter species of contrasting quality (Pereira et al. 2021).

Table 5 Summary table for the two-way SIMPER analysis performed on aquatic hyphomycetes communities, showing the percentage dissimilarity between comparison pairs and the aquatic hyphomycete species that most contributed to that dissimilarity (up to 90% dissimilarity is explained)

Green tea × Alder leaf litter			Rooibos tea × Alder leaf litter			Green tea × Rooibos tea			Múceres stream × Caramulo stream		
Average dissimilarity = 63%			Average dissimilarity = 55%			Average dissimilarity = 63%			Average dissimilarity = 56%		
Species	Contr%	Cum%	Species	Contr%	Cum%	Species	Contr%	Cum%	Species	Contr%	Cum%
A. tetracladia	8.5	8.5	*H. chaetocladia*	8.0	8.0	*C. longibrachiata*	9.0	9.0	*F. curvula*	10.1	10.1
F. curvula	8.4	16.9	*T. acuminatus*	7.7	15.8	*T. acuminatus*	8.9	17.9	*C. longibrachiata*	8.1	18.2
C. longibrachiata	8.0	24.9	*F. curvula*	7.6	23.4	*H. chaetocladia*	8.7	26.6	*H. chaetocladia*	7.5	25.7
A. acuminata	7.6	32.5	*A. tetracladia*	7.3	30.7	*T. elegans*	6.6	33.3	*A. tetracladia*	6.6	32.3
T. elegans	6.9	39.3	*A. acuminata*	6.7	37.4	*A. crassa*	6.5	39.8	*N. lugdunensis*	6.5	38.8
A. pulchella	6.6	45.9	*C. aquatica*	6.3	43.7	*N. lugdunensis*	6.5	46.2	*C. aquatica*	6.4	45.2
C. aquatica	5.9	51.9	*A. crassa*	6.1	49.8	*C. aquatica*	6.3	52.5	*A. acuminata*	6.4	51.5
N. lugdunensis	5.7	57.6	*A. pulchella*	5.5	55.3	*A. tetracladia*	6.0	58.5	*A. crassa*	5.2	56.8
H. chaetocladia	5.2	62.8	*T. elegans*	5.3	60.7	*A. acuminata*	6.0	64.5	*T. elegans*	5.2	62.0
H. stellata	4.9	67.7	*C. longibrachiata*	5.0	65.7	*F. curvula*	5.8	70.3	*T. acuminatus*	5.0	67.0
A. crassa	4.3	72.0	*N. lugdunensis*	3.8	69.5	*H. stellata*	5.2	75.5	*H. stellata*	4.8	71.8
S. neglecta	3.9	75.9	*L. curvula*	3.6	73.1	*S. neglecta*	3.5	79.0	*S. neglecta*	3.7	75.5
L. curvula	3.7	79.6	*S. neglecta*	3.4	76.6	*A. filiformis*	2.9	81.9	*A. pulchella*	3.1	78.6
T. acuminatus	2.9	82.6	*A. filiformis*	3.2	79.8	*A. pulchella*	2.8	84.7	*T. marchalianum*	3.0	81.5
T. marchalianum	2.8	85.3	*D. foliicola*	3.1	82.9	*L. curvula*	2.7	87.3	*L. curvula*	2.9	84.4
D. foliicola	2.7	88.0	*T. marchalianum*	3.1	86.0	*T. marchalianum*	2.6	89.9	*A. filiformis*	2.9	87.3
A. filiformis	2.4	90.4	*H. stellata*	2.6	88.6	*D. foliicola*	2.4	92.3	*D. foliicola*	2.9	90.2
			L. aquatica	2.5	91.1						

On the contrary, green tea had lower to similar aquatic hyphomycete sporulation rates and lower species richness than alder leaf litter, but showed similar decomposition rates. As mentioned above, microbial colonization was likely inhibited on green tea by the presence of antimicrobial compounds (Hamilton-Miller 1995). The similar decomposition rates between green tea and alder leaf litter suggests that the former substrate has a higher fraction of easily degradable compounds than the latter or that species dominating in green tea (e.g., *C. longibrachiata*, *Neonectria lugdunensis*, which generally do not contribute much to conidial production in temperate streams) are efficient decomposers. Also, other organisms may have compensated for the low species richness and activity of aquatic hyphomycetes on green tea: positive relationships have been found between eumetazoa invertebrates, protozoa and bacteria biomass and diversity and tea decomposition rates (Peralta-Maraver et al. 2019).

Aquatic hyphomycetes community composition varied among substrates and between streams as expected, given differences in substrate characteristics and environmental conditions (Gulis and Suberkropp 2003; Ferreira et al. 2016; Pereira et al. 2021). The dominant aquatic hyphomycete species were clearly different among the tree substrates, but comparisons with the literature are limited since substrate colonization by aquatic hyphomycetes in streams in spring has been addressed by only a few studies in central Portugal (Duarte et al. 2016; Ferreira and Graça 2016; Abril et al. 2021); most studies have been carried out in autumn/winter. *Hydrocina chaetocladia*, which is generally an important species in more recalcitrant substrates as chestnut or oak leaves, but not alder, was a dominant species in rooibos tea (21–27% relative contribution to total conidial production) compared with the other substrates (5–14%). *Triscelophorus acuminatus* generally does not contribute much to conidial production on leaf litter, but it was an important species on rooibos tea (13–15%), but not on alder leaf litter or green tea (~ 1%). *Tetrachaetum elegans*, generally an important species, especially on more recalcitrant substrates, was also important in rooibos tea in Caramulo stream (15%). *Clavatospora longibrachiata*, which generally does not contribute much conidial production, was a major contributor in green tea (20–34% vs. < 5% on the other substrates). *Neonectria lugdunensis*, another species generally with low conidial production (but common in *Eucalyptus globulus* Labill. leaves, rich in secondary compounds; Canhoto and Graça 1996), was an important species in green tea in Caramulo stream (28% vs. < 10% in other substrates). *Flagelospora curvula*, which generally contributes more to conidial production on more palatable substrates, here contributed more to conidial production on alder leaf litter (e.g., 40% vs. < 24% in other substrates in Múceres stream). Therefore, it seems that aquatic hyphomycete species colonized teas as expected based on their characteristics.

Although streams differed in environmental conditions known to affect microbial decomposer activity and litter decomposition (i.e., water nutrient concentrations and temperature), only the decomposition of alder leaf litter differed between streams. The decomposition of alder leaf litter was higher in Múceres than in Caramulo stream, indicating that it was being driven more by temperature (higher in Múceres) than by nutrient concentration (higher in Caramulo). Although nutrient concentrations were higher in Caramulo than in Múceres stream, nutrient concentrations were already high in Múceres stream (738 µg DIN/L and 30 µg SRP/L), and microbial activity was likely not nutrient limited there. In this scenario (i.e., no nutrient limitation), water temperature might have become more important, stimulating microbial activities (Ferreira and Chauvet 2011; Ferreira et al. 2016). Additionally, microbial activity might have been phosphorus-limited in Caramulo stream, where the DIN:SRP ratio was higher than in Múceres stream (39 vs. 24). Still, identifying the environmental factors responsible for differences in substrate decomposition rates between streams is difficult as only two streams were considered, which had water temperature and nutrient concentration varying in opposite directions.

In conclusion, tea may be used as a surrogate of natural leaf litter in studies addressing leaf litter decomposition in streams, as microbial colonization and decomposition followed patters similar to that of natural leaf litter. However, tea is supplied in fine mesh bags (~ 0.25 mm mesh opening) that exclude macroinvertebrates, and therefore it does not allow for the assessment of total substrate decomposition, which can substantially underestimate decomposition rates in settings where detritivores are abundant (Eggleton et al. 2020). In streams where detritivores are rare, e.g., some tropical and insular streams, leaf litter decomposition is mostly carried out by microbes (Boyero et al. 2011; Ferreira et al. 2016), and tea bags could allow an accurate estimation of total decomposition rates.

The use of tea bags as surrogates for natural leaf litter may be exploited in stream bioassessment programs. Stream bioassessment in Europe, complying with the Water Framework Directive, relies on measures of community structural parameters, which allow assessing ecosystem structural integrity only (e.g., number of species, diversity, species replacement; European Commission 2000). However, the same legislation suggests that functional metrics should be considered to fully characterize the ecosystem ecological status, which depends on both structural and functional integrity (European Commission 2000). Leaf litter decomposition has been suggested as an indicator of stream functional integrity and it has been shown to be sensitive to several environmental stressors (Gessner and Chauvet 2002; Ferreira et al. 2020). However, preparing leaf litter for decomposition experiments is time-consuming, and prone to

a large source of variability. Tea bags could be a standardized alternative proxy to assess ecosystem functioning.

Acknowledgements We thank Ana Pereira and Olímpia Sobral for the help in the field, and two anonymous reviewers for their comments on an earlier version of the manuscript.

Author contributions VF and JC conceived the study; VF and MASG provided funding; VF, JS and JC carried out the work; VF performed data analysis and wrote the first version of the manuscript; JS, JC and MASG revised the manuscript.

Funding This work was supported by the Portuguese Foundation for Science and Technology through project UIDB/04292/2020 granted to MARE, contracts IF/00129/2014 and CEEIND/02484/2018 granted to VF, and postdoc fellowship SFRH/BPD/108779/2015 granted to JC.

Availability of data and material Data are available from the corresponding author upon reasonable request.

Code availability Not applicable.

Declarations

Conflict of interest We further declare that there are no conflicts of interest.

References

Abdel-Raheem AM, Ali EH (2004) Lignocellulolytic enzyme production by aquatic hyphomycetes species isolated from the Nile's delta region. Mycopathologia 157:277–286

Abril M, Menendez M, Ferreira V (2021) Decomposition of leaf litter mixtures in streams: effects of component litter species and current velocity. Aquat Sci 83:54

APHA (1995) Standard methods for the examination of water and wastewater. American Public Health Association, Washington

Bärlocher F, Schweizer M (1983) Effects of leaf size and decay rate on colonization by aquatic hyphomycetes. Oikos 41:205–210

Biasi C, Graça MAS, Santos S, Ferreira V (2017) Nutrient enrichment in water more than in leaves affects aquatic microbial litter processing. Oecologia 184:555–568

BICON (2021) Australian biosecurity import conditions. Dried or preserved flowers and foliage. Australian Government. Department of Agriculture, Water and the Environment. https://bicon.agriculture.gov.au/BiconWeb4.0/ImportConditions/Questions/EvaluateCase?elementID=0000067983&elementVersionID=268. Accessed on 7 September 2021

Boyero L, Pearson RG, Gessner MO, Barmuta LA, Ferreira V, Graça MAS, Dudgeon D, Boulton AJ, Callisto M, Chauvet E, Helson JE, Bruder A, Albariño RJ, Yule CM, Arunachalam M, Davies JN, Figueroa R, Flecker AS, Ramírez A, Death RG, Iwata T, Mathooko JM, Mathuriau C, Gonçalves JF Jr, Moretti MS, Jingut T, Lamothe S, M'Erimba C, Ratnarajah L, Schindler MH, Castela J, Buria LM, Cornejo A, Villanueva VD, West DC (2011) A global experiment suggests climate warming will not accelerate litter decomposition in streams but might reduce carbon sequestration. Ecol Lett 14:289–294

Brosed M, Jabiol J, Gessner MO (2017) Nutrient stoichiometry of aquatic hyphomycetes: interstrain variation and ergosterol conversion factores. Fungal Ecol 29:97–102

Canhoto C, Graça MAS (1996) Decomposition of *Eucalyptus globulus* leaves and three native leaf species (*Alnus glutinosa*, *Castanea sativa* and *Quercus faginea*) in a Portuguese low order stream. Hydrobiologia 333:79–85

Cebrian J (1999) Patterns in the fate of production in plant communities. Am Nat 154:449–468

Chamier AC (1985) Cell-wall-degrading enzymes of aquatic hyphomycetes: a review. Bot J Linn Soc 91:67–81

Cornut J, Elger A, Lambrigot D, Marmonier P, Chauvet E (2010) Early stages of leaf decomposition are mediated by aquatic fungi in the hyporheic zone of woodland streams. Freshw Biol 55:2541–2556

Djukic I, Kepfer-Rojas S, Schmidt IK, Larsen KS, Beier C, Berg B, Verheyen K, Caliman A, Paquette A, Gutiérrez-Girón A, Humber A, Valdecantos A, Petraglia A, Alexander H, Augustaitis A, Saillard A, Fernández ACR, Sousa AI, Lillebø AI, Gripp AR, Francez A-J, Fischer A, Bohner A, Malyshev A, Andrić A, Smith A, Stanisci A, Seres A, Schmidt A, Avila A, Probst A, Ouin A, Khuroo AA, Verstraeten A, Palabral-Aguilera AN, Stefanski A, Gaxiola A, Muys B, Bosman B, Ahrends B, Parker B, Sattler B, Yang B, Juráni B, Erschbamer B, Ortiz CER, Christiansen CT, Adair EC, Meredieu C, Mony C, Nock CA, Chen C-L, Wang C-P, Baum C, Rixen C, Delire C, Piscart C, Andrews C, Rebmann C, Branquinho C, Polyanskaya D, Delgado DF, Wundram D, Radeideh D, Ordóñez-Regil E, Crawford E, Preda E, Tropina E, Groner E, Lucot E, Hornung E, Gacia E, Lévesque E, Benedito E, Davydov EA, Ampoorter E, Bolzan FP, Varela F, Kristöfel F, Maestre FT, Maunoury-Danger F, Hofhansl F, Kitz F, Sutter F, Cuesta F, Lobo FA, Souza FL, Berninger F, Zehetner F, Wohlfahrt G, Vourlitis G, Carreño-Rocabado G, Arena G, Pinha GD, González G, Canut G, Lee H, Verbeeck H, Auge H, Pauli H, Nacro HB, Bahamonde HA, Feldhaar H, Jäger H, Serrano HC, Verheyden H, Bruelheide H, Meesenburg H, Jungkunst H, Jactel H, Shibata H, Kurokawa H, Rosas HL, Villalobos HLR, Yesilonis I, Melece I, van Halder I, Quirós IG, Makelele I, Senou I, Fekete I, Mihal I, Ostonen I, Borovská J, Roales J, Shoqeir J, Lata JC, Theurillat J-P, Probst J-L, Zimmerman J, Vijayanathan J, Tang J, Thompson J, Doležal J, Sanchez-Cabeza J-A, Merlet J, Henschel J, Neirynck J, Knops J, Loehr J, von Oppen J, Þorláksdóttir JS, Löffler J, Cardoso-Mohedano J-G, Benito-Alonso J-L, Torezan JM, Morina JC, Jiménez JJ, Quinde JD, Alatalo J, Seeber J, Stadler J, Kriiska K, Coulibaly K, Fukuzawa K, Szlavecz K, Gerhátová K, Lajtha K, Käppeler K, Jennings KA, Tielbörger K, Hoshizaki K, Green K, Yé L, Pazianoto LHR, Dienstbach L, Williams L, Yahdjian L, Brigham LM, van den Brink L, Rustad L, Zhang L, Morillas L, Xiankai L, Carneiro LS, Martino LD, Villar L, Bader MY, Morley M, Lebouvier M, Tomaselli M, Sternberg M, Schaub M, Santos-Reis M, Glushkova M, Torres MGA, Giroux M-A, Graaff M-A, Pons M-N, Bauters M, Mazón M, Frenzel M, Didion M, Wagner M, Hamid M, Lopes ML, Apple M, Schädler M, Weih M, Gualmini M, Vadeboncoeur MA, Bierbaumer M, Danger M, Liddell M, Mirtl M, Scherer-Lorenzen M, Růžek M, Carbognani M, Musciano MD, Matsushita M, Zhiyanski M, Pușcaș M, Barna M, Ataka M, Jiangming M, Alsafran M, Carnol M, Barsoum N, Tokuchi N, Eisenhauer N, Lecomte N, Filippova N, Hölzel N, Ferlian N, Romero O, Pinto OB, Peri P, Weber P, Vittoz P, Turtureanu PD, Fleischer P, Macreadie P, Haase P, Reich P, Petřík P, Choler P, Marmonier P, Muriel P, Ponette Q, Guariento RD, Canessa R, Kiese R, Hewitt R, Rønn R, Adrian R, Kanka R, Weigel R, Gatti RC, Martins RL, Georges R, Meneses RI, Gavilán RG, Dasgupta S, Wittlinger S, Puijalon S, Freda S, Suzuki S, Charles S, Gogo S, Drollinger S, Mereu S, Wipf S, Trevathan-Tackett S, Löfgren S, Stoll S, Trogisch S, Hoeber S, Seitz S, Glatzel S, Milton SJ, Dousset S, Mori T, Sato T, Ise T, Hishi T, Kenta T, Nakaji T, Michelan TS, Camboulive T, Mozdzer TJ, Scholten T, Spiegelberger T, Zechmeister T, Kleinebecker T, Hiura T, Enoki T, Ursu T-M,

Cella U-M, Hamer U, Klaus VH, Rêgo V-M, Cecco V, Busch V, Fontana V, Piscová V, Carbonell V, Ochoa V, Bretagnolle V, Maire V, Farjalla V, Zhou W, Luo W, McDowell WH, Hu Y, Utsumi Y, Kominami Y, Zaika Y, Rozhkov Y, Kotroczó Z, Tóth Z (2018) Early stage litter decomposition across biomes. Sci Total Environ 628–629:1369–1394

Duarte S, Cássio F, Ferreira V, Canhoto C, Pascoal C (2016) Seasonal variability may affect microbial decomposers and leaf decomposition more than warming in streams. Microb Ecol 72:263–276

Duarte S, Cássio F, Pascoal C, Bärlocher F (2017) Taxa-area relationship of aquatic fungi on deciduous leaves. PLoS ONE 12:e0181545

Eggleton P, Griffiths H, Ashton L, Law S, Evans TA, Pass K (2020) Not our cup of tea: The Tea Bag Index (Keuskamp et al. 2013) for assessing decomposition is problematic in most environments, due to macrofauna. Ecol Evol. https://doi.org/10.22541/au.160502884.44867586/v1

European Commission (2000) Directive 2000/60/EC of the European Parliament and of the Council of 23 October 2000 Establishing a Framework for Community Action in the Field of Water Policy. Official Journal 22 December 2000 L 327/1. European Commission, Brussels

Fanin N, Bezaud S, Sarneel JM, Cecchini S, Nicolas M, Augusto L (2020) Relative importance of climate, soil and plant functional traits during the early decomposition stage of standardized litter. Ecosystems 23:1004–1018

Ferreira V, Chauvet E (2011) Synergistic effects of water temperature and dissolved nutrients on litter decomposition and associated fungi. Glob Change Biol 17:551–564

Ferreira V, Graça MAS (2016) Effects of whole-stream nitrogen enrichment and litter species mixing on litter decomposition and associated fungi. Limnologica 58:69–77

Ferreira V, Elosegi A, Gulis V, Pozo J, Graça MAS (2006a) Eucalyptus plantations affect fungal communities associated with leaf litter decomposition in Iberian streams. Arch Hydrobiol 166:467–490

Ferreira V, Gulis V, Graça MAS (2006b) Whole-stream nitrate addition affects litter decomposition and associated fungi but not invertebrates. Oecologia 149:718–729

Ferreira V, Encalada AC, Graça MAS (2012) Effects of litter diversity on decomposition and biological colonization of submerged litter in temperate and tropical streams. Freshw Sci 31:945–962

Ferreira V, Raposeiro PM, Pereira A, Cruz AM, Costa AC, Graça MAS, Gonçalves V (2016) Leaf litter decomposition in remote oceanic island streams is driven by microbes and depends on litter quality and environmental conditions. Freshw Biol 61:783–799

Ferreira V, Boyero L, Calvo C, Correa F, Figueroa R, Gonçalves JF Jr, Goyenola G, Graça MAS, Hepp LU, Kariuki S, Lopez-Rodriguez A, Mazzeo N, M'Erimba C, Peil A, Pozo J, Rezende R, Teixeira-de-Mello F (2019) A global assessment of the effects of eucalyptus plantations on stream ecosystem functioning. Ecosystems 22:629–642

Ferreira V, Elosegi A, Tiegs SD, von Schiller D, Young R (2020) Organic matter decomposition and ecosystem metabolism as tools to assess the functional integrity of streams and rivers—a systematic review. Water 12:3523

Ferreira V, Silva J, Cornut J, Sobral O, Bachelet Q, Bouquerel J, Danger M (2021) Organic-matter decomposition as a bioassessment tool of stream functioning: a comparison of eight decomposition-based indicators exposed to different environmental changes. Environ Pollut 290:118111

Gessner MO, Chauvet E (1994) Importance of stream microfungi in controlling breakdown rates of leaf litter. Ecology 75:1807–1817

Gessner MO, Chauvet E (2002) A case for using litter breakdown to assess functional stream integrity. Ecol Appl 12:498–510

Gessner MO, Swan CM, Dang CK, McKie BG, Bardgett RD, Wall DH, Hättenschwiler S (2010) Diversity meets decomposition. Trends Ecol Evol 25:372–380

Graça MA, Poquet JM (2014) Do climate and soil influence phenotypic variability in leaf litter, microbial decomposition and shredder consumption? Oecologia 174:1021–1032

Gulis V, Suberkropp K (2003) Leaf litter decomposition and microbial activity in nutrient-enriched and unaltered reaches of a headwater stream. Freshw Biol 48:123–134

Gulis V, Marvanová L, Descals E (2020) An illustrated key to the common temperate species of aquatic hyphomycetes. In: Bärlocher F, Gessner MO, Graça MAS (eds) Methods to study litter decomposition—a practical guide, 2nd edn. Springer, pp 223–240

Hamilton-Miller JMT (1995) Antimicrobial properties of tea (*Camellia sinensis* L.). Antimicrob Agents Chemother 39:2375–2377

Hieber M, Gessner MO (2002) Contribution of stream detrivores, fungi, and bacteria to leaf breakdown based on biomass estimates. Ecology 83:1026v1038

Jabiol J, Lecerf A, Lamothe S, Gessner MO, Chauvet E (2019) Litter quality modulates effects of dissolved nitrogen on leaf decomposition by stream microbial communities. Microb Ecol 77:959–966

Keuskamp JA, Dingemans BJJ, Lehtinen T, Sarneel JM, Hefting MM (2013) Tea Bag Index: a novel approach to collect uniform decomposition data across ecosystems. Methods Ecol Evol 4:1070–1075

Lalimi FY, Silvestri S, D'Alpaos A, Roner M, Marani M (2018) The spatial variability of organic matter and decomposition processes at the marsh scale. J Geophys Res Biogeosci 123:3713–3727

Lecerf A, Chauvet E (2008) Intraspecific variability in leaf traits strongly affects alder leaf decomposition in a stream. Basic Appl Ecol 9:598–605

LeRoy CJ, Wooley SC, Lindroth RL (2012) Genotype and soil nutrient environment influence aspen litter chemistry and in-stream decomposition. Freshw Sci 31:1244–1253

Marks JC (2019) Revisiting the fates of dead leaves that fall into streams. Annu Rev Ecol Evol Syst 50:547–568

Marley ARG, Smeaton C, Austin WEN (2019) An assessment of the Tea Bag Index method as a proxy for organic matter decomposition in intertidal environments. J Geophys Res Biogeosci 124:2991–3004

Martínez A, Larrañaga A, Pérez J, Descals E, Pozo J (2014) Temperature affects leaf litter decomposition in low-order forest streams: field and microcosm approaches. FEMS Microbiol Ecol 87:257–267

Molinero J, Pozo J (2004) Impact of a eucalyptus (*Eucalyptus globulus* Labill.) plantation on the nutrient content and dynamics of coarse particulate organic matter (CPOM) in a small dytrsm. Hydrobiologia 528:143–165

Mori T (2021) Tea Bags—standard materials for testing impacts of nitrogen addition on litter decomposition in aquatic ecosystems? Nitrogen 2:259–267

Mori T, Ono K, Sakai Y (2021) Testing the Tea Bag Index as a potential indicator for assessing litter decomposition in aquatic ecosystems. BioRxiv. https://doi.org/10.1101/2021.04.26.441560

MPI (2021) New Zealand Ministry for Primary Industries. Importing dried and preserved plant products. https://www.mpi.govt.nz/import/plants-flowers-seeds-plant-growing-products/dried-preserved-plant-products. Accessed on 7 September 2021

Mueller P, Schiler-Beers LM, Mozdzer TJ, Chmura GL, Dinter T, Kuzyakov Y, de Groot AV, Esselink P, Smit C, D'Alpaos A, Ibáñez C, Lazarus M, Neumeier U, Johnsson BJ, Baldwin AH, Yardood SA, Montemayor DI, Yang Z, Wu J, Jensen K, Nolte S (2018) Global-change effects on early-stage decomposition processes in tidal wetlands—implications from a global survey using standardized litter. Biogeosciences 15:3189–3202

Ostrofsky ML (1997) Relationship between chemical characteristics of autumn-shed leaves and aquatic processing rates. J N Am Benthol Soc 16:750–759

Pascoal C, Cássio F (2004) Contribution of fungi and bacteria to leaf litter decomposition in a polluted river. Appl Environ Microbiol 70:5266–5273

Peralta-Maraver I, Perkins DM, Thompson MSA, Fussmann K, Reiss J, Robertson AL (2019) Comparing biotic drivers of litter breakdown across stream compartments. J Anim Ecol 88:1146–1157

Pereira A, Geraldes P, Lima-Fernandes E, Fernandes I, Cássio F, Pascoal C (2016) Structural and functional measures of leaf-associated invertebrates and fungi as predictors of stream eutrophication. Ecol Ind 69:648–656

Pereira A, Figueiredo A, Ferreira V (2021) Invasive instream litter decomposition through changes in water nitrogen concentration and litter characteristics. Microb Ecol 83:257–273

Petraglia A, Cacciatori C, Chelli S, Fenu G, Calderisi G, Gargano D, Abeli T, Orsenigo S, Carbognani M (2019) Litter decomposition: effects of temperature driven by soil moisture and vegetation type. Plant Soil 435:187–200

Ramos SM, Graça MAS, Ferreira V (2021) A comparison of decomposition rates and biological colonization of leaf litter from tropical and temperate origins. Aquat Ecol 55:925–949

Sanfuentes E, Fajardo S, Sabag M, Hansen E, González M (2016) *Phytophthora kernoviae* isolated from fallen leaves of *Drymis winteri* in native forest of southern Chile. Aust Plant Dis Notes 11:19

Seelen LMS, Flaim G, Keuskamp J, Teurlincx S, Font RA, Tolunay D, Fránková M, Sumberová K, Temponeras M, Lenhardt M, Jennings E, Domis LNS (2019) An affordable and reliable assessment of aquatic decomposition: tailoring the Tea Bag Index to surface waters. Water Res 151:31–43

Suberkropp K, Arsuffi TL (1984) Degradation, growth, and changes in palatability of leaves colonized by six aquatic hyphomycete species. Mycologia 76:398–407

Trevathan-Tackett SM, Brodersen KE, Macreadie PI (2020) Effects of elevated temperature on microbial breakdown of seagrass leaf and tea litter biomass. Biogeochemistry 151:171–185

Trevathan-Tackett SM, Kepfer-Rojas S, Engelen AH, York PH, Ola A, Li J, Kelleway JJ, Jinks KI, Jackson EL, Adame MF, Pendall E, Lovelock CE, Connolly RM, Watson A, Visby I, Trethowan A, Taylor B, Roberts TNB, Petch J, Farrington L, Djukic I, Macreadie PI (2021) Ecosystem type drives tea litter decomposition and associated prokaryotic microbiome communities in freshwater and coastal wetlands at a continental scale. Sci Total Environ 782:146819

Webber JF, Rose J (2008) Dissemination of aerial and root infecting *Phytophthoras* by human vectors. In: Frankel SJ, Kliejunas JT, Palmieri KM (eds) Proceof the sudden oak death third science symposium. US Department of Agriculture, Forest Service, Pacific Southwest Research Station, pp 195–198

Woodward G, Gessner MO, Giller PS, Gulis V, Hladyz S, Lecerf A, Malmqvist B, McKie BG, Tiegs SD, Cariss H, Dobson M, Elosegi A, Ferreira V, Graça MAS, Fleituch T, Lacoursiere J, Nistorescu M, Pozo J, Risnoveanu G, Schindler M, Vadineanu A, Vought LB-M, Chauvet E (2012) Continental-scale effects of nutrient pollution on stream ecosystem functioning. Science 336:1438–1440

Zemek J, Marvanová L, Kuniak L, Kadlecikova B (1985) Hydrolytic enzymes in aquatic hyphomycetes. Folia Microbiol 30:363–372