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Leaf litter decomposition in remote oceanic island streams is driven by microbes and depends on litter quality and environmental conditions

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SUMMARY

1. Leaf litter decomposition is an important process in many streams. The flow of carbon and nutrients to higher trophic levels generally depends on litter characteristics and environmental conditions, and is driven by the activities of microbes and invertebrate shredders. However, little is known about what drives litter decomposition in oceanic islands, where invertebrate communities are species-poor.

2. In this study, we assessed the relative importance of litter quality and environmental conditions on the biological colonisation and decomposition of litter exposed to and protected from macroinvertebrates, in the Azores archipelago, North Atlantic Ocean. Three leaf litter species with distinct physical and chemical characteristics (*Acacia melanoxylon*, *Clethra arborea* and *Pittosporum undulatum*) were incubated in six streams with distinct water characteristics. Coarse and fine mesh bags were used to isolate the relative role of macroinvertebrates on litter decomposition. Incubation of litter took place in late spring – early summer and lasted for up to 56 days.

3. No significant differences in litter decomposition rates were found between coarse and fine mesh bags suggesting that microbes, especially aquatic hyphomycetes, are the key players in litter decomposition in these island streams.

4. Litter decomposition rates were inversely proportional to initial lignin concentration: *A. melanoxylon* 0.0080 day^{-1}, *C. arborea* 0.0121 day^{-1}, *P. undulatum* 0.0292 day^{-1}, on average across streams.

5. Litter decomposition rates and associated decomposers differed among streams, suggesting that environmental conditions (e.g. nutrient concentration) may be important moderators of biological activities in these streams, as found for continental streams.

6. Species richness, fungal biomass and reproductive activity of aquatic hyphomycetes on decomposing litter were recorded in Atlantic islands for the first time and were at levels similar to those found for continental streams.

7. High microbial activities in Atlantic island streams ensure litter decomposition when shredder abundance is low.

Keywords: aquatic hyphomycetes, Azores, dissolved nutrients, invertebrate shredders, litter chemistry

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Introduction

Aquatic communities in forest streams derive a substantial portion of their energy, carbon and nutrients from litter of terrestrial origin (Wallace *et al.*, 1997). The incorporation of this organic matter into the food web is mediated by the activities of microbial decomposers, especially aquatic hyphomycetes, and invertebrate shredders (Petersen & Cummins, 1974; Hieber & Gessner, 2002). The relative contribution of microbes to litter decomposition partially depends on shredder abundance, with microbes accounting up to 39% of the litter carbon loss when shredders are abundant (Hieber & Gessner, 2002; Pascoal & Cássio, 2004) and much more (>46%) when shredders are less abundant or absent (Benstead *et al.*, 2009; Raposeiro *et al.*, 2014; Taylor & Chauvet, 2014). The relative contribution of shredders and microbes to litter decomposition can determine the response of this process to environmental change (Gulis, Ferreira & Graça, 2006; Ferreira *et al.*, 2015c).

The rate at which the litter is decomposed generally depends on litter intrinsic characteristics. Soft litter, with low concentration of structural (e.g. lignin, hemicelluloses) and secondary compounds (e.g. polyphenols) and high concentration of nutrients (e.g. nitrogen) are generally colonised and decomposed faster than tough litter, with high concentration of structural and secondary compounds and low concentration of nutrients (Ostrofsky, 1997; Lecerf & Chauvet, 2008; Ferreira, Encalada & Graça, 2012). Litter toughness and the concentration in structural compounds may even be more important, determining biological colonisation and litter decomposition rates than nutrient concentrations (Li, Ng & Dudgeon, 2009; Schindler & Gessner, 2009; Frainer *et al.*, 2015).

The rate at which litter decomposes is also sensitive to environmental conditions (Suberkropp & Chauvet, 1995). Moderate levels of dissolved inorganic nutrients generally stimulate litter colonisation and decomposition (reviewed by Ferreira *et al.*, 2014, 2015a). Microbes can retrieve nutrients from both the organic substrate and the water, but nutrients in the water are already in their inorganic form and require less energy to be retrieved than the nutrients in the organic matrix (Gulis & Suberkropp, 2003). Higher microbial colonisation in nutrient richer conditions increases litter palatability to shredders that can further promote litter decomposition (Bärlocher & Sridhar, 2014).

Oceanic islands ecosystems may be especially sensitive to environmental change as they generally have less diverse communities and are rich in endemic taxa highly adapted to local conditions, when compared with continental ecosystems (Kier *et al.*, 2009). Human-induced environmental change, e.g. due to agriculture, forestry, urbanisation and invasion by exotic species, is of particular concern in oceanic islands (e.g. Vitousek, Andersen & Loope, 1998; Silva *et al.*, 2008), mainly on those whose aquatic systems are under great pressure while they still have to provide important ecosystem services such as high quality water. Despite these conflicting interests, our knowledge on how stream communities and processes respond to environmental change in species poor oceanic islands is still scarce, compromising efficient management of freshwater resources.

Here, we assessed the biological colonisation and decomposition of three leaf species across six streams differing in water characteristics in the Azores archipelago (North Atlantic). We predicted that biological colonisation and litter decomposition would differ among streams (Suberkropp & Chauvet, 1995), and would depend on litter species (Fernandes *et al.*, 2014) and presence of macroinvertebrates (Gulis *et al.*, 2006). A special effort was placed on the assessment of the colonisation of decomposing litter by aquatic hyphomycetes and a list of species is presented for the first time for these oceanic islands.

Materials and methods

Study region

This study was done in São Miguel, Azores archipelago, an oceanic island in the North Atlantic Ocean (36°55′–39°43′N and 25°00′–31°71W), in the Middle Atlantic Ridge, where the North American, Eurasian and African lithospheric plates join. Mean annual temperatures in the Azores range from 14 to 18 °C and mean annual precipitation from 740 to 2400 mm. Most of the precipitation (65–70%) falls between October and March (Louvat & Allègre, 1998; DROTRH/INAG, 2001).

Native forests (i.e. laurel forests) are composed mostly of evergreen species and are dominated by species endemic to the Macaronesia as *Laurus azorica* (Lauraceae), *Ilex azorica* (Aquifoliaceae) and *Morella faya* (Myricaceae) (Elias & Dias, 2009). In the highlands, large areas of native forests have been invaded by the exotic *Pittosporum undulatum* (Pittosporaceae) or converted into commercial plantations of the conifer *Cryptomeria japonica* (Cupressaceae), while in the lowlands they have been converted to agricultural land, pastures and urbanisation (Raposeiro, Costa & Hughes, 2011).

Streams

Azorean streams are typically narrow and short, with steep and shallow channels. The higher reaches run mostly through forests while middle and lower reaches are affected by agriculture and urbanisation (Raposeiro *et al.*, 2011; Gonçalves, Marques & Raposeiro, 2015). We selected six permanent forest streams with no visible human impacts and similar geomorphology (low order, 0.4–2.9 km long, <3 m wide, 10–25% slope, substrate composed mainly of gravel and cobbles) (Table 1). These streams are part of a regional biomonitoring program since 2003 and naturally have different dissolved nutrient concentration (2 orders of magnitude difference) and temperature (up to 2 °C) (Gonçalves *et al.*, 2013). Water temperature was recorded hourly for the duration of the experiment (11/12 June – 6/7 August, 2014) using data loggers (Hobo Pendant UA-001-08, Onset Computer Corp. MA, U.S.A.) and hourly values were averaged to produce daily means. On five occasions during this period, electrical conductivity and pH were recorded with a multiparametric field probe (CyberScan 600, Eutech instruments, Nijkerk, the Netherlands) and dissolved oxygen with a dissolved oxygen data logger (Hobo U26, Onset Computer Corp., MA, U.S.A.). On the same occasions, 1 L of stream water was collected in acid washed plastic bottles, transported to the laboratory in a cooler, filtered (47 mm diameter, 1.2 μm pore size; Whatman GF/C, GE Healthcare Europe GmbH, Little Chalfont, U.K.) and analysed for nitrate (ion chromatography; APHA, 1995), ammonium (atomic absorption; AHPA, 1995) and phosphate (ion chromatography; APHA, 1995).

Benthic macroinvertebrates

Benthic macroinvertebrates were collected according to the Portuguese macroinvertebrate sampling method (INAG, 2008). A benthic sample was taken from each stream on four occasions between 4/5 June (day –7) and 6/7 August (day 56). A sample was composed of six substrate kicks over 1 m each, taken with a kick-net (30 × 30 cm opening, 500 μm pore size); the kicks were distributed according with the existing habitats (e.g. riffles, pools), along a 10 m reach. Samples were preserved in the field with 70% ethanol. In the laboratory, macroinvertebrates were identified and counted under a stereo microscope (Zeiss Stemi, Göttingen, Deutschland). Identification was made to the lowest possible taxonomic level using identification keys (e.g. Tachet, Bournand & Richoux, 2002; Oscoz, Galicia & Miranda, 2011), and individuals were assigned to functional feeding groups (FFG) following Schmidt-Kloiber & Hering (2012, 2015). Abundance was expressed as the number of (no.) individuals sample^{-1} and taxa richness was expressed as the number of (no.) taxa sample^{-1}.

Leaf litter

Mature leaves of nine tree and shrub species commonly found in the riparian vegetation of Azorean streams were collected directly from single trees/shrubs on March 2014 and air-dried at room temperature until used for physical and chemical characterisation (Table S1). Ten air-dry leaves per species were soaked in distilled water for 1 h and leaf toughness was determined using a penetrometer (Graça, Bärlocher & Gessner, 2005). Leaves were secured between two acrylic

Table 1 Location and physical and chemical characteristics of the six Azorean streams during the letter decomposition experiment (11/12 June–6/7 August, 2014). Values are means ± 1 SE. Streams are ranked by nitrogen concentration. Streams with the same letter do not significantly differ (one-way ANOVA followed by Tukey's HSD test, $P > 0.050$).

Water variables	*n*	AFG2	Lom4	AFG1	Lom1	Lom2	Lom3
Latitude (N)		37°45′25.6″	37°46′36.5″	37°46′00.0″	37°46′34.6″	37°44′26.8″	37°46′36.6″
Longitude (W)		25°27′49.3″	25°27′09.4″	25°29′19.0″	25°11′10.1″	25°28′07.9″	25°27′08.4″
Elevation (m a.s.l.)		608	632	619	401	310	632
Order (Strahler system)		2	2	2	3	3	3
Temperature (°C)	56	14.3 ± 0.1^{a}	15.1 ± 0.1^{d}	14.6 ± 0.1^{a}	15.6 ± 0.1^{b}	15.5 ± 0.1^{b}	13.4 ± <0.1^{c}
Conductivity (μS cm^{-1})	5	101 ± 2^{a}	84 ± 2 ^{a}	105 ± 1^{ab}	136 ± 18^{b}	99 ± 1^{a}	94 ± 4^{a}
Oxygen (mg L^{-1})	5	9.1 ± 0.1^{a}	8.4 ± 0.1^{b}	8.6 ± 0.2^{b}	8.6 ± 0.1^{b}	8.5 ± 0.1^{b}	8.6 ± 0.1^{b}
pH	5	8.1 ± 0.1^{a}	8.0 ± 0.1^{ab}	8.0 ± 0.1^{ab}	7.7 ± 0.1^{b}	7.9 ± <0.1^{ab}	8.0 ± 0.1^{ab}
NO_3 (μg L^{-1})	4	28 ± 21^{a}	73 ± 14^{a}	238 ± 6^{b}	238 ± 24^{b}	588 ± 19^{c}	1033 ± 34^{d}
NH_4 (μg L^{-1})	5	16 ± 5^{a}	18 ± 6^{a}	16 ± 5^{a}	18 ± 5^{a}	18 ± 5^{a}	22 ± 5^{a}
PO_4 (μg L^{-1})	5	53 ± 9^{a}	30 ± 5^{a}	43 ± 9^{a}	171 ± 12^{b}	175 ± 8^{b}	164 ± 9^{b}

sheets and the mass required to punch a 0.79 mm diameter iron rod through the mesophyll, avoiding major veins, was recorded three times per leaf (g; Graça *et al.*, 2005). Ten additional leaves per species were moistened with distilled water to render them soft and allow the extraction of a 12 mm diameter disc without breaking, avoiding major veins when possible. Discs were oven-dried at 105 °C for 48 h and the specific leaf area (SLA) was determined as the ratio between the disc area and mass ($mm^2\ mg^{-1}$). Three batches of leaves per species were ground to a fine powder (<0.5 mm size), and the powder was oven-dried at 105 °C for 48 h and analysed for lignin (Goering & Van Soest, 1970), polyphenols (Graça *et al.*, 2005), phosphorus (P; APHA, 1995), nitrogen (N) and carbon (C) (CNH auto analyser IRMS Thermo Delta V advantage with a Flash EA, 1112 series) concentrations (all as percentage dry mass, % DM).

Three plant species, *Acacia melanoxylon* (Fabaceae), *Clethra arborea* (Clethraceae) and *P. undulatum*, were selected to provide a gradient of toughness, SLA, lignin and N concentrations (Fig. S1), as these leaf characteristics most often correlate with litter decomposition rates and associated biological activities (Lecerf & Chauvet, 2008; Li *et al.*, 2009; Schindler & Gessner, 2009; Ferreira *et al.*, 2012). From the selected leaf species, *A. melanoxylon* was the toughest and had the highest lignin, N and C concentrations, but had the lowest phenol concentration and SLA (Table 2). *P. undulatum* had the lowest lignin concentration, while *C. arborea* was the softest and had the highest SLA and polyphenols concentration (Table 2). Leaves were collected directly from the trees/shrubs and not after natural abscission because in these evergreen species it is difficult to collect sufficient naturally abscised leaves in a short period (to avoid large variation in leaf characteristics). Leaves were air-dried and not used fresh for logistic reasons, but a large portion of the leaf litter entering streams, including those within evergreen forests, arrives from lateral inputs (Molinero & Pozo, 2004, 2006), and thus using air-dry leaves is ecologically relevant.

Litter decomposition

Air-dry leaves were weighed into 2.90–3.10 g portions, sprayed with distilled water to render them soft and less susceptible to breakage due to handling, and enclosed in mesh bags (10 × 15 cm). Two mesh sizes were used to assess the relative contribution of macroinvertebrates and microbes to litter decomposition: coarse mesh (5 mm mesh with 10 mm holes; CM), which allows macroinvertebrate access to leaf litter and thus decomposition is carried out by the combined activities of macroinvertebrates and microbes, and fine mesh (0.5 mm mesh; FM), which prevents macroinvertebrate access to the leaf litter and thus litter decomposition is carried out mainly by the microbial community. Twelve litter bags of each mesh size and leaf species were deployed in each stream on 11 or 12 June, 2014 (432 mesh bags total). Three replicate litter bags were retrieved from each stream after 7, 21, 35 and 56 days, enclosed individually in zip lock bags and transported to the laboratory in a cooler.

In the laboratory, litter from FM bags was gently rinsed with distilled water into a 0.5 mm mesh sieve to retain small leaf fragments, two sets of five leaf discs (12 mm diameter) were cut out from individual leaves for microbial determinations (see below), and the bulk remaining litter was saved into aluminum pans. Litter from CM bags was gently rinsed with tap water into a 0.5 mm mesh sieve to retain small leaf fragments and adherent macroinvertebrates (see below) and the remaining litter was also saved. The remaining litter was oven-dried (70 °C, 48 h) and weighed (0.1 mg precision) for determination of dry mass (DM) remaining. DM remaining was ignited (500 °C, 8 h) and reweighed (0.1 mg precision) for determination of ash mass. The remaining ash-free dry mass (AFDM) was estimated as the difference between DM and ash mass, taking into account the AFDM of the leaf discs taken from FM bags (see below). The fraction of the remaining AFDM was estimated as the ratio between the remaining AFDM and the initial AFDM.

Table 2 Initial physical and chemical characteristics of the three leaf litter species used in the decomposition experiment. Values are means ± 1 SE. Species with the same letter do not significantly differ (one-way ANOVA followed by Tukey's HSD test, $P > 0.050$).

Litter variables*	*n*	*Acacia melanoxylon*	*Clethra arborea*	*Pittosporum undulatum*
Toughness (g)	10	310.24 ± 13.84[a]	97.71 ± 6.89[b]	176.90 ± 8.86[c]
SLA ($mm^2\ mg^{-1}$)	10	9.18 ± 0.38[a]	16.74 ± 0.93[b]	10.60 ± 0.67[a]
Lignin (% DM)	3	40.95 ± 0.19[a]	27.78 ± 1.40[b]	21.95 ± 1.54[c]
Phenols (% DM)	3	5.41 ± 0.27[a]	13.09 ± 0.22[b]	9.39 ± 0.38[c]
P (% DM)	3	0.09 ± 0.01[a]	0.09 ± 0.01[a]	0.10 ± 0.01[a]
N (% DM)	3	2.14 ± 0.06[a]	0.71 ± 0.03[b]	0.81 ± 0.06[b]
C (% DM)	3	50.26 ± 0.60[a]	45.07 ± 0.56[b]	48.98 ± 0.22[b]

*SLA, specific leaf area; P, phosphorus; N, nitrogen; C, carbon.

Initial AFDM was estimated by multiplying the initial air-dry mass by a conversion factor derived from extra sets of five mesh bags for each mesh size and leaf species. These extra bags were taken to the field on day 0, immersed in water for approximately 10 min, and taken back to the laboratory for determination of DM and AFDM as described above. The initial air-dry mass to initial AFDM conversion factor, given by the ratio between initial AFDM and initial air-dry mass, was 0.90 for *A. melanoxylon* (both mesh sizes), 0.85 for *C. arborea* in CM, 0.86 for *C. arborea* in FM, and 0.83 for *P. undulatum* (both mesh sizes).

Fungal biomass

One set of five leaf discs was used for the determination of ergosterol concentration as a surrogate of fungal biomass (Graça *et al.*, 2005). Leaf discs were frozen at −20 °C until ergosterol extraction, lyophilised overnight (LY3TE, Snijders Scientific, Tilburg, The Netherlands), weighed (0.1 mg precision) to determine discs DM and placed in tightly closed tubes with 10 mL of alkaline methanol (8 g KOH L^{-1}). Lipids were extracted in a water bath (80 °C, 30 min), purified by solid phase extraction (Waters Sep-Pak® Vac RC tC_{18} cartridges; Waters Corp., Milford, MA, U.S.A.) and the ergosterol eluted with isopropanol. Ergosterol was quantified by high-performance liquid chromatography (HPLC) by measuring absorbance at 282 nm. The HPLC system (Dionex DX-120, Sunnyvale, CA, U.S.A.) was equipped with the Thermo Scientific Syncronis C18 column (250 × 4 mm, 5 μm particle size; Thermo, Waltham, MA, U.S.A.) and the Thermo Universal Uniguard holder 4/4.6 mm ID3 + Syncronis C18 (10 × 4 mm, 5 μm particle size) drop in guard pre column (Thermo), maintained at 33 °C; the mobile phase was 100% methanol, flowing at 1.4 mL min^{-1}. Ergosterol was converted into mycelial biomass assuming 5.5 μg ergosterol mg^{-1} mycelial DM (Gessner & Chauvet, 1993). Discs DM was converted into AFDM, using the ash fraction derived from the discs used for sporulation (see below) and the results were expressed as mg mycelial biomass g^{-1} leaf litter AFDM.

Conidial production by aquatic hyphomycetes

Another set of five leaf discs was used to induce conidial production by aquatic hyphomycetes. Due to fast decomposition rates, insufficient leaf litter existed on day 56 for *P. undulatum* incubated in stream Lom2 to induce conidial production. Leaf discs were incubated in 100 mL Erlenmeyer flasks with 25 mL of filtered stream water (47 mm diameter, 1.2 μm pore size; Whatman GF/C, GE Healthcare Europe GmbH, Little Chalfont, U.K.), on an orbital shaker (100 rotations min^{-1}) at 13 °C for 48 h, under a 10 h light :14 h dark photoperiod. The conidial suspensions were poured into 50 mL Falcon tubes, the flasks were rinsed twice, the suspensions fixed with 2 mL of 37% formalin and the final volume adjusted to the next mark in the Falcon tube with distilled water. Leaf discs were saved, oven-dried, weighed, ignited and reweighed as for the remaining bulk mass to determine the ash fraction of the disc and the AFDM.

When preparing the filters for conidial identification and counting, 150 μL of 0.5% Triton X-100 were added to the suspensions and gently mixed with a magnetic stirring bar to ensure a uniform distribution of conidia. Aliquots of 1–35 mL were filtered through cellulose nitrate filters (25 mm diameter, 5 μm pore size; Sartorius Stedim Biotech GmhH, Göttingen, Germany) with gently vacuum and the filters were stained with 0.05% trypan blue in 60% lactic acid. Conidia were identified and counted at 320× magnification (Graça *et al.*, 2005). Sporulation rates were expressed as the number of (no.) conidia mg^{-1} leaf litter AFDM day^{-1} and species richness as the number of (no.) species $sample^{-1}$. Cumulative conidial production by the last sampling date was estimated by linearly interpolating the values for each day between sampling dates and summing up.

Litter macroinvertebrates

Macroinvertebrates associated with decomposing litter were saved and preserved in 70% ethanol until identified and counted as described for benthic macroinvertebrates. The abundance was expressed as the number of (no.) individuals g^{-1} leaf litter AFDM and taxa richness was expressed as the number of (no.) taxa $sample^{-1}$.

Data analyses

Water characteristics were compared among streams and leaf characteristics were compared among the selected species by one-way analysis of variance (ANOVA), followed by Tukey's honest significant difference (HSD) test when significant effects were detected in ANOVA. Ordination of the nine tree and shrub species based on leaf characteristics was done by principal component analysis (PCA) as the length of the gradient of axis one was <3 SD as determined by detrended correspondence analysis (DCA) (CANOCO v4.5; ter Braak & Smilauer, 1998).

Exponential decomposition rates (k, day^{-1}) were estimated as the slope of linear regressions of fraction of AFDM remaining (ln-transformed) against time (days), with the intercept fixed at ln(1) = 0. Fraction of AFDM remaining (ln-transformed) was compared among treatments by homogeneity-of-slopes model to test whether the covariate (time) had different effects at different levels of categorical variables (stream, leaf species and mesh size). This was the case as significant interactions were found between time and each categorical variable (data not shown). Thus, fraction of AFDM remaining (ln-transformed) was compared among treatments by the separate slopes model, followed by Tukey's unequal HSD test when significant effects were detected. No significant effect of mesh size was found and thus exponential decomposition rates per leaf species and stream were estimated by pooling CM and FM bags together. Fraction of AFDM remaining (ln-transformed) (CM and FM bags pooled) was compared among treatments by homogeneity-of-slopes model to test whether the covariate (time) had different effects at different levels of categorical variables (stream and leaf species). This was the case (data not shown) and so fraction of AFDM remaining (ln-transformed) was compared among treatments by separate slopes model, followed by Tukey's unequal HSD test when significant effects were detected.

Fungal biomass [log(x + 1) transformed], aquatic hyphomycetes sporulation rates [log(x + 1) transformed], aquatic hyphomycete species richness, total macroinvertebrate abundance [log(x + 1) transformed], total macroinvertebrate taxa richness and shredder abundance [log(x + 1) transformed] associated with decomposing litter were compared among treatments by repeated measures (RM) ANOVA, followed by Tukey's unequal HSD test when significant effects were detected in RM ANOVA. Four sampling dates were considered in all analyses, except for aquatic hyphomycetes sporulation rates and species richness where only two sampling dates (day 21 and day 35) were considered as no conidial production was observed for *A. melanoxylon* on day 7 (all streams) and not enough leaf material existed on day 56 for *P. undulatum* incubated in Lom2 to induce conidial production.

Aquatic hyphomycete communities and macroinvertebrate communities (in terms of taxa and functional feeding groups) were compared among litter species and streams by permutational multivariate analysis of variance (PERMANOVA) (Anderson, 2001; McArdle & Anderson, 2001) based on the Bray–Curtis similarity matrix. As PERMANOVA is sensitive to differences in dispersion, PERMDISP was performed to test for homogeneity of multivariate dispersion across groups (Anderson, Gorley & Clarke, 2008). PERMANOVA and PERMDISP were performed using PRIMER 6 v6.1.11 & PERMANOVA+ v1.0.1 (Primer-E Ltd, Plymouth, U.K.).

Normal distribution was checked by the Shapiro–Wilk's test and homogeneity of variances was checked by the Bartlett's chi-square test, and data were transformed when necessary to attain normality and homoscedasticity. All statistical analyses were performed on STATISTICA 7 software (StatSoft Inc., Tulsa, Oklahoma, U.S.A.), unless indicated otherwise.

Results

Streams

During the study period in late spring – early summer, streams were cool (13.4–15.6 °C), well oxygenated (8.4–9.1 mg O_2 L^{-1}), slightly alkaline (pH 7.7–8.1), displayed low conductivity (84–136 μS cm^{-1}) and low to moderate nutrient concentrations (28–1033 μg NO_3 L^{-1} and 30–175 μg PO_4 L^{-1}) (Table 1).

Benthic macroinvertebrates

A total of 32 716 macroinvertebrates were collected from the benthos, with an average number of individuals per stream sample of 503 (Lom2) – 2960 (Lom1). Macroinvertebrates were distributed by 32 taxa, with taxa richness per stream varying between 17 (Lom2) and 23 (Lom1) (Table 3). Stream AFG2 was dominated by grazer/scraper taxa (54% relative abundance), the caddis flies *Oxyethira falcata* (43%) and *Hydroptila* sp. (10%), but also had an important contribution from gatherer/collector taxa (40%), mainly Orthocladiinae midges (24%) (Table 3). Most streams were, however, dominated by gatherer/collector taxa (60–79%), mainly Orthocladiinae (19–61%) and Tanytarsini midges (up to 28%) and the oligochaeta *Nais* sp. (13–30%) (Table 3). The grazer/scraper *Hydroptila* sp. (up to 21%) and the filter feeder *Simulium azorense* (up to 20%) also made important contributions to benthic communities across streams (Table 3). Shredders were represented by four taxa only, the crane fly *Dicranomyia* sp., the isopod *Jaera insulana*, the caddis fly *Limnephilus atlanticus* and Tipulidae crane flies, which contributed with up to 0.1–6% to the total number of individuals in samples.

Litter decomposition

Litter mass remaining decreased exponentially over the incubation period and after 56 days it varied between 72

Table 3 Relative abundance (%) of benthic macroinvertebrate taxa in six Azorean streams between 4/5 June – 6/7 August, 2014 (n = 4). Functional feeding group (FFG) for each taxon (according to Schmidt-Kloiber & Hering, 2012, 2015) and total taxa richness are also shown. Streams are ranked by nitrogen concentration.

Family	Taxa	FFG	AFG2	Lom4	AFG1	Lom1	Lom2	Lom3
Anthomydae		Gatherer/collector						0.1
Ceratopogoninae		Predator		*	*	0.1		
Chironomidae	Tanypodinae	Predator		0.9		*		0.2
Chironomidae	Tanytarsini	Gatherer/collector	1.4	28.2	9.5	16.3	1.8	2.6
Dryopidae	*Dryops* sp.	Predator	*		*	*	0.1	
Dugesiidae	*Dugesia* sp.	Predator	0.3	1.4	3.4	2.4	1.6	0.1
Empididae	Clinocerinae	Gatherer/collector	0.2	1.9	0.8	0.7		1.1
Ephydridae		Filter feeder						0.1
Hydrachnidae		Gatherer/collector	0.2	*	0.1	0.2	1	
Hydroptilidae	*Hydroptila* sp.	Grazer/scraper	10.2	5.6	3.7	20.8	12.2	5.6
Hydroptilidae	*Oxyethira falcata*	Grazer/scraper	43.3	4.3	3.9	5.5	1.1	3.1
Hydrozetidae	*Hydrozetes* sp.	Gatherer/collector	1.1	0.1	1.5	0.3	0.8	*
Isotomidae		Gatherer/collector					0.1	
Janiridae	*Jaera insulana*	Shredder			0.2	*	0.1	
Limnephilidae	*Limnephilus atlanticus*	Shredder	0.1	0.1				
Limoniidae	*Dicranomyia* sp.	Shredder	1.9	0.5	6.0	0.8	3.6	2.5
Lumbricidae		Gatherer/collector	0.1	0.1		*	0.1	
Lumbriculidae		Gatherer/collector						*
Lymnaeidae	*Galba truncatula*	Grazer/scraper				0.4		
Lymnaeidae	*Radix peregra*	Grazer/scraper				0.1		0
Malaconothridae	*Trimalaconothrus* sp.	Gatherer/collector	0.3	0.1	0.1	0.2	0.4	0.1
Naididae	*Nais* sp.	Gatherer/collector	12.3	25.4	14.8	21.8	30	13.1
Onychiuridae		Gatherer/collector				*		
Orthocladiinae		Gatherer/collector	23.6	22.5	32.5	19.2	32.8	61.2
Physidae	*Physella acuta*	Grazer/scraper				0.2		
Poduridae		Gatherer/collector						*
Psychodidae		Gatherer/collector		0.1	0.1	0.1	*	0.2
Simuliidae	*Simulium azorense*	Filter feeder	4.5	7.7	20.1	9.5	13.6	9.6
Sminthuridae	*Stenacidia violacea*	Gatherer/collector	0.1	*	*			
Sperchonidae	*Sperchon brevirostris*	Gatherer/collector	0.5	0.9	3.3	1.3	0.6	0.4
Tipulidae		Shredder			0.1			
Trichoniscidae	*Trichoniscus* sp.	Gatherer/collector	*					
Total taxa richness (no. species stream^{-1})			18	19	19	23	17	19

*relative abundance < 0.1%.

and 54% of initial mass for *A. melanoxylon*, 67 and 33% for *C. arborea*, and 44 and 2% for *P. undulatum* across streams and mesh sizes (Fig. 1). Litter decomposition significantly differed among litter species (separate-slopes model, $P = 0.005$) and streams ($P < 0.0001$), with a significant interaction between both factors ($P < 0.0001$) (Table S2). No significant effect of mesh size was found, either when considered individually or interacting with litter species or streams (separate-slopes model, $P > 0.371$; Table S2). Thus, CM and FM bags were pooled for the estimation of new decomposition rates per leaf species and stream (Table S3).

Litter decomposition rates were in the order *A. melanoxylon* (average across streams: 0.0080, day^{-1}) < *C. arborea* (0.0121, day^{-1}) < *P. undulatum* (0.0292, day^{-1}) (Table 4), following a lignin concentration gradient that decreases from *A. melanoxylon* to *P. undulatum* (Table 2). Decomposition rates significantly differed among litter species and streams (separate slopes model, $P = 0.006$ and <0.001 respectively; Table S3). However, there was a significant interaction between both factors ($P < 0.0001$; Table S3), indicating that differences among species depended on environmental conditions and differences among streams depended on litter species. Indeed, decomposition rates significantly differ among streams only for *C. arborea* (Lom3 differed from AFG2; Tukey test, $P = 0.004$) and *P. undulatum* (Lom2 differed from all other streams; Tukey test, $p < 0.0001$) while differences among litter species only occurred between *P. undulatum* and the other two species (Tukey test, $p < 0.001$), except in stream Lom3 where all the three species significantly differed (Tukey test, $P < 0.001$)

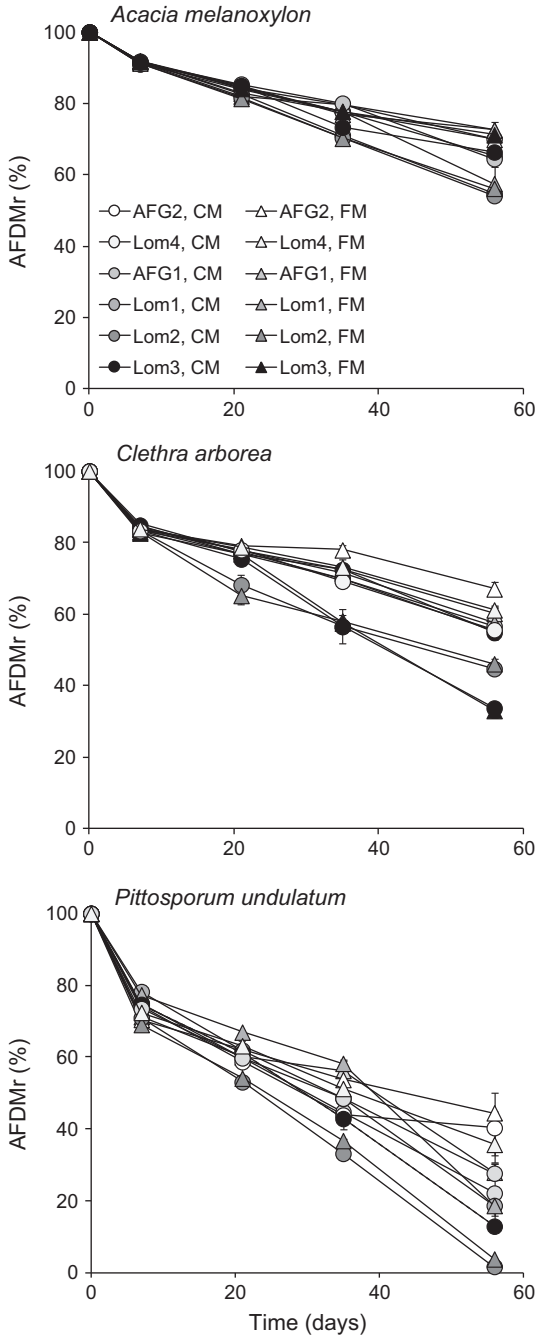

Fig. 1 Ash-free dry mass remaining (AFDMr) of three leaf litter species incubated in coarse mesh (CM) and fine mesh (FM) bags in six Azorean streams over 56 days (11/12 June–6/7 August, 2014). Values are means ± 1SE; 1SEs are smaller than symbols in most cases. Streams are ranked by nitrogen concentration (symbols with increased darkness).

(Table 4). A significant interaction was also found between litter species, stream and time (separate-slopes model, $P < 0.0001$), suggesting that effects of litter species and stream are time dependent.

Table 4 Exponential decomposition rates (k) of three leaf litter species incubated in six Azorean streams over 56 days (11/12 June – 6/7 August, 2014), standard error (SE) and coefficient of determination of the regression (R^2) ($P < 0.0001$ in all cases). Data from coarse mesh and fine mesh bags were merged for each treatment (see Methods). Streams are ranked by nitrogen concentration. Treatments with the same letter do not significantly differ (separate-slopes model followed by Tukey's unequal HSD test, $P > 0.050$).

Litter species	Stream	k (day^{-1})	SE	R^2	Separate-slopes model
Acacia melanoxylon	AFG2	0.0066	0.0002	0.85	a
	Lom4	0.0072	0.0002	0.91	a
	AFG1	0.0070	0.0003	0.85	a
	Lom1	0.0098	0.0004	0.91	ab
	Lom2	0.0104	0.0001	0.99	ab
	Lom3	0.0072	0.0003	0.86	a
Clethra arborea	AFG2	0.0088	0.0006	0.35	ab
	Lom4	0.0100	0.0004	0.73	abc
	AFG1	0.0101	0.0005	0.69	abc
	Lom1	0.0105	0.0003	0.86	abc
	Lom2	0.0152	0.0005	0.88	bcd
	Lom3	0.0183	0.0006	0.92	cd
Pittosporum undulatum	AFG2	0.0177	0.0012	0.39	de
	Lom4	0.0210	0.0008	0.84	de
	AFG1	0.0253	0.0020	0.63	e
	Lom1	0.0264	0.0014	0.84	e
	Lom2	0.0534	0.0046	0.73	f
	Lom3	0.0316	0.0019	0.82	e

Fungal biomass

Fungal biomass associated with leaf litter in streams AFG1, Lom4 and AFG2 (lower nutrient concentrations) generally increased over the incubation period, while fungal biomass associated with leaf litter in streams Lom1, Lom2 and Lom3 (higher nutrient concentrations) either rapidly increased to a plateau or attained a peak before the end of the experiment (Fig. 2). Fungal biomass significantly differed among litter species and streams (RM ANOVA, $P < 0.0001$ for both factors) (Table S4). However, the significant interaction between factors ($P < 0.0001$) indicates that there are exceptions (Table S4). Nevertheless, *A. melanoxylon* generally had a higher fungal biomass than the other two litter species, and litter in stream Lom2 generally had a higher fungal biomass than litter in other streams (Fig. 2).

Aquatic hyphomycete conidial production

Similarly to fungal biomass, sporulation rates by aquatic hyphomycetes presented distinct dynamics in streams with lower and higher nutrient concentrations. In streams Lom1 and Lom2, sporulation rates increased after day 7 and attained a peak by day 21–35 and decreased thereafter, while in stream Lom3 (for *P. undu-*

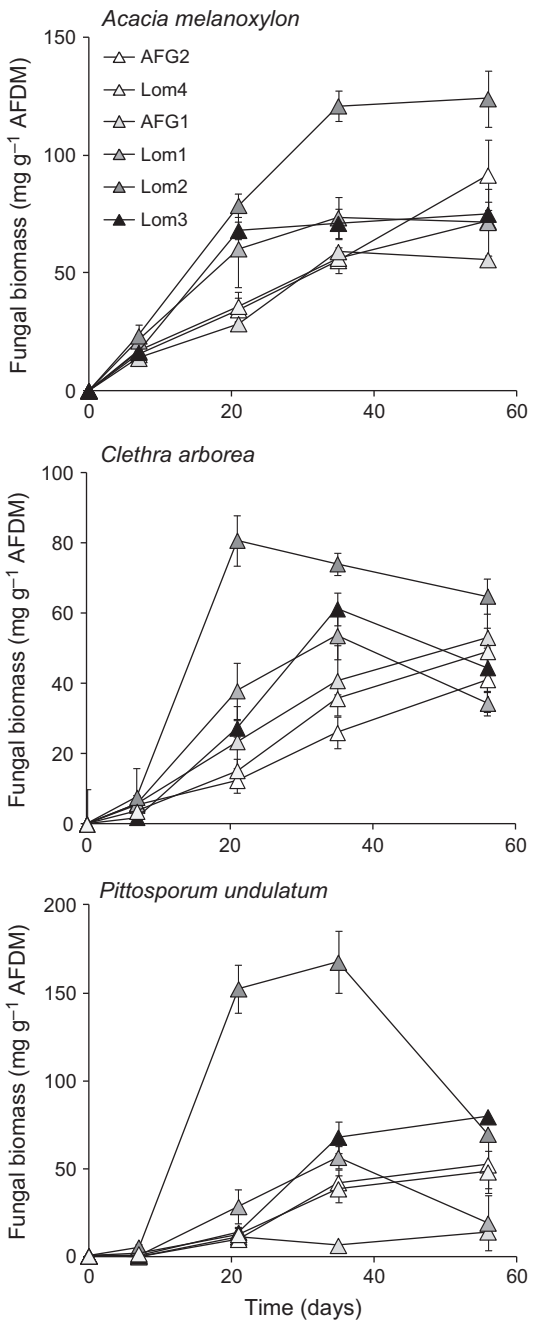

Fig. 2 Fungal biomass associated with three leaf litter species incubated in fine mesh bags in six Azorean streams over 56 days (11/12 June–6/7 August, 2014). Values are means ± 1SE. Streams are ranked by nitrogen concentration (symbols with increased darkness).

latum) it attained a plateau by day 35 (Fig. 3). In particular, sporulation rates in stream Lom2 were very high, with peaks of 2706, 4620 and 15502 conidia mg^{-1} AFDM day^{-1} for *A. melanoxylon*, *C. arborea* and *P. undulatum* respectively (Fig. 3). In streams with lower nutrient concentration (AFG1, Lom4 and AFG2), sporulation rates increased over the incubation period and attained maximum values of 257–890 conidia mg^{-1} AFDM day^{-1} (across species and streams) by day 56 (Fig. 3). Sporulation rates significantly differed among litter species and streams (RM ANOVA, $P < 0.0001$ for both factors), but the significant interaction between both factors ($P < 0.0001$) indicates that differences among litter species depended on environmental conditions and differences among streams depended on litter species (Table S4). Cumulative conidial production by day 56 was 2–17-fold higher in stream Lom2 than on any other stream, and in this stream it was in the order *A. melanoxylon* < *C. arborea* < *P. undulatum* (Fig. 3 insert).

Aquatic hyphomycete species richness generally increased over the incubation period or it attained a plateau between days 21 and 56, with maximum number of species per date of 4–10 (across litter species and streams) (Fig. 3). Species richness per date did not significantly differ among litter species (RM ANOVA, $P = 0.353$), but significantly differed among streams ($P < 0.0001$) (Table S4). However, the significant interaction between litter species and streams ($P = 0.002$) indicates exceptions (Table S4). Total species richness over the incubation period varied between 8–13 for *A. melanoxylon*, 8–15 for *C. arborea* and 9–14 for *P. undulatum*, with a total of 30 species being recorded in the entire experiment (Table S5).

Aquatic hyphomycete communities were dominated by *Lunulospora curvula* (43–93% relative abundance across litter species and streams), except in *A. melanoxylon* and *C. arborea* in stream Lom3 (Fig. 4, Table S5). *Tricladium chaetocladium* and *Tetrachaetum elegans* contributed most to the total conidial production in stream Lom3 (up to 54 and 14% respectively). *Anguillospora pseudolonguissima* was important in streams Lom1 and Lom2 (up to 26%) and *Dimosphospora foliicola* was important in stream Lom3 (up to 13%) and to a lesser extent in stream Lom4. *Triscelophorus monosporus* was notably present in *A. melanoxylon* (up to 13%) (Fig. 4, Table S5). Aquatic hyphomycete community structure significantly differed among litter species (PERMANOVA, $P = 0.001$) and streams ($P = 0.001$) (Table 5).

Litter macroinvertebrates

Total macroinvertebrates. Macroinvertebrate abundance was generally stabilised after day 7 or it attained a peak before the experiment ended, with values generally in the order *A. melanoxylon* < *C. arborea* < *P. undulatum* (Fig. 5). Values for stream Lom3 were particularly high with peaks of 180, 314 and 935 individuals g^{-1} AFDM for *A. melanoxylon*, *C. arborea* and *P. undulatum*

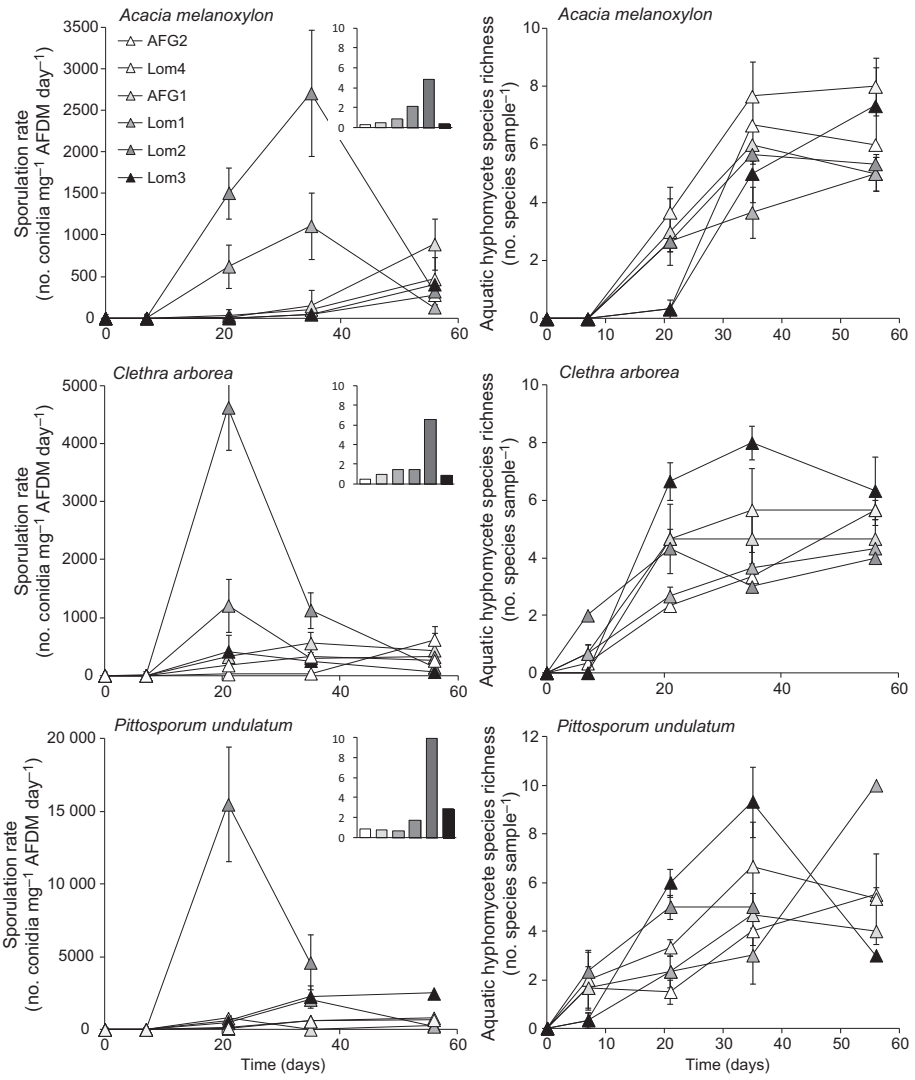

Fig. 3 Aquatic hyphomycete sporulation rates and species richness associated with three leaf litter species incubated in fine mesh bags in six Azorean streams over 56 days (11/12 June – 6/7 August, 2014) or 35 days (12 June–17 July, 2014, for *P. undulatum* incubated in Lom2). Values are means ± 1SE. Insert shows cumulative conidial production over 56 days (11/12 June–6/7 August, 2014) or 35 days (12 June–17 July, 2014, for *P. undulatum* incubated in Lom2) as millions of conidia 5 leaf discs^{-1}. Streams are ranked by nitrogen concentration (symbols with increased darkness).

respectively (Fig. 5). Macroinvertebrate abundance significantly differed among litter species and streams (RM ANOVA, $P < 0.0001$ for both factors), but the significant interaction between both factors ($P = 0.022$) indicates exceptions (Table S6).

Macroinvertebrate taxa richness stabilised after day 7 or it attained a peak before the experiment ended, with maximum number of taxa per date of 4–11 (across litter species and streams) (Fig. 5). Taxa richness per date significantly differed among litter species (RM ANOVA, $P = 0.039$) and streams ($P < 0.0001$), with no significant interaction between both factors ($P = 0.755$) (Table S6). Total taxa richness over the incubation period varied between 9–16 for *A. melanoxylon*, 9–14 for *C. arborea* and 11–17 for *P. undulatum*, with a total of 26 taxa being recorded in the entire experiment (Table S7).

Macroinvertebrate communities were dominated by Orthocladiinae midges (43–89% relative abundance across litter species and streams; Table S7). The caddis flies *Hydroptila* sp. (up to 23%) and *Oxyethira falcata* (up to 25%, mostly in *C. arborea* and *P. undulatum*), the blackfly *Simulium azorense* (up to 18%, mostly in *C. arborea*), and the Tanytarsini (up to 17%, mostly in *C. arborea* and *P. undulatum*) and Tanypodinae midges (up to 13%, mostly in *P. undulatum*) also had important relative abundances (Table S7). Macroinvertebrate community structure did not significantly differ among litter species (PERMANOVA, $P = 0.149$), but it significantly differed among streams ($p = 0.001$) (Table 5).

Functional feeding groups (FFG). Most macroinvertebrates were gatherers/collectors (54–92% relative abundance across litter species and streams), followed by the grazers/scrapers (up to 44%) (Fig. 6, Table S7). Shredders, the FFG directly involved on litter decomposition, were represented by four taxa (the isopod *Jaera insulana*, the caddisfly *Limnephilus atlanticus* and the crane flies *Dicranomyia* sp. and Tipulidae) and only accounted for up to 5% of

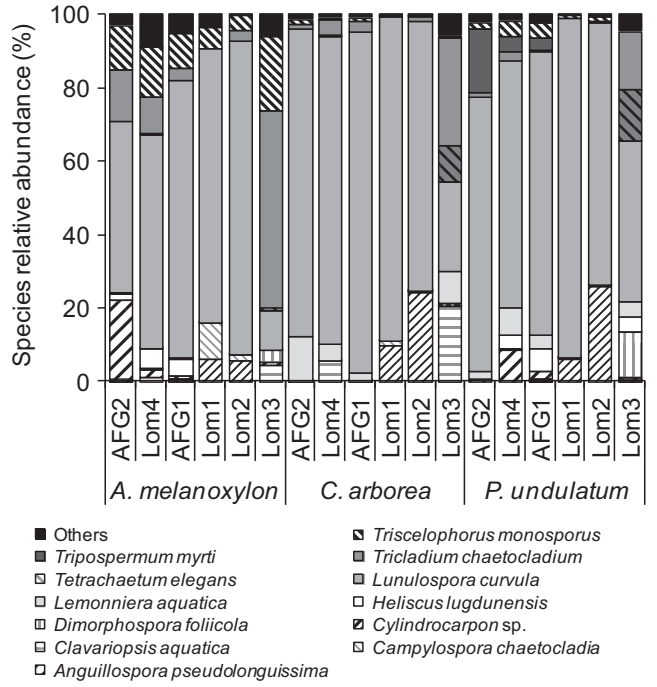

Fig. 4 Relative abundance (based on conidial production) of aquatic hyphomycete species associated with three leaf litter species incubated in fine mesh bags in six Azorean streams over 56 days (11/12 June–6/7 August, 2014) or 35 days (12 June–17 July, 2014, for *P. undulatum* incubated in Lom2). Streams are ranked by nitrogen concentration.

individuals (Fig. 6, Table S7). Shredder abundance did not significantly differ among litter species (RM ANOVA, $P = 0.547$), but did significantly differ among streams ($P < 0.0001$), with no significant interaction between both factors ($P = 0.126$) (Table S6). Macroinvertebrate FFG structure did not significantly differ among litter species (PERMANOVA, $P = 0.408$), but it significantly differed among streams ($P = 0.001$) (Table 5).

Correlation among variables

Strong and significant correlations were found among litter decomposition rates and fungal biomass and conidial production for the three litter species (Table 6). Macroinvertebrate abundance (average across sampling dates) did not significantly correlate with any biological variable (Pearson correlation, $P = 0.070$ for *C. arborea* litter decomposition and $P > 0.453$ for all other cases).

Discussion

In this study we show that leaf litter decomposition in North Atlantic oceanic island streams not strongly affected by human activities is driven by microbes and depends on litter quality and environmental conditions.

Table 5 Summary table for two-way PERMANOVA and PERMDISP tests performed on total aquatic hyphomycete abundance (based on conidial production), total macroinvertebrate abundance and abundance of macroinvertebrate functional feeding [standardised and log(x + 1) transformed] associated with three leaf litter species incubated in six Azorean streams over 56 days.

	PERMANOVA				PERMDISP	
Source of variation	d.f.	MS	Pseudo-F	*P*	*F*	*P*
Aquatic hyphomycete communities						
Litter species	2	10348	9.950	0.001	2.033	0.252
Stream	5	14933	14.359	0.001		
Litter species × Stream	10	1465	1.408	0.036		
Residual	150	1040				
Total	167					
Macroinvertebrate communities						
Litter species	2	2319	1.357	0.149	2.222	0.059
Stream	5	27365	16.012	0.001		
Litter species × Stream	10	1444	0.845	0.795		
Residual	193	1709				
Total	210					
Functional feeding groups						
Litter species	2	244	1.070	0.408	1.495	0.188
Stream	5	9283	40.783	0.001		
Litter species × Stream	10	252	1.108	0.347		
Residual	193	228				
Total	210					

In North Atlantic oceanic islands, litter decomposition is driven by microbes

No significant differences were observed in litter decomposition rates between fine mesh and coarse mesh bags, independently of litter species and stream, suggesting that macroinvertebrates have a negligible role in litter decomposition in Azorean streams. The low contribution of stream macroinvertebrates to litter decomposition in oceanic islands has been reported before, and is attributed to the low abundance and richness of shredders in these isolated systems (Larned, 2000; Benstead *et al.*, 2009; MacKenzie *et al.*, 2013; Raposeiro *et al.*, 2014). Age, area and distance to the mainland are key factors ruling the colonisation of oceanic islands by organisms (Raposeiro, Hughes & Costa, 2009, 2013), and explain why remote islands have low diversity and abundance of shredders (Benstead *et al.*, 2009; MacKenzie *et al.*, 2013; Raposeiro *et al.*, 2013). In Azorean streams, in particular, only a few shredder taxa have been identified: the iso-

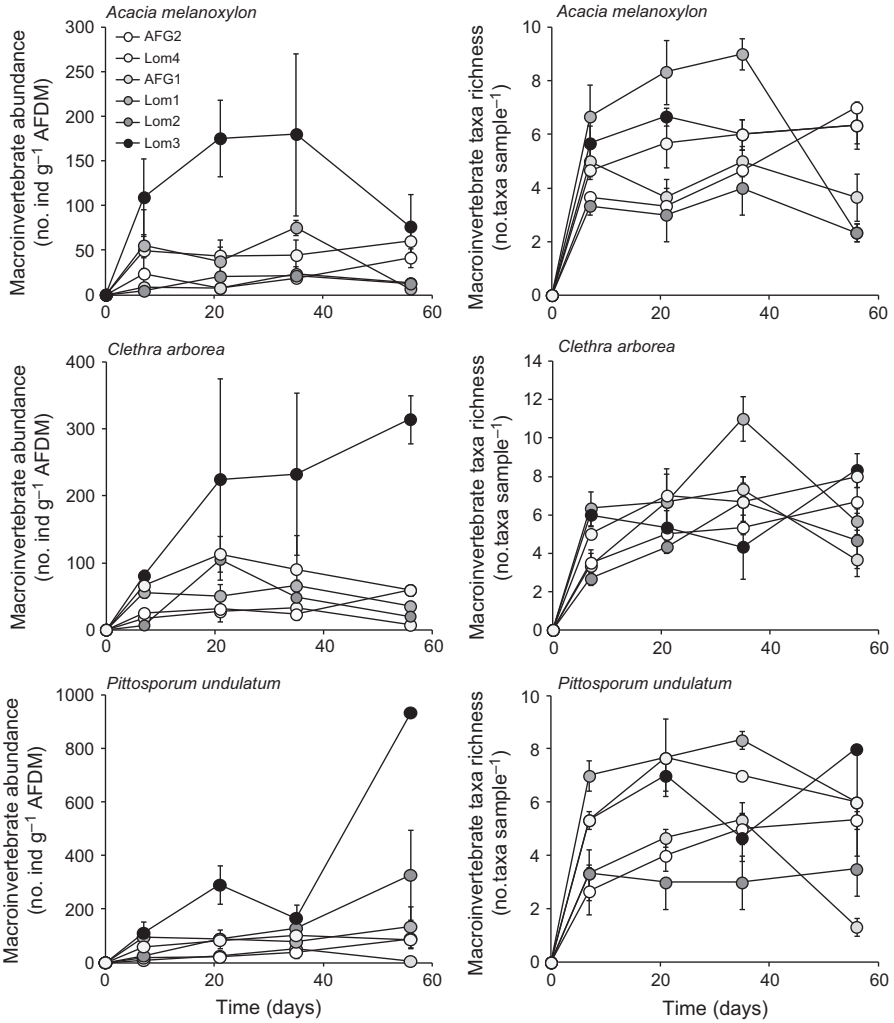

Fig. 5 Total macroinvertebrate abundance and taxa richness associated with three leaf litter species incubated in coarse mesh bags in six Azorean streams over 56 days (11/12 June–6/7 August, 2014). Values are means ± 1SE. Streams are ranked by nitrogen concentration (symbols with increased darkness).

pod *Jaera insulana*, the caddis fly *Limnephilus atlanticus* and the crane flies *Dicranomyia* sp., *Tipula macaronesica* and *T. oleracea*, but they accounted for less than 5% of total macroinvertebrates colonising the decomposing litter in this study. The, sometimes high, colonisation of leaf litter by other macroinvertebrates did not compensate for this lack of shredders on litter decomposition. Macroinvertebrate communities were dominated by gatherer/collectors who could have been taking advantage from the fine particulate organic matter released from the litter as a result from microbial activities, and grazer/scrapers who could have been feeding on the biofilm on the leaves surface.

Leaf litter decomposition in Azorean streams is thus driven by microbes, especially aquatic hyphomycetes as shown by the efficient colonisation of litter by these fungi and strong correlations between leaf litter decomposition rates and fungal biomass accumulation and conidial production (Gessner & Chauvet, 1994). The high species richness of aquatic hyphomycetes in Azorean streams (30 species recorded in this first survey) and efficient colonisation of decomposing substrates was surprising, and contrasted with what was observed for shredders. In fact, decomposing leaves were colonised by aquatic hyphomycetes at levels similar to those found in continental streams; maybe only species richness per treatment was slightly lower than observed for continental streams not strongly affected by human activities (Gulis & Suberkropp, 2003; Ferreira, Gulis & Graça, 2006; Gulis *et al.*, 2006), but comparisons may be confounded by differences in litter species used (see below). The sporulation rate by aquatic hyphomycetes associated with *P. undulatum* in stream Lom2 (15502 conidia mg^{-1} AFDM day^{-1}) was even among the highest sporulation rates reported in the literature (Gulis & Suberkropp, 2003).

All aquatic hyphomycete species found in this study are also frequently found in continental streams (Gulis &

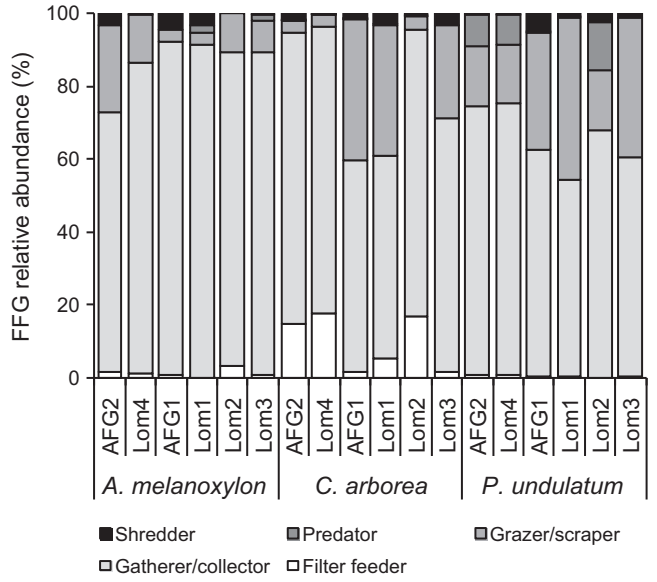

Fig. 6 Relative abundance of macroinvertebrate functional feeding groups (FFG) associated with three leaf litter species incubated in coarse mesh bags in six Azorean streams over 56 days (11/12 June–6/7 August, 2014). Streams are ranked by nitrogen concentration.

Table 6 Correlations (Pearson *r*) between exponential decomposition rates, fungal biomass (average across sampling dates) and cumulative conidial production. *P* values are also shown.

	Decomposition rates		Fungal biomass	
Variables	*r*	*P*	*r*	*P*
Acacia melanoxylon				
Fungal biomass	0.81	0.052		
Cumulative conidial production	0.92	0.010	0.89	0.017
Clethra arborea				
Fungal biomass	0.59	0.214		
Cumulative conidial production	0.39	0.447	0.96	0.003
Clethra arborea without Lom3				
Fungal biomass	0.99	0.001		
Cumulative conidial production	0.99	0.001	0.98	0.002
Pittosporum undulatum				
Fungal biomass	0.92	0.009		
Cumulative conidial production	0.97	0.001	0.98	<0.001

Suberkropp, 2003; Pascoal, Cássio & Marvanová, 2005; Ferreira *et al.*, 2006). Differences in relative abundances are likely due to differences in litter characteristics and environmental conditions between studies (see below). For instance, *L. curvula* generally dominated aquatic hyphomycete communities in this study, but this is a known 'warm-water' species (Ferreira *et al.*, 2014), and the litter decomposition experiment was performed in Spring/Summer. The high number of aquatic hyphomycete species in Azorean streams may suggest that the biogeographical filters that restrict shredders colonisation of these streams do not apply to smaller organisms. In fact, a recent review has shown that aquatic hyphomycetes are quite ubiquitous worldwide (Duarte *et al.*, 2016).

Despite being driven by microbes, the litter decomposition rates in Azorean streams were within the range reported for continental streams (Webster & Benfield, 1986; Abelho, 2001), and other island streams (Larned, 2000; Benstead *et al.*, 2009; MacKenzie *et al.*, 2013; Raposeiro *et al.*, 2014), and ranged from medium ($0.010 > k > 0.005$, day^{-1}) to fast ($k > 0.010$, day^{-1}) (Petersen & Cummins, 1974). This suggests that, even in the absence of shredders, litter inputs to Azorean streams are effectively processed and nutrients are cycled at high rates.

In North Atlantic oceanic islands, litter decomposition depends on litter characteristics

Leaf litter decomposition rates were in the order *A. melanoxylon* < *C. arborea* < *P. undulatum*, inversely proportional to initial lignin concentration. Lignin has been shown before to be a strong inhibitor of litter decomposition (Lecerf & Chauvet, 2008; Schindler & Gessner, 2009; Frainer *et al.*, 2015). *A. melanoxylon* also had the highest initial toughness and C concentration and the lowest SLA, which contribute to describe this litter as recalcitrant, despite its high N concentration. It has been also shown before that toughness may be more important than N concentration in determining litter decomposition rates (Li *et al.*, 2009). *P. undulatum* had intermediate values for all litter characteristics evaluated, except that it had the lowest lignin concentration, which compared with *C. arborea* may have rendered it more labile.

The effects of differences in litter characteristics on litter decomposition rates were mediated through its effects on biological activities. Similarly to what was observed for litter decomposition rates, conidial production was generally in the order *A. melanoxylon* < *C. arborea* < *P. undulatum*. Conidial production has been shown to lead to important losses of initial litter mass (Ferreira *et al.*, 2006, 2012; Baldy *et al.*, 2007; Cornut *et al.*, 2010). Contrary to expected, however, *A. melanoxylon* generally had the highest fungal biomass accumulation. This is probably due to *A. melanoxylon* higher structural stability as a substrate for fungal mycelia;

after 56 days of incubation, there were still 72–54% initial mass remaining for *A. melanoxylon*, compared with 67–33% for *C. arborea* and 44–2% for *P. undulatum*.

Litter species maintained the same relative order regarding decomposition rates in all streams suggesting that the same litter characteristic (i.e. initial lignin concentration) controlled decomposition rates across streams. Conversely, the magnitude of the difference in decomposition rates between litter species varied across streams suggesting that changes in environmental conditions may reduce or increase differences in decomposition rates between litter species (Irons *et al.*, 1994; Gulis & Suberkropp, 2003; Ferreira *et al.*, 2006; Fernandes *et al.*, 2012; see below).

In North Atlantic oceanic islands, litter decomposition responds to changes in environmental conditions

Moderate increases in dissolved nutrient concentrations generally stimulate microbial activities and decomposition of submerged litter (reviewed by Ferreira *et al.*, 2014, 2015a). In this field study, the increase in nutrients concentrations (28–588 μg NO_3 L^{-1} and 53–175 μg PO_4 L^{-1}; between streams AFG2 and Lom2) was accompanied by an increase in microbial activities and litter decomposition, as anticipated (Suberkropp & Chauvet, 1995; Gulis *et al.*, 2006; Fernandes *et al.*, 2014). The stimulation of litter decomposition between streams AFG2 and Lom2 was generally stronger for *P. undulatum* (k_{Lom2}/k_{AFG2} = 3.0) than for the other two litter species (k_{Lom2}/k_{AFG2} = 1.6 for *A. melanoxylon* and 1.7 for *C. arborea*). This agrees with previous studies that have shown a stronger response of nutrient poor than of nutrient-rich litter to increases in nutrient availability due to nutrient limitation of microbial activities in the former substrates (Gulis & Suberkropp, 2003; Ferreira *et al.*, 2006; Gulis *et al.*, 2006). Stream Lom3 had the highest nutrient concentration (1033 μg NO_3 L^{-1} and 164 μg PO_4 L^{-1}), but not the highest microbial activities and litter decomposition, which suggests that other factors could be limiting the microbial activity in this stream.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Figure S1. Principal component analysis (PCA) of leaf characteristics of nine tree and shrub species commonly found in the riparian vegetation of Azorean streams.

Table S1. Physical and chemical characteristics of leaves of nine tree and shrub species commonly found in the riparian vegetation of Azorean streams.

Table S2. Summary table for separate-slopes model performed on proportion of litter mass remaining of three leaf litter species incubated in coarse and fine mesh bags in six Azorean streams over 56 days.

Table S3. Summary table for separate-slopes model performed on proportion of litter mass remaining (ln-transformed) of three leaf litter species incubated in six Azorean streams over 56 days.

Table S4. Summary table for RM ANOVAs performed on fungal biomass, aquatic hyphomycete sporulation rate and aquatic hyphomycete species richness associated with three leaf litter species incubated in fine mesh bags in six Azorean streams over 56 days.

Table S5. Relative abundance of aquatic hyphomycete species (based on conidial production) associated with

three leaf litter species incubated in fine mesh bags in six Azorean streams over 35–56 days. Total species richness is also shown.

Table S6. Summary table for RM ANOVAs performed on total macroinvertebrate abundance, total macroinvertebrate taxa richness and shredder abundance associated with three leaf litter species incubated in coarse mesh bags in six Azorean streams over 56 days.

Table S7. Relative abundance of macroinvertebrate taxa associated with three leaf litter species incubated in coarse mesh bags in six Azorean streams over 56 days. Functional feeding group (FFG) for each taxon and total taxa richness are also shown.

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