Replacement of native forests by conifer plantations affects fungal decomposer community structure but not litter decomposition in Atlantic island streams

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ABSTRACT

Forest change occurs worldwide, especially on islands where space is a limiting factor for human activities. The replacement of diverse native forests by tree monocultures and subsequent changes in litter input characteristics can have strong effects on stream communities and processes. Aquatic decomposers and litter decomposition can be particularly sensitive to forest change due to their dependence on terrestrial litter supply and on litter characteristics. However, studies addressing the effects of forest changes, and conifer plantations in particular, on stream litter decomposition and associated decomposers are scarce. Here, we assessed the effects of the replacement of native laurel forests in São Miguel island, Azores archipelago, by commercial conifer plantations (*Cryptomeria japonica* (L. f.) D. Don) on litter decomposition and associated fungal decomposers. Leaves of the native broadleaf tree *Ilex perado* Aiton and conifer needles were enclosed in mesh bags and incubated in three streams flowing through conifer plantations and three streams flowing through native laurel forests in winter 2015 for determination of litter decomposition rates, and aquatic hyphomycete community structure and reproductive activity (one stream per type). Aquatic hyphomycete communities' structure strongly differed between native and conifer streams as a result from differences in the total number of species recorded (higher in the native stream), species identity (only 10 species, out of 26, were common to both streams) and species evenness (higher in the native stream). These differences in communities between streams likely result from changes in litter inputs characteristics between native and conifer streams and from aquatic hyphomycetes having substrate preferences. Differences in aquatic hyphomycete communities were not accompanied by differences in litter decomposition rates between native and conifer streams, which likely results from functional redundancy between decomposer communities and suggests that community structure and function may not always be tightly coupled. Litter decomposition rates did not differ between native and conifer species because *I. perado* leaves were tough and had a tick waxy cuticle, which may have limited microbial development, as for the conifer needles. Thus, the replacement of native laurel forests by conifer plantations did not affect litter decomposition in Azorean streams likely because litter is recalcitrant and decomposition is driven by microbes. However, changes in aquatic hyphomycete communities arriving from forest change may have unpredictable effects in case of additional environmental change. Hence, to prevent unwanted effects of changes in microbial communities, a riparian buffer of native vegetation should be maintained in conifer plantations.

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1. Introduction

Forest change constitutes a major environmental transformation worldwide (FAO, 2015). This is especially true for islands, where human pressure is high due to space and resources limitations (Keppel et al., 2014; Calado et al., 2016). The replacement of diverse native forests by tree monocultures is generally accompanied by decreases in litter diversity, changes in litter quantity and chemical composition (the direction and magnitude depending on species identity) and changes in the timing of litter fall (Graça et al., 2002; Inoue et al., 2012; Martínez et al., 2013). These changes can have strong impacts on stream ecosystems due to their large terrestrial-aquatic interface and strong dependence on

terrestrial organic matter (Graça et al., 2002; Hladyz et al., 2011; Ferreira et al., 2016b).

Aquatic communities in forest streams derive most of their energy and carbon from the litter provided by the riparian vegetation (Wallace et al., 1997). Litter decomposition is thus a fundamental ecosystem process in forest streams, where it fuels aquatic communities and contributes to nutrient cycling (Wallace et al., 1997). The incorporation of litter carbon and nutrients into secondary production is driven by microbial decomposers, primarily aquatic hyphomycetes (Hieber and Gessner, 2002; Gulis and Suberkropp, 2003; Pascoal and Cássio, 2004; Cornut et al., 2010), especially on island streams, where macroinvertebrate detritivores can be rare (Benstead et al., 2009; Ferreira et al., 2016c). Since aquatic hyphomycetes have different substrate preferences as a result from different nutrient requirements and enzymatic capabilities (Arsuffi and Suberkropp, 1984, 1988; Gulis, 2001), changes in forest composition may severely affect aquatic communities' composition (Bärlocher and Graça, 2002; Ferreira et al., 2006a). However, this is not always the case with some studies finding no major differences in aquatic hyphomycete communities between streams flowing through native forests and similar streams flowing through tree plantations (Kominoski et al., 2011; Martínez et al., 2013). Additionally, community structure is not always strongly coupled with ecosystem functioning (Bärlocher and Graça, 2002; Ferreira et al., 2006a; Mckie and Malmqvist, 2009; Riipinen et al., 2009) and thus it is difficult to anticipate the effects of forest change on litter decomposition.

The effects of the replacement of native broadleaf forests by conifer plantations, in particular, on litter decomposition and associated decomposers have been rarely addressed and results are conflicting, suggesting that effects may depend on different factors such as stream characteristics, litter identity, type of conifer plantation, type of native forest and type of decomposer community involved (i.e. microbes only or microbes + macroinvertebrates) (reviewed by Ferreira et al. (2016b), Chauvet et al. (2016)).

Here, we assessed the effects of the replacement of native forests in São Miguel island, Azores archipelago, by commercial conifer plantations (*Cryptomeria japonica* (L. f.) D. Don) on native (*Ilex perado* Aiton) and conifer (*C. japonica*) litter decomposition, as a surrogate for stream functioning, and associated fungal decomposer reproductive activity and community structure. We tested the null hypothesis that forest change would not affect microbial decomposer communities' structure and litter decomposition.

2. Materials and methods

2.1. Study region

This study was done in São Miguel island, the largest (760 km^2) of the nine islands that make the Azores archipelago. This archipelago is located in the North Atlantic Ocean, in the Middle Atlantic Ridge where the North American, Eurasian and African lithospheric plates join, about 1500 km off Portugal mainland. Mean annual temperatures in the Azores range between 14 and 18 °C and mean annual precipitation between 740 and 2400 mm, depending on elevation, with most of the precipitation (70–75%) falling between October and March (Marques et al., 2008; Hernández et al., 2016).

Since the Portuguese settlement in the 15th century, human activities changed completely the island's landscape (Constância, 1963; Moreira, 1987). Due to unregulated and uncontrolled exploitation of the forest resources for wood and charcoal, and clearance for agriculture and urbanization, the original dense forests were almost completely destroyed by the beginning of the 19th century (Constância, 1963). In 1951, the forest area in São

Miguel island was <6% (Dias et al., 2007a). Planting measures were undertaken to counterbalance this situation and *C. japonica*, a conifer that originates from Japan and south China and was introduced into the Azores as an ornamental tree in the mid-19th century (Albergaria, 2000), progressively became an important species once its commercial interest and its ability to adapt and resist to strong winds (a limiting factor in the Azores) were recognized (Dias et al., 2007a).

Nowadays, forests cover about 22% of the land area in the Azores (SRAM/DROTRH, 2007) with *C. japonica* plantations representing 26% of the forest area and 60% of forest plantation area, especially at elevations >400 m a.s.l. (DRRF, 2014). Native forests cover less than 10% of the total area, mostly at elevations >800 m a.s.l. (Borges et al., 2010; DRRF, 2014). Laurel forests develop in areas of high humidity and exposed to humid winds that promote an arboreal stratum composed by ombrophilous species as *I. perado*, *Laurus azorica* (Seub.) Franco, *Frangula azorica* V. Grubov and *Vaccinium cylindraceum* Sm. (Dias et al., 2007b).

2.2. Streams

The six study streams were located in the volcanic complexes of Nordeste and Fogo, in the northeast and central area of São Miguel island, respectively (Table 1). Streams were perennial, small (1st–3rd order, <1 m wide and <20 cm deep) and with the substrate composed mainly by sand, gravel and cobbles. Three streams were surrounded by native vegetation ('native streams', NAT1–NAT3) and three streams flowed through mature (45–50 years old) conifer (*C. japonica*) plantations ('conifer streams', CON1–CON3) (Table 1). Streams were not affected by any visible human activity, besides commercial forestry in the conifer streams, and thus differences in water chemistry can be attributed to forest change.

2.3. Water variables

Water temperature was recorded hourly during the litter decomposition experiment (7/8 January – 3/4 March 2015) using submerged data loggers (Hobo Pendant UA-001-08, Onset Computer Corp., MA, USA); hourly values were averaged to produce daily means (n = 55). Electrical conductivity and pH were recorded at the beginning and on each sampling date with a multiparametric field probe (CyberScan 600, Eutech Instruments, Nijkerk, the Netherlands) (n = 5). On the same occasions (n = 5), 1 L of stream water was collected in acid washed plastic bottles, transported to the laboratory cold, filtered through glass microfiber filters (47 mm diameter, 1.2 μm pore size; Whatman GF/C, GE Healthcare Europe GmbH, Little Chalfont, UK) and analyzed for phosphate, nitrate and ammonium according to Skalar methods M503-555R (Standard Method 450-P I), M461-318 (EPA 353.2) and M155-008R (EPA 350.1), respectively (Skalar, 2004).

2.4. Litter species and initial characteristics

Two litter species were selected for this study: the native broadleaf species *I. perado*, which is abundant in areas of native vegetation and is present in the surroundings of the three native streams, and the conifer species *C. japonica*, as the species used in conifer plantations. *I. perado* is an evergreen species, which precluded the collection of enough naturally abscised leaves in the short period necessary to avoid differences in leaf characteristics. Thus, *I. perado* leaves were directly collected from trees in October 2014. To reduce the variability in leaf characteristics, mature leaves of similar size and with no signs of damage or herbivory were collected from trees grown in close proximity and with similar characteristics (e.g. similar size and vitality). Although the conifer species is also evergreen, enough freshly fallen twigs were

Table 1
Location, size and vegetation characteristics of the six Azorean streams used in the litter decomposition experiment.

Streams	Acronym	Latitude (N)	Longitude (W)	Elevation (m a.s.l.)	Order	Distance from source (m)	Basin area (km²)	Area covered (%)[a]	
								Native forests	Conifer plantations
Native streams									
Ribeira da Achada	NAT1	37°48′3.686″	25°14′29.587″	925	1	63	0.02	85.7/100	0.0/0.0
Ribeira Grande	NAT2	37°46′32.135″	25°27′41.414″	628	3	867	0.21	100/100	0.0/0.0
Unnamed stream	NAT3	37°48′5.850″	25°14′33.331″	920	1	155	0.02	51.8/100	0.0/0.0
Conifer streams									
Ribeira do Folhado	CON1	37°48′52.747″	25°14′36.290″	729	1	983	0.26	0.0/0.0	51.7/100
Ribeira da Mulher	CON2	37°48′50.695″	25°14′23.834″	736	1	1244	0.41	0.0/0.0	61.7/100
Unnamed stream	CON3	37°48′51.250″	25°14′16.062″	738	1	464	0.11	0.0/0.0	66.9/100

[a] The first value indicates the area covered (%) in the basin and the second value indicates the area covered (%) in the riparian area of the experimental site, which was defined as a strip 50 m long and 10 m wide on each stream bank.

picked from the plantation floor and the tip (∼10 cm), corresponding to the last growth period, was cut. Leaves and conifer shoots were air dried at room temperature in the dark and stored dry until needed. The use of air-dried green leaves is ecologically relevant since the strong winds typical in this region can lead to premature leaf fall, and litter can later enter the streams by lateral transport.

Before the experiment started, three batches of each litter species were grinded to <5 mm powder, oven dried (105 °C, 48 h) and analyzed for initial carbon, nitrogen (auto analyzer, IRMS Thermo Delta V advantage with a Flash EA, 1112 series; Thermo Fisher Scientific Inc., Waltham, MA, USA), phosphorus (spectrophotometrically after basic digestion with sodium persulphate and sodium hydroxide; APHA, 1995), lignin (Goering and van Soest method; Goering and van Soest, 1970) and polyphenols (Folin-Ciocalteu's method; Graça et al., 2005) concentration following standard protocols. Results were expressed as percentage of dry mass (% DM).

2.5. Litter bags and litter decomposition

Batches of 2.45–2.55 g of air dried litter were sprayed with distilled water to render them soft and less susceptible to breakage, and enclosed individually into fine mesh bags (10 × 12 cm, 0.5 mm mesh pore). Fine mesh bags were used as we were interested in assessing microbial-driven litter decomposition; previous studies have shown that the contribution of macroinvertebrates to litter decomposition is negligible in Azorean streams (Raposeiro et al., 2014; Ferreira et al., 2016c). Also, fine mesh bags prevent litter mass loss due to physical abrasion from increases in current velocity and in suspended sediments that follow heavy rains.

On the 7th and 8th January 2015, 12 litter bags of each species were incubated in each of the six streams (12 bags × 2 litter species × 6 streams = 144 bags in total). Litter bags were tied with lines to iron nails anchored to the streambed or secured to submerged rocks. Extra 20 litter bags (10 bags per species) were submerged at one stream for ∼10 min and returned to the laboratory for determination of an initial air dry mass to initial ash-free dry mass (AFDM) conversion factor taking into account mass loss due to handling. The litter was rinsed with tap water, placed into pre-weighed aluminium pans, oven dried (60 °C, >48 h) and weighed (±0.1 mg) to determine initial dry mass (DM). Dry samples were ignited (500 °C, 4 h) and the ashes were weighed (±0.1 mg). The initial AFDM was determined as the difference between DM and ash mass. The initial air dry mass to initial AFDM conversion factor, estimated as the ratio between initial AFDM and initial air dry mass, was 0.86 for *I. perado* and 0.84 for *C. japonica*.

After 6, 13, 34 and 55 days, three litter bags of each species were retrieved from each stream, placed individually into plastic zip lock bags and returned to the laboratory in an ice box. Once in the laboratory, the litter was rinsed on top of a sieve (0.5 mm mesh pore) to allow the removal of fine sediments while retaining small litter fragments. Litter samples from streams NAT1, NAT2,

CON1 and CON2 were processed for the determination of mass remaining. The litter was rinsed with tap water, placed into pre-weighed aluminium pans and processed as described above for the conversion factor. AFDM remaining was determined by the difference between DM and ash mass and results were expressed as percentage of initial AFDM.

Litter samples from streams NAT3 and CON3, as representatives of each type of stream, were processed for the determination of mass remaining and spore production by aquatic hyphomycetes. Five leaves were selected from each *I. perado* sample and five conifer shoots from each *C. japonica* sample, gently rinsed with distilled water and five leaf discs (12 mm diameter, one from each leaf) or five shoot tips (needles henceforth) were cut, respectively, and used to promote sporulation by aquatic hyphomycetes (see below). The remaining litter in the samples was rinsed with tap water, placed into pre-weighed aluminium pans and processed as described above for the conversion factor. AFDM remaining was determined by the difference between DM and ash mass, taking into account the leaf discs or needles taken to induce spore production (see below), and results were expressed as percentage of initial AFDM.

2.6. Spore production by aquatic hyphomycetes

Spore production by aquatic hyphomycetes was induced in the laboratory. This was the microbial variable selected for this study since it provides information on both reproductive activity (function) and community composition (structure) of aquatic hyphomycetes, which are key microbial decomposers in forest streams, also on islands (Ferreira et al., 2016c). Additionally, reproductive activity is expected to be most sensitive to changes in environmental conditions compared with growth or metabolism variables (Gulis and Suberkropp, 2003; Ferreira et al., 2006b; Lecerf and Chauvet, 2008).

Leaf discs and needles were incubated in 100 mL Erlenmeyer flasks with 25 mL of filtered stream water (glass microfiber filters, 47 mm diameter, 1.2 μm pore size; Whatman GF/C, GE Healthcare Europe GmbH, Little Chalfont, UK), displayed on an orbital shaker (75 rpm) for 48 h at 10 °C, with 16 h dark: 8 h light regime. The spore suspension was transferred into 50 mL graduated Falcon tubes, fixed with 2 mL of 37% formalin and the final volume adjusted to 35 mL with distilled water. Tubes were stored in the dark until used for slides preparation. Leaf discs and needles were placed in pre-weighed aluminium cups, oven dried (60 °C, >24 h) and weighed (±0.1 mg) to determine DM. Dry samples were ignited (500 °C, 4 h) and ashes were weighed (±0.1 mg). Leaf discs and needles AFDM was determined by the difference between DM and ash mass, and added to the bulk AFDM above.

Each tube was gently shaken and the spore suspension was transferred into a beaker. An aliquot (100 μL) of Triton X-100 (0.5%) was added and the suspension was gently stirred with a

magnetic bar to ensure a uniform distribution of spores. Aliquots of the suspension were filtered through cellulose nitrate filters (Sartorius Stedim, 5 µm pore size; Sartorius Stedim Biotech GmbH, Goettingen, Germany) and the filters were stained with cotton blue in 60% lactic acid (0.05%) and mounted on a slide. Spores were identified and counted with a compound microscope (Leica DM2500, Leica Microsystems CMS GmbH, Wetzlar, Germany) at 200× magnification. Sporulation rates were expressed as number of conidia released/mg litter AFDM/day and aquatic hyphomycete species richness as number of species/sample.

2.7. Statistical analysis

Elevation and water characteristics were compared between stream types (i.e., native and conifer) by one-way analysis of variance (ANOVA). Initial litter chemical characteristics were compared between litter species by one-way ANOVA.

Litter decomposition rates on a per day basis (k, d^{-1}) were estimated assuming a negative exponential decay model, as the slope of the linear regression between the fraction of AFDM remaining (ln(x)-transformed) and time (days), with the intersect fixed at ln(1) = 0. Since streams differed in water temperature, litter decomposition rates were also estimated on a degree-day basis (k, dd^{-1}) by replacing time in the model above by the cumulative daily temperature average (°C) by the sampling date. Litter decomposition rates were compared between stream types and litter species by two-way ANOVA.

Sporulation rates and aquatic hyphomycete species richness (both log(x + 1)-transformed) were compared among streams, litter species and time by three-way ANOVAs. Aquatic hyphomycete communities were compared among streams and litter species by analysis of similarity (ANOSIM), based on a Bray-Curtis similarity matrix of spore production (log(x + 1)-transformed).

Tukey's honest significant difference (HSD) test was used as the multiple comparison post-hoc test when significant differences were identified in the ANOVAs. Normality (Shapiro-Wilk test) and homoscedasticity (Bartlet test) were checked and data were transformed when needed to attain normal distribution and homogeneity of variances. Univariate analyses were performed on Statistica 7 (StatSoft Inc., Tulsa, Oklahoma, USA) and Minitab 17 (Minitab Inc., State College, Pennsylvania, USA), and community analyses were performed on Primer 6 (Primer-E Ltd, Plymouth, UK).

3. Results

3.1. Streams

During the litter decomposition experiment, streams were cool, slightly acidic, had low conductivity, low to moderate nitrate and ammonium concentrations and moderate to high phosphate concentrations (Table 2). Native and conifer streams did not significantly differ in any of the measured characteristics (Table 2).

3.2. Litter initial characteristics

I. perado had lower polyphenols, lignin and carbon concentrations and higher nitrogen and phosphorus concentrations when compared with C. japonica (Table 3). Differences between litter species were significant in all cases (Table 3).

3.3. Litter decomposition

Litter decomposed slowly and after 55 days incubation there were still 82–91% of initial mass remaining across litter species and streams. This translated into decomposition rates (d^{-1}) of 0.0025–0.0039 for I. perado and 0.0018–0.0041 for C. japonica across streams. Taking into account differences in temperature among streams when estimating decomposition rates (dd^{-1}) led to values between 0.0002–0.0004 for I. perado and 0002–0.0003 for C. japonica across streams. No significant differences in decomposition rates (either expressed per day or per degree-day) were observed between streams types or litter species (Fig. 1).

3.4. Spore production by aquatic hyphomycetes

Sporulation rates by aquatic hyphomycetes were low (⩽163 conidia/mg AFDM/day) and did not significantly differ between stream types or litter species (three-way ANOVA, p = 0.540 and p = 0.349, respectively; Fig. 2a). Sporulation rates dynamics tended to differ between native and conifer streams with rates increasing after day 34 for litter incubated in the conifer stream, while remaining low or peaking on day 34 for litter incubated in the native stream (Fig. 2a), although no stream × time interaction was observed (three-way ANOVA, p = 0.068).

Aquatic hyphomycetes species richness attained an average maximum of six species per sample and did not significantly differ between stream types or litter species (three-way ANOVA, p = 0.882 and p = 0.161, respectively; Fig. 2b). Species richness per sample increased over time, except for I. perado litter in the native stream where it peaked by day 35 (Fig. 2b). No stream × time interaction was, however, observed (three-way ANOVA, p = 0.389).

Although the average aquatic hyphomycete species richness per sample was low (⩽6 species; Fig. 2b), a total of 26 species (20 in the native stream and 16 in the conifer stream) was recorded (Table 4) as a result of high variation in the number and identity of species among replicates within each sampling date and high increase in the number of new colonizing species between days 13 and 34 and to a lesser extent between days 34 and 55 (Fig. 2c). Out of the 26 aquatic hyphomycete species recorded, only 10 species were shared by both streams; 10 species were exclusive of the native stream and 6 species of the conifer stream (Table 4). Considering the four treatments, total species richness was in the order: I. perado in the native stream (18 species) > both litter species in the conifer stream (16 species) > C. japonica in the native stream (7 species) (Table 4). Aquatic hyphomycete communities in the conifer stream where clearly dominated by Heliscus lugdunensis, while communities in the native stream were more even, especially in I. perado litter (Table 4). Thus, aquatic hyphomycetes communities' structure significantly differed between streams (ANOSIM, Global R = 0.467, p = 0.001) but not between litter species (Global R = 0.011, p = 0.353).

4. Discussion

Conifer plantations cover a large area worldwide (∼60 million ha already in 2000; FAO, 2001), but information on their effects on stream ecosystem functioning is still limited (Ferreira et al., 2016b; Chauvet et al., 2016). Here we addressed the effects of C. japonica plantations on litter decomposition and associated decomposers in island streams. Conifer plantations affected decomposer communities' structure. However, changes in decomposer communities did not translate into altered litter decomposition rates in conifer streams.

4.1. Decomposers community structure differed between conifer and native streams

Changes in forest composition generally translate into changes in litter inputs to streams, either in specific composition, quantity,

Table 2

Water physical and chemical characteristics (mean ± SE) of native and conifer streams (three streams per type) during the litter decomposition experiment (7th January – 4th March 2015). Average values (mean ± SE) per stream type are also given. Comparisons between stream types were made by one-way ANOVA.

Streams	Temperature (°C)	Conductivity (µS/cm)	pH	PO_4^{3-} (µg/L)	NH_4^+ (µg/L)	NO_3^- (µg/L)
Native streams						
NAT1	9.0 ± 0.1	32.5 ± 0.9	5.7 ± 0.4	29.9 ± 8.1	14.7 ± 3.5	20.5 ± 7.0
NAT2	10.7 ± 0.1	81.0 ± 0.9	6.5 ± 0.8	190.8 ± 97.4	10.7 ± 0.8	55.7 ± 21.1
NAT3	9.0 ± 0.1	34.6 ± 0.8	6.0 ± 0.2	21.6 ± 6.1	11.0 ± 2.1	4.0 ± 3.0
Conifer streams						
CON1	9.8 ± 0.1	48.4 ± 2.3	6.4 ± 0.3	70.7 ± 34.5	15.1 ± 3.0	118.9 ± 16.7
CON2	11.8 ± 0.1	65.1 ± 3.3	5.6 ± 0.5	77.8 ± 30.5	12.3 ± 2.7	510.1 ± 12.8
CON3	9.6 ± 0.1	48.9 ± 3.8	6.4 ± 0.8	93.3 ± 42.5	52.2 ± 38.2	118.6 ± 67.3
Average						
NAT	9.6 ± 0.6	49.4 ± 15.8	6.0 ± 0.2	80.8 ± 55.0	12.1 ± 1.3	26.8 ± 15.2
CON	10.4 ± 0.7	54.1 ± 5.5	6.1 ± 0.3	80.6 ± 6.7	26.5 ± 12.8	249.2 ± 130.4
p value	0.427	0.790	0.828	0.997	0.327	0.166

Table 3

Initial chemical characteristics (mean ± SE) of the native broadleaf and conifer litter species used in the decomposition experiment. Comparisons between litter species were made by one-way ANOVA.

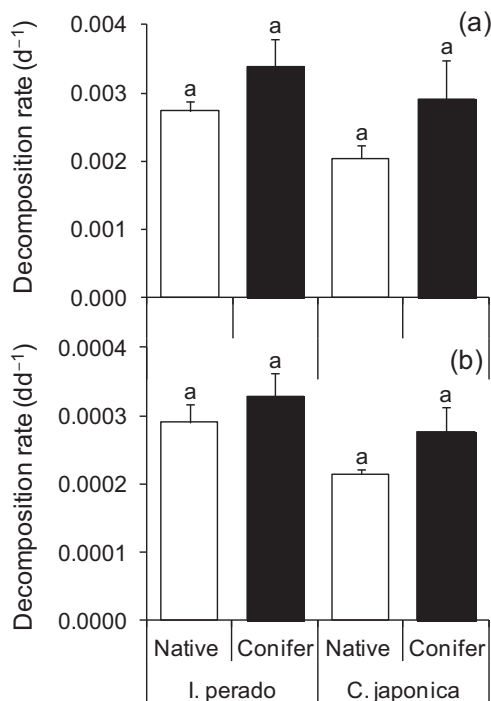| Litter variables	*I. perado* (native litter)	*C. japonica* (conifer litter)	p value
Polyphenols (% DM)	4.6 ± 0.3	7.7 ± 0.2	0.001
Lignin (% DM)	28.9 ± 0.5	44.2 ± 0.3	<0.001
Carbon (% DM)	49.4 ± 0.1	51.3 ± 0.2	0.001
Nitrogen (% DM)	1.08 ± 0.04	0.34 ± 0.02	<0.001
Phosphorus (% DM)	0.054 ± 0.003	0.031 ± 0.003	0.006

Fig. 1. Decomposition rates on a per day (a) and on a per degree-day (b) basis for *I. perado* and *C. japonica* litter incubated in native and conifer streams (three streams per type). Values are means ± SE. Comparisons between stream types and litter species were made by two-way ANOVA; treatments with the same letter did not significantly differ (p > 0.050).
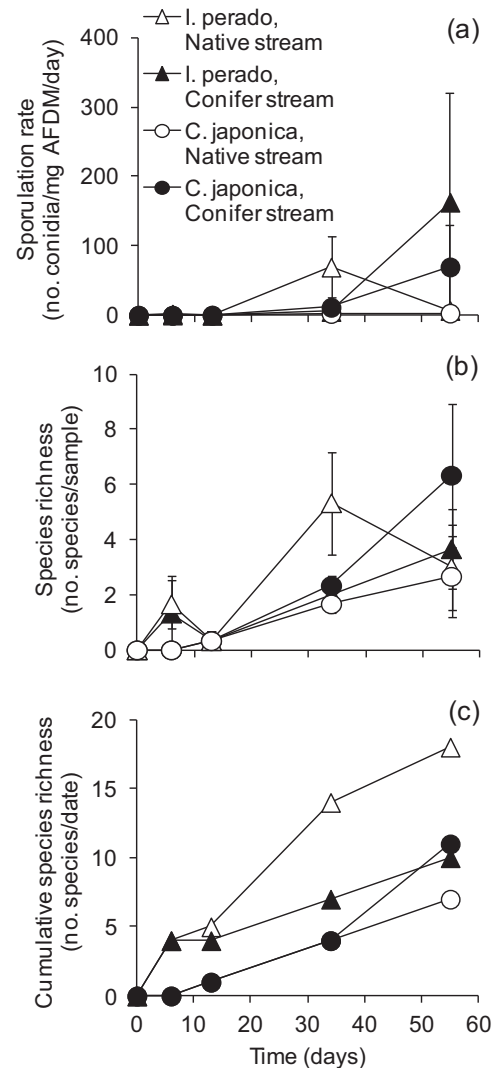

Fig. 2. Aquatic hyphomycete sporulation rates (a), species richness (b) and cumulative species richness (c) associated with *I. perado* and *C. japonica* litter incubated in native and conifer streams (one stream per type) over 55 days. Values are means ± SE.

quality (defined by chemical and physical characteristics) or phenology (Graça et al., 2002; Inoue et al., 2012; Martínez et al., 2013). Since stream decomposers depend on litter inputs from the riparian vegetation, changes in the characteristics of the latter likely affect decomposers' communities. Indeed, aquatic hyphomy-

cete communities' structure differed between native and conifer streams. This difference was profound and resulted from differences in the total number of species recorded (20 in the native vs. 16 in the conifer stream), identity of the species present (only 10 species, out of a total of 26, were shared by both streams) and

Table 4
Relative abundance (%) of aquatic hyphomycete species associated with *I. perado* and *C. japonica* litter incubated in native (NAT) and conifer (CON) streams (one stream per type) over 55 days. Most abundant species are highlighted in bold. Total species richness is also shown.

Aquatic hyphomycete	*I. perado*		*C. japonica*	
Species	NAT	CON	NAT	CON
Alatospora pulchella	1.5		**33.3**	
Anguillospora longissima	0.1			
Articulospora tetracladia	**15.0**	4.6	4.8	0.1
Clavariopsis aquatica				1.9
Flagellospora curvula				0.5
Fontanospora eccentrica		0.1		
Heliscus lugdunensis		**66.0**		**70.0**
Lemonniera aquatica	3.3	1.4		
Lemonniera filiformis	7.9	1.4		0.9
Lemonniera pseudofloscula	0.1			0.2
Lemonniera sp.	8.3	2.2		
Lunulospora curvula	**13.4**		9.5	4.0
Mycocentrospora sp.	0.2			
Pleuropedium multiseptatum			4.2	
Stenocladiella neglecta	0.3			
Tetrachaetum elegans		4.3	1.2	12.0
Tetracladium maxilliforme	**25.0**			
Tetracladium setigerum	2.9			
Tricellula aquatica	0.2			
Tricladium chaetocladium	**19.0**		**43.8**	9.8
Tricladium patulum	1.2			
Tricladium splendens				0.3
Triscelophorus acuminatus	0.5	10.7		
Triscelophorus monosporus		7.1		
Unidentified tetraradiate 1	1.2	2.1	3.3	0.3
Unidentified tetraradiate 2	0.1			
Total species richness (no. species/stream)	18	10	7	11

in species evenness (higher in the native stream). Higher aquatic hyphomycete species richness in the native than in the conifer stream may reflect the higher substrate diversity in the former stream, which flows through a diverse native laurel forest compared with the conifer stream that flows through a monospecific plantation of *C. japonica*. Positive correlations between aquatic hyphomycete and riparian tree or benthic leaf litter species richness in native streams have been reported before (Rajashekhar and Kaveriappa, 2003; Laitung and Chauvet, 2005; Lecerf et al., 2005; Ferreira et al., 2016a). Our results also agree with previous studies showing differences in aquatic hyphomycete communities between native streams and streams flowing through monospecific plantations (Bärlocher and Graça, 2002; Ferreira et al., 2006a). High litter species richness allows for high aquatic hyphomycete species richness due to niche complementarity because aquatic hyphomycete have substrate preferences (Gulis, 2001).

The native and conifer streams compared for aquatic hyphomycete variables also differed in dissolved nutrient availability, with the conifer stream showing 4×, 5× and 29× higher average concentrations of phosphate, ammonium and nitrate, respectively. Higher nutrient availability has been reported to promote higher aquatic hyphomycete species richness and activity (e.g. higher sporulation rates) (Gulis and Suberkropp, 2003; Pascoal et al., 2005; Ferreira et al., 2006b). This was not our case, though, which suggests that either differences in dissolved nutrient concentration between native and conifer streams were of low importance for aquatic hyphomycetes or that higher nutrient concentrations in the conifer stream mitigated to some extent the potential negative effects of conifer plantations on aquatic hyphomycetes. Interestingly, there was a tendency for higher aquatic hyphomycete species richness on the litter species natural from each stream than on the 'introduced' litter species, i.e. total species richness was higher on *I. perado* than on *C. japonica* litter in the native stream (18 vs. 10) and higher (although to a lesser extent) on *C. japonica*

than on *I. perado* in the conifer stream (11 vs. 7), which could suggest some adaptation of the aquatic hyphomycete communities in each stream to the substrate they most often encounter.

Our conclusions regarding fungal communities associated with decomposing litter in conifer streams are, however, limited by the reduced number of streams used and should be considered carefully.

4.2. Litter decomposition rates were not affected by changes in the riparian vegetation

The profound changes observed in aquatic hyphomycete communities' structure did not translate into differences in litter decomposition rates between native and conifer streams. This may suggest some functional redundancy between microbial communities, i.e. different microbial communities are able to keep litter decomposition at similar rates (Bärlocher and Graça, 2002; Pascoal et al., 2005; Ferreira et al., 2006a). Ecosystem functioning (i.e. litter decomposition) and structure (i.e. decomposers communities) were thus not tightly coupled in Azorean streams.

Microbes, aquatic hyphomycetes in particular, are the prime decomposers in Azorean streams, where invertebrate detritivores are rare (Raposeiro et al., 2014; Ferreira et al., 2016c). Microbial induced litter decomposition (as in our case) may be less sensitive to forest change than overall (microbial + macroinvertebrate induced) litter decomposition as demonstrated for the replacement of native mixed deciduous forests by *Eucalyptus globulus* Labill. plantations (Ferreira et al., 2016b). Also, previous studies addressing the replacement of native forests by conifer plantations reported strong alterations in the benthic macroinvertebrate detritivore community with changes in community structure, size-spectra, density and biomass (Whiles and Wallace, 1997; Riipinen et al., 2009, 2010; Hisabae et al., 2011; Martínez et al., 2013, 2016), although not always this was translated into differences in litter decomposition rates (e.g. due to density compensation among taxa; Riipinen et al., 2009, 2010). Thus, the lack of macroinvertebrate detritivores in Azorean streams may render ecosystem functioning less susceptible to forest change compared with streams where macroinvertebrate detritivores are abundant and drive litter decomposition.

We should note, however, that litter lost <20% of initial mass during the 55 days incubation period. Maybe, at more advanced stages of decomposition, when litter becomes softer and better colonized by microbial decomposers, the effects of changes in riparian forest on litter decomposition rates become more evident.

4.3. Litter decomposition rates did not differ between native and conifer species

While *I. perado* leaves were of higher nutritional value for decomposers than *C. japonica* needles (higher nutrient concentrations and lower concentrations of structural and secondary compounds in the former substrate), litter derived from native laurel forests (*I. perado* included) is dominated by leathery leaves with a thick layer of wax (Rosa et al., 2010) and thus it is of general poor quality for decomposers (microbes and macroinvertebrates) that generally prefer soft litter (Li et al., 2009; Graça and Cressa, 2010). Indeed, microbial activity was low on both substrates and decomposition rates did not significantly differ between litter species.

The literature generally reports faster decomposition rates for broadleaf than for conifer litter as a result from the general better quality of the former compared with the latter substrate (Whiles and Wallace, 1997; Hisabae et al., 2011; Martínez et al., 2013). However, *I. perado* leaves are of relatively low quality compared with most commonly used deciduous broadleaf species (Ferreira

et al., 2012) owing to its toughness and waxy cuticle (Rosa et al., 2010), which likely limited microbial conditioning and activity (Foucreau et al., 2013; Newman et al., 2015). Nevertheless, *I. perado* leaves harbored a higher number of aquatic hyphomycete species than *C. japonica* needles, suggesting that not all fungal species may be able to cope with the recalcitrant characteristics of the needles.

The fact that the litter in native streams is dominated by poor quality species may contribute to protect Azorean native streams from severe changes in ecosystem functioning due to the replacement of native forests by conifer plantations, which also provide low quality litter to streams, as compared with streams where the native forest and the conifer plantations have drastically different litter traits (e.g. replacement of native deciduous forests by conifer (*Pinus radiata* D. Don) plantations; Martínez et al., 2013). In fact, Kominoski et al. (2013) forecasted that effects of forest change on stream functioning would be strongest when native and 'replacement' species have more dissimilar functional traits.

5. Conclusions

The replacement of native laurel forests by conifer plantations did not affect early stage litter decomposition in Azorean streams likely owing to the lack of macroinvertebrate detritivores and to the absence of high quality leaf litter (Kominoski et al., 2011). The decomposition of the tough leaf litter contributed by native forests and conifer plantations to streams is driven by microbes, whose communities are highly redundant (Bärlocher and Graça, 2002; Pascoal et al., 2005; Ferreira et al., 2006a) and thus able to buffer changes in litter decomposition that could arrive from changes in microbial communities' structure. However, the strong changes in aquatic hyphomycete communities' structure arriving from forest change may have unpredictable effects in case of additional environmental change. It is interesting to note that a higher number of aquatic hyphomycete species was found in native than in conifer streams. To protect high species richness and prevent unwanted effects of changes in microbial communities' structure, a riparian buffer of native vegetation could be maintained in conifer plantations.

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