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Ponta Delgada

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Dissertação apresentada ao Departamento de Biologia para a obtenção do título de Mestre em Biodiversidade e Biotecnologia Vegetal.

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2016

Acknowledgments

The concretization of this thesis wouldn't be possible without the faultless efforts and priceless mentorship from Professor Doctor Vítor Gonçalves and Doctor Verónica Ferreira. I am sincerely grateful for all the sharing of knowledge, inestimable help and the opportunity to develop this study within this research group.

I am also thankful to Doctor Pedro Raposeiro, Elia Peña, Roberto Resendes and João Brum for all their collaboration in the preparation, field and laboratory work of this study, which were essential for achieving the objectives.

I deeply appreciate to MARE, University of Coimbra, for having been very well received during my formation in aquatic hyphomycetes. It was an unforgettable experience, mostly due to the nice and welcoming people involved.

Special thanks to my friends Filipe Bernardo and Jorge Frias for accompanying me during my difficult times, always providing good advice.

Immensely grateful to my sweetheart Mariana Câmara for all her contribution in the practical work of this study and mainly for personal support at all levels during the whole time.

And of course to my family, particularly my parents, brother, little sister and godmother for the unconditional sustenance, protection, help and inspiration, specially the one provided by my little sister child's innocence during my lifetime.

This work also is dedicated to my relatives who have passed away, particularly my godfather Alberto Faustino, who deeply marked my life with their wise and fully inspired counsels.

This study was financed by the European Fund for Economic and Regional Development (FEDER) through the Program Operational Factors of Competitiveness (COMPETE; FCOMP-01-0124-FEDER-041055) and by National Funds through the Portuguese Foundation of Science and Technology (FCT) through the project 'PROSTREAM – Ecological experimentation in island ecosystems: vegetal litter PROcessing in STREAMS' (EXPL/AAG-GLO/0189/2013) granted to Verónica Ferreira and through the strategic project UID/MAR/04292/2013 granted to MARE.

Abstract

Streams are intimately connected with their surrounding vegetation, which provides shading and fuels aquatic food webs with litter, and are therefore very vulnerable to changes in the riparian vegetation. The replacement of native forest by commercial tree plantations and invasions by exotic tree species can affect the existent biologic dynamics despite the fact that the heterotrophic nature of streams is maintained. However, changes in riparian vegetation often show contrasting results on ecosystem functioning, with stimulation, inhibition or no major effect on litter decomposition rates, suggesting that the effects of forest change could be context dependent. The present study evaluates the effects of forest change on stream communities and activities, in São Miguel island (Azores archipelago), by comparing decomposition rates of native (*Ilex perado*), conifer (*Cryptomeria japonica*) and exotic (*Pittosporum undulatum*) litter, and reproduction and community structure of litter associated aquatic hyphomycetes, among streams (n=9) flowing through native vegetation (laurel forest), conifer plantations and forests invaded by exotic woody species. Decomposition of *I. perado*, *C. japonica* and *P. undulatum* litter, and sporulation rates and species richness of litter associated aquatic hyphomycetes, did not significantly differ between native and conifer streams, while aquatic hyphomycetes community structure was strongly affect by forest change. In contrast, decomposition of *I. perado* and *P. undulatum* litter was significant higher in invaded streams than in native streams. This study shows that forest changes can impact stream ecosystem functioning, but the effects were moderated by litter identity and type of forest change.

Keywords: aquatic hyphomycetes; conifer plantations; litter decomposition; native forest; stream ecosystem functioning; woody species invasion.

Resumo

Os ribeiros estão intimamente ligados à vegetação circundante, responsável pelo ensombramento e abastecimento das cadeias alimentares aquáticas com detritos orgânicos, sendo assim muito vulneráveis a alterações na vegetação ribeirinha. A substituição da floresta nativa pela floresta de produção ou a sua invasão por espécies arbóreas exóticas pode afetar as dinâmicas biológicas existentes, apesar da natureza heterotrófica dos ribeiros ser mantida. Contudo, alterações na vegetação ribeirinha muitas vezes apresentam resultados contrastantes sobre o funcionamento do ecossistema, como estimulação, inibição ou ausência de diferenças significativas em termos de taxas de decomposição dos detritos orgânicos, sugerindo que os efeitos da alteração das florestas podem ser dependentes do contexto em que se inserem. O presente estudo avalia os efeitos da alteração das florestas sobre as comunidades e atividades biológicas dos ribeiros na ilha de São Miguel (Arquipélago dos Açores), através da comparação das taxas de decomposição de detritos orgânicos com origem nativa (*Ilex perado*), conífera (*Cryptomeria japonica*) e exótica (*Pittosporum undulatum*), bem como das taxas de reprodução e da estrutura das comunidades dos hifomicetes aquáticos associados aos detritos, entre ribeiros (n=9) circundados por floresta nativa (floresta laurissilva), por plantações de coníferas e por florestas invadidas por espécies exóticas. A decomposição de *I. perado*, *C. japonica* e *P. undulatum*, assim como a taxa de esporulação e riqueza específica dos hifomicetes aquáticos associados não diferiram significativamente entre os ribeiros nativos e os de coníferas, enquanto a estrutura das comunidades dos hifomicetes aquáticos foi fortemente afetada pela alteração da floresta. Contrariamente, a decomposição de *I. perado* e *P. undulatum* foi significativamente maior nos ribeiros invadidos comparativamente aos nativos. Este estudo demonstra que as alterações das florestas podem ter impacto no funcionamento dos ecossistemas, porém os efeitos foram moderados pela identidade dos detritos orgânicos e pelo tipo de alteração da floresta.

Palavras-chave: decomposição; floresta de produção; floresta invadida; floresta nativa; funcionamento do ecossistema ribeirinho; hifomicetes aquáticos.

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Introduction

In low-order forest streams, primary production is limited by the closed canopy that prevents solar irradiation from reaching the stream and contributes to keep low water temperature (Vannote et al., 1980). In these streams, food webs obtain energy mostly from the decomposition of allochthonous organic matter provided by the riparian vegetation (Vannote et al., 1980; Wallace et al., 1997). Allochthonous organic matter (litter henceforth) inputs can be composed by many different plant parts as leaves, floral parts, wood, fruits, bark, cones, and nuts, with leaves usually comprising the largest component with 41 – 98% of total litterfall (Abelho, 2001). Litter inputs depend on forest composition and thus the replacement of native forests by commercial tree plantations or their invasion by exotic woody species may affect litter input characteristics and consequently litter decomposition in streams (Graça et al., 2002; Kominoski et al., 2013).

In fact, the composition of riparian forests around the world is changing in response to global changes in climate (e.g. temperature augmentation), variation in precipitation and hydrology, plant epidemics, altered land use, habitat fragmentation, unsustainable human water use, and biotic homogenization (Poff et al., 2007). These changes certainly will continue and even increase with the human population growth and their demand for forest and water resources, associated with changes in global climate (Tilman et al., 2001; Richardson et al., 2007; Sabo et al., 2010).

Litter inputs may vary in phenology, identity, diversity, quantity and quality with changes in forest composition, especially if tree species in native forests and those replacing or invading them have different characteristics (Graça et al., 2002; Kominoski et al., 2013). For instance, the replacement of native mixed broadleaf deciduous forests by eucalyptus (*Eucalyptus globulus* Labill.) monocultures may lead to changes in litterfall composition with a dominance of eucalyptus litter in monocultures versus a diverse litter mixture in native forests (Abelho and Graça, 1996), seasonality with high litterfall in summer in eucalyptus monocultures and in autumn/winter in native forests (Abelho and Graça, 1996; Pozo et al., 1997; Bärlocher & Graça, 2002), quantity with reduction of total litter inputs (20 – 32%) on eucalyptus monocultures (Pozo et al., 1997; Molinero

& Pozo, 2004, 2006), and quality with lower litter nutritional value on eucalyptus monocultures as eucalyptus litter is highly recalcitrant (Pozo et al., 1997; Molinero & Pozo, 2004, 2006). In another example, the replacement of native forests by conifer plantations may lead to changes in litter quality with lower litter nutritional value and higher level of toxic compounds on conifer litter compared with litter from native forests (Whiles and Wallace, 1997; Hisabae et al., 2011; Martínez et al., 2013), and seasonality with seasonal stability of conifer litterfall while in native forests seasonality in litterfall is high (Hisabae et al., 2011), although annual amount of litter input may be similar between stream types (Martínez et al., 2013).

In water, leaf decomposition proceeds in three phases that are interdependent and overlap in time: (1) leaching, (2) microbial conditioning and (3) fragmentation (Fig. 1) (Gessner et al., 1999; Abelho, 2001). Leaching of soluble compounds (e.g. simple sugars, polyphenolics) is generally rapid and can contribute to a substantial decrease in initial litter mass. The extent of leaching depends on various factors as stream water temperature and leaf species (Webster and Benfield, 1986; Abelho, 2001). Once litter has lost most of its defensive compounds it is colonized by the microbial community, fungi, particularly aquatic hyphomycetes, and bacteria (Gulis and Subberkrop, 2003; Pascoal and Cássio, 2004). Microbes promote litter decomposition through respiration, i.e. mineralization of organic carbon, and incorporation of organic carbon into biomass and spores (Gulis and Subberkrop, 2003; Cornut et al., 2010). Also, fungi produce enzymes that give them the capability to depolymerize pectin, xylan and cellulose, resulting in the maceration of cellular walls and the release of fine particulate organic matter (FPOM) (Suberkropp et al., 1983). The fragmentation phase refers to the fragmentation of leaf litter by physical abrasion and shredding by invertebrate detritivores. However, in places were detritivores are rare or absent, as in many island streams, biotic fragmentation is reduced and litter decomposition is mediated mostly by microbial activities (Malmqvist et al., 1993; Larned, 2000; Hughes and Malmqvist, 2005; Raposeiro et al., 2014; Ferreira et al., 2016b).

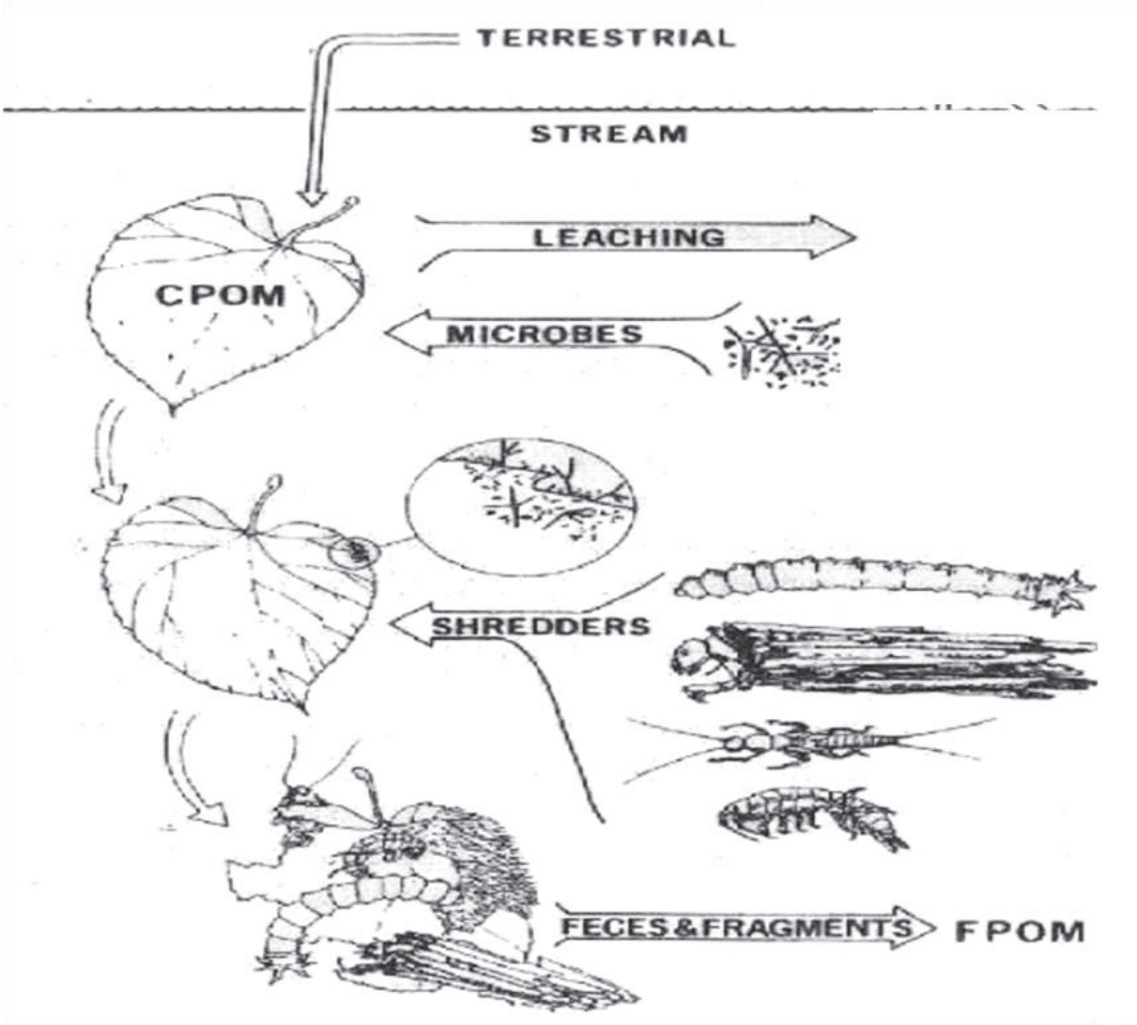

Fig. 1. Diagrammatic representation of leaf decomposition in streams, through their three phases leaching, microbial conditioning and fragmentation by shredders (CPOM, coarse particulate organic matter; FPOM, fine particulate organic matter; adapted from Cummins and Klug, 1979).

Microbial colonization and activity on submerged litter depend on water temperature, water chemistry, leaf chemistry, and structural characteristics of leaves. Aquatic hyphomycete activity on submerged litter (e.g. reproductive activity) is generally stimulated by increases in water and litter nutrient concentration and water temperature (Gulis and Suberkropp, 2003; Ferreira et al., 2006b; Ferreira and Chauvet, 2011). Litter colonization by aquatic hyphomycetes can be delayed by 1 – 2 weeks when the concentration of polyphenolics and tannins is high (Bärlocher and Oertli, 1978; Bärlocher et al., 1995; Chauvet et al., 1997) and aquatic hyphomycetes activity is higher in soft and nutrient rich litter than in more recalcitrant litter (Gulis and Suberkropp, 2003; Ferreira et al., 2006b).

Stream and litter characteristics may be affected by changes in forest composition. Different tree species may have different shapes and thus induce different shading which may affect water temperature (Ferreira et al., 2006a). Nitrogen-fixing trees or trees with nutrient rich litter may induce increases in water nutrient concentration (Goldstein et al., 2009; Mineau et al., 2011; MacKenzie et al., 2013; Wiegner et al., 2013). Different tree species produce litter with distinct chemical and physical characteristics (Ostrofski, 1997; Ferreira et al.,2016b). The replacement of native forest by commercial tree plantations and invasions by exotic tree species can affect biologic dynamics despite the fact that the heterotrophic nature of streams is maintained (Graça et al., 2002; Kominoski et al., 2013). However, these changes in riparian vegetation often show contrasting results on litter decomposition, with stimulation (Whiles and Wallace, 1997; Lecerf et al., 2007; Menéndez et al., 2013), inhibition (Abelho and Graça, 1996; Kennedy and Hobbie, 2004; Lecerf and Chauvet, 2008a) or no major differences (Bärlocher and Graça, 2002; Lecerf et al., 2005; Riipinen et al., 2010; Hisabae et al., 2011) in decomposition rates. This inconsistency between native and altered forests is also visible for aquatic hyphomycetes activity on decomposing litter. For instance, the replacement of native deciduous forests by eucalyptus monocultures was found to stimulate aquatic hyphomycete sporulation and biomass in one study (Ferreira et al., 2006a), while other studies found no major difference between stream types (Chauvet et al., 1997; Pozo et al., 1998). Aquatic hyphomycete community structure can also be affected by forest change (Bärlocher and Graça, 2002; Ferreira et al., 2006a).

These inconsistent results suggest that the effects of forest change on litter decomposition could be moderated by factors such as forest type, physical and chemical characteristics of litter, type of decomposers involved in processes (microbes and/or invertebrates), and stream characteristics (Ferreira et al., 2016a).

The present study evaluates the effects of forest change on stream fungal communities and activities, in São Miguel island (Azores archipelago), by comparing decomposition rates of native, conifer and exotic litter, and reproduction and community structure of litter associated aquatic hyphomycetes, among streams flowing through native vegetation (laurel forest), conifer plantations and forests invaded by exotic wood species. The following hypothesis were tested: (1) litter decomposition, and reproduction and community structure of litter associated aquatic hyphomycetes, differ among streams flowing through distinct forests as a result from changes in litter inputs and environmental conditions driven by forest change (Ferreira et al., 2006a; Hladyz et al., 2011; Martínez et al., 2013), (2) litter decomposition, and reproduction and community structure of associated aquatic hyphomycetes, vary among litter species as a result from differences in litter physical and chemical characteristics (Gessner and Chauvet, 1994; Ferreira et al., 2006b), (3) there is an interaction between litter species and stream type for litter decomposition and associated aquatic hyphomycetes since litter characteristics often moderate the response of microbial activities to environmental change (Gulis and Suberkropp, 2003; Ferreira et al., 2006b).

Materials and methods

Study region

This study was done in São Miguel island, Azores archipelago. The Azores archipelago is an autonomous region of Portugal, 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 (Fig. 2A). The archipelago is composed of nine volcanic islands (ranging 17 – 760 km^2), with São Miguel island being the largest (Fig. 2B). Mean annual temperatures in the Azores range from 14 to 18 ºC and mean annual precipitation from 740 to 2400 mm, with most of the precipitation (65 – 70%) falling between October and March (Louvat & Allègre, 1998; DROTRH/INAG, 2001).

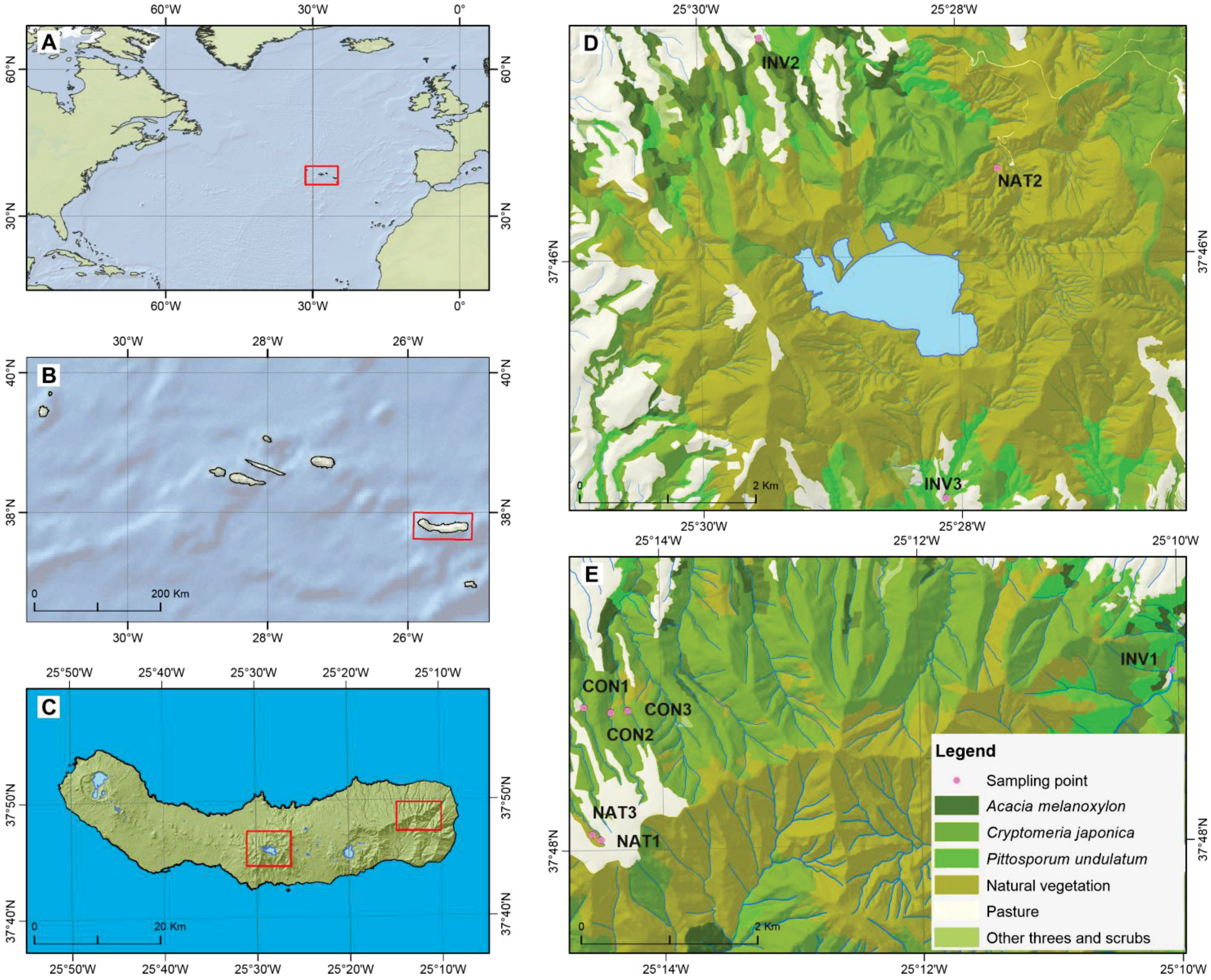

Fig. 2. Location of the study streams. A, Azores archipelago in the Atlantic Ocean. B, São Miguel island in the Azores archipelago. C, Study areas in São Miguel island. D and E, Detail of the study areas. NAT1 – NAT3, streams flowing through native vegetation; CON1 – CON3, streams flowing through conifer plantations; INV1 – INV3, streams with riparian vegetation invaded by exotic species.

In the Azores, land uses are highly influenced by human activities. Occupation by pastures and agricultural fields prevails (56% of total area), followed by non-native forests (conifer plantations and forests invaded by exotic vegetation; 22%) and native vegetation (13%), which shows the largest variation among the islands (Cruz et al., 2007) (Fig. 3). In São Miguel island, pastures and agricultural fields occupy 61% of land area, followed by non-native forests (21%) and native vegetation (9%), which is below regional average (Cruz et al., 2007) (Fig. 3). These data suggest that native vegetation in the Azores, and particularly in São Miguel island, is under pressure and points to the importance of studying the impacts of its replacement by commercial plantations or invasion by exotic species in lotic systems.

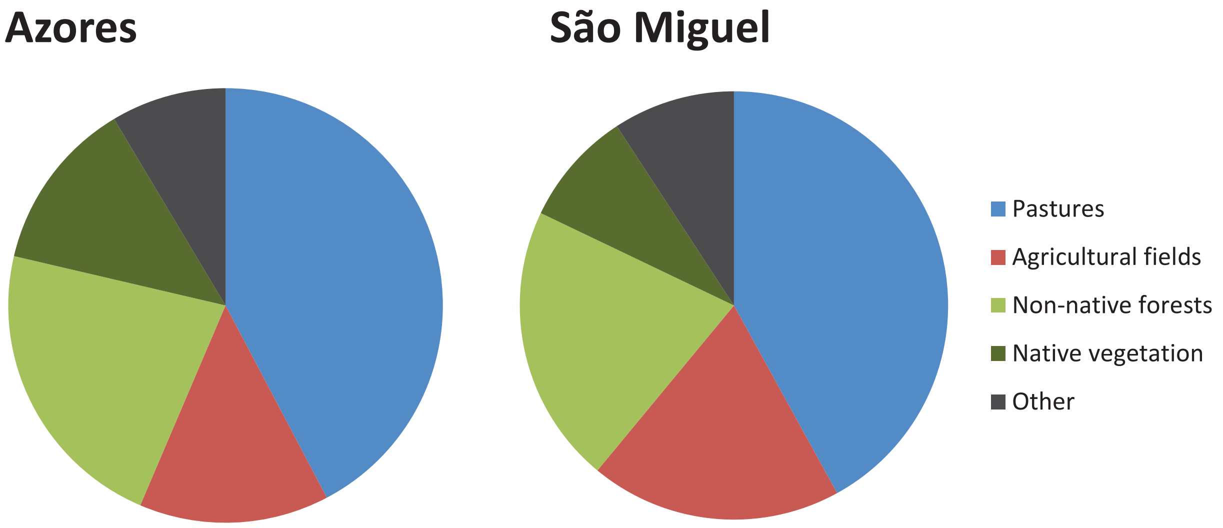

Fig. 3. Land uses (% cover) in the Azores archipelago and São Miguel island. 'Other' indicate land cover (%) by urban, industrial, uncovered areas and lakes. *Cryptomeria japonica* is included in non-native forests. Data from Cruz et al. (2007).

At the present, vascular flora of São Miguel island is mostly composed by exotic plants that represent about 66% of the approximately 1000 species found in the island (Silva & Smith 2004, 2006). Between those exotic species several aggressive invaders, including trees or shrubs such as *Pittosporum undulatum*, *Clethra arborea* and *Acacia melanoxylon* have expanded their distribution on the island, replacing native species (Sjögren, 1973; Schaefer, 2003). Most of the forest patches are now dominated by *P. undulatum*, *A. melanoxylon* and *Eucalyptus globulus* (Hortal et al., 2010).

There is no concrete information about the destruction of the native forest over the decades, but it is possible to understand that the native forest of São Miguel island was characterized by different types, including the hyper-humid laurel forest that was selected for this study and that is one of the most threatened (Dias et al., 2007a). This type of laurel forest develops in areas of intense fogs and exposed to humid winds, with wet substrate due to their impermeability (Dias et al., 2007a). These conditions promote an arboreal stratum composed by ombrophilous species as *Laurus azorica* (Seub.) Franco, *Frangula azorica* V. Grubov, *Ilex perado* Aiton and *Vaccinium cylindraceum* Sm (Dias et al., 2007a).

It is difficult to precisely define the limits of the different types of native forests because these are identified according with species composition and environmental conditions, ranging gradually from the coast until higher elevations. Obviously, human colonization of the littoral zones and the necessity to have agricultural fields led to the destruction of native forest types present at low elevations, increasing in elevation over the years as population density increased and so did the need for more agricultural fields, timber and charcoal. Thus, it is not surprising that nowadays native forests are mostly restricted to higher elevations and only there they are associated with streams (riparian vegetation) (Dias et al., 2007a, b).

Cryptomeria japonica D. Don., a conifer originary from Japan and areas of south China, was introduced into the Azores islands in the middle 19th century (Palhinha, 1966) for wood production necessary to build boxes for orange exportation (Moreira, 1987). At that time, the floristic structure of Azores islands, and of São Miguel in particular, suffered large changes with the introduction of exotic species (Dias et al., 2007b). In 1951, the forest area in São Miguel island was < 6%, a bad situation that resulted from the lack of proper planning, with the unregulated and uncontrolled exploitation of the resources not being offset by corresponding planting (Dias et al., 2007b). Planting measures were undertaken to counterbalance this situation, with *C. japonica* progressively becoming an important species once its commercial interest and its ability to adapt to strong winds (a limiting factor in the Azores) were recognized (Dias et al., 2007b). Nowadays, *C. japonica* occupies 26% of the total forested area in the Azores and dominates production forests in the region (60%), especially at elevations > 400 m a.s.l., being cut by law at 30 years, when the trees reach a mean height of 21 m and a mean diameter of 28 – 130 cm (Dias et al., 2007b).

Pittosporum undulatum Vent is a tree species native to Australia and was introduced into the Azores in the 19th century too, first as an ornamental plant and after as a hedgerow species for the protection of orange tree plantations (Dias et al., 2007b; Hortal et al., 2010; Lourenço et al., 2011; Costa et al., 2012). This species later invaded a wide range of habitats throughout the Azores islands, changing the landscape dramatically (Dias et al., 2007b; Hortal et al., 2010; Lourenço et al., 2011). *P. undulatum* is a species with low nutrient requirements and fast growth rate, it is adapted to dry and windy conditions, and forms very dense clusters that prevent the growth of other species, which make it an aggressive invader (Dias et al., 2007b). *P. undulatum* is presently the most widespread woody invader in the Azores with 49% of forested area occupancy, especially between 100 – 600 m a.s.l., and it is one of the priority species for control (Dias et al., 2007b; Hortal et al., 2010; Lourenço et al., 2011; Costa et al., 2012). It is also ranked in the top 10 of the worst invasive species in Macaronesia, (Silva et al., 2008).

Stands of *P. undulatum* often have an understory dominated by the herbaceous *Hedychium gardnerianum* Sheppard *ex* Ker Gawl., which is a rhizomatous perennial herb of the Zingiberaceae family introduced into the Azores from its native Himalayas also in the middle 19th century (Medeiros et al., 2003). This species is widespread in the Azores islands, except in Corvo island, and it is extensively distributed throughout São Miguel island. It also spreads rapidly whenever the native forest becomes degraded, as well as being scattered in the dense laurel forest of the island (Medeiros et al., 2003).

Other important exotic woody invaders, especially at low elevations, include *Acacia melanoxylon* R. Br., native from Southeast Australia and Tasmania, which is very appreciated by its ornamental characteristics and wood value. It can propagate vegetatively or through seeds but its effects on ecosystems are limited by competition with *P. undulatum* (Dias et al., 2007b).

Streams

Nine perennial small streams were selected in the volcanic complexes of Nordeste and Fogo, in the northeast and central area of São Miguel island, respectively (Fig. 2C). Streams ranged 1 – 3 order (Strahler, 1957), and were < 8 m wide and < 60 cm deep. Substrate was composed mainly by sand, gravel and cobbles. Streams were selected to have distinct riparian vegetation: three streams were surrounded by native vegetation (‘native streams’, NAT1 – NAT3), three streams flowed through conifer (*C. japonica*) plantations (‘conifer streams’, CON1 – CON3) and three streams flowed through forests invaded by the exotic tree *P. undulatum*, with an understory dominated by the exotic herbaceous *H. gardnerianum* (‘invaded streams’, INV1 – INV3; in INV2 the arboreal stratum in the riparian vegetation is co-dominated by *A. melanoxylon* and *P. undulatum*) (Fig. 2D, 2E, 4). Due to the location of the different forest types, native streams were at an average elevation of 824 m a.s.l., conifer streams were at 734 m a.s.l. and invaded streams at 271 m a.s.l.. Streams were not affected by any visible human activity, besides changes in forest composition in the conifer and invaded streams, and thus differences in water characteristics can be attributed to forest change and elevation.

Fig. 4. Selected stream types according with their surrounding vegetation. A, Native stream. B, Conifer stream. C, Invaded stream.

Water variables

On five occasions between January – April 2015, electrical conductivity and pH were recorded with a multiparametric field probe (CyberScan 600, Eutech instruments, Nijkerk, the Netherlands). 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, ammonium and phosphate according to Skalar methods M461-318 (EPA 353.2), M155-008R (EPA 350.1), and M503-555R (Standard Method 450-P I), respectively (Skalar, 2004).

Litter species

Three litter species were selected for this study: the native broadleaf species *I. perado* (Fig. 5A), which is dominant in areas of native vegetation and is present in the surroundings of the three native streams; the conifer species *C. japonica* (Fig. 5B), as the species used in conifer plantations; and the exotic broadleaf species *P. undulatum* (Fig. 5C), as a dominant exotic species. As the broadleaf species are evergreen, leaves were directly collected from trees in October 2014. To reduce the variability in leaf characteristics within each species, 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). Conifer twigs were picked from the ground freshly fallen and the 5 – 10 cm tip, corresponding to the last growth period, was cut. Leaves and needles were air-dried at room temperature in the dark and stored dry until needed.

Fig. 5. Litter species used in this study. A, *Ilex perado* Aiton (photo by: Rui Elias). B, *Cryptomeria japonica* D. Don (photo by: Rita Varela). C, *Pittosporum undulatum* Vent (photo by: Luís Silva).

Before the experiment started, three batches of litter of each species were grinded to < 5 mm powder, oven dried (105ºC, 48h) and analyzed for initial phosphorus, nitrogen, carbon, lignin and polyphenols concentration following standard protocols (Graça et al., 2005) at MARE – Marine and Environmental Sciences Centre, University of Coimbra. Additionally, three 12 mm diameter discs were extracted from each of five leaves of the broadleaf species and used to assess initial leaf toughness and specific leaf area (Graça et al., 2005).

Litter bags and litter decomposition

Litter portions of 2.49 g (± 0.03 g, SD) 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 microbial-driven litter decomposition since previous studies have shown that the contribution of macroinvertebrates to litter decomposition was negligible in Azorean streams (Raposeiro et al., 2014; Ferreira et al., 2016b). Also, fine mesh bags would minimize litter mass loss due to physical fragmentation, especially in the case of spates that are common in winter.

On the 7th and 8th of January 2015, 12 litter bags of each species were incubated in each of the nine streams (36 litter bags per stream, 324 bags in total). Litter bags were tied with lines to iron bars anchored to the streambed or secured to submerged rocks. Extra 30 litterbags (10 per species) were submerged at one stream (INV1) 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 (60ºC, > 48h; WTB BINDER 572F, Tuttlingen, Germany) and weighed (0.1 mg precision) to determine initial dry mass (DM). Dry samples were ignited (500ºC, 4h; Lenton EF 11/8B, Hope Valley, U.K.) and the ashes were weighed (0.1 mg precision). 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 both the broadleaf species and 0.84 for the conifer species.

After 6, 13, 34 and 55 days, three litter bags of each species were retrieved from each stream, placed individually into plastic ziplock bags and returned to the laboratory in an ice box. Once in the laboratory, litter bags were opened and 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, CON2, INV1 and INV2 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, CON3 and INV3, as representatives of each type of stream, were processed for the determination of mass remaining and spore production by aquatic hyphomycetes. Five different leaves were selected from each broadleaf sample and five conifer shoots from each conifer sample, gently rinsed with distilled water, and five leaf discs (12 mm diameter, one from each leaf) or five shoot tips 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 litter mass taken for spore production (see below), and results were expressed as percentage of initial AFDM.

Spore production by aquatic hyphomycetes

Spore production by aquatic hyphomycetes was induced under laboratory conditions. Leaf discs and needles were incubated in 100 mL Erlenmeyer flasks with 25 mL of filtered stream water (fibre glass filters, 1.2 μm pore; Whatmam® GF/C, GE Healthcare Europe GmbH, Little Chalfont, U.K.), on an orbital shaker (75 rpm) for 48h at 10 ºC, with 8h of light per day. The spore suspension was transferred to 50 mL graduated centrifuge tubes, fixed with 2 ml of 37% formalin and the volume adjusted to 35 mL with distilled water. Tubes were stored in the dark until used for slides preparation. Litter discs and needles were placed in pre-weighed aluminium cups, oven dried (60ºC, > 24h; WTB BINDER 572F, Tuttlingen, Germany) and weighed (0.1 mg precision) to determine DM. Dry samples were ignited (500ºC, 4h; Lenton EF 11/8B, Hope Valley, U.K.) and ashes were weighed (0.1 mg precision). Leaf discs and needles AFDM was determined by the difference between DM and ash mass, and added to the bulk AFDM above.

Spore suspensions were gently shaken and transferred into a beaker, where 100 μL of Triton X-100 (0.5%) solution were added to ensure a uniform distribution of conidia. After the suspension was homogenised on a magnetic stirrer, aliquots were filtered through cellulose nitrate filters (Sartorius Stedim, 5 μm pore size; Sartorius Stedim Biotech GmbH, Goettingen, Germany). 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^{-1} AFDM day^{-1} and aquatic hyphomycete species richness as number of species $sample^{-1}$.

Statistical analysis

Water characteristics were compared among streams and stream types (i.e., native, conifer and invaded) by nested analysis of variance (ANOVA) (streams nested within stream types). Initial litter chemical and physical variables were compared among litter species by one-way ANOVA.

Litter decomposition rates on a per day basis (k, d^{-1}) were estimated by the slope of the linear regression between the fraction of AFDM remaining (ln-transformed) and time (days), with the intersect fixed at ln(1) = 0 (negative exponential decay model). The fraction of AFDM remaining (ln-transformed) was compared among streams, stream types and litter species by nested analysis of covariance (ANCOVA; streams nested within stream types).

Sporulation rates and aquatic hyphomycete species richness (both log(x+1)-transformed) were compared among streams, litter species and time by 3-way ANOVAs (time, stream and litter species as categorical variables); day 6 was not considered in the analyses as no spore production was observed for conifer streams. Ordination of aquatic hyphomycete communities was done by non-metric multidimensional scaling (NMDS) based on a Bray-Curtis similarity matrix of spore production (log(x+1)-transformed) and comparison among streams and litter species was done by analysis of similarity (ANOSIM) (PRIMER 6 v6.1.11 & PERMANOVA+ v1.0.1; Primer-E Ltd, Plymouth, UK).

Tukey honest significant difference (HSD) test was used as the multiple comparison post-hoc test when significant differences were identified in the ANOVAs. Tukey unequal HSD test was used as the multiple comparison post-hoc test after the ANCOVA. Normality (Shapiro-Wilk test) and homoscedasticity (Bartlet test) were checked and data were transformed when needed to attain normal distribution and homogeneity of variances. Analyses were performed on STATISTICA 7 software (StatSoft Inc., Tulsa, Oklahoma, USA), unless otherwise indicated.

Results

Streams

During the study period, stream water was cool (Table 1), but water temperature significantly differed among stream types (nested ANOVA, $p < 0.001$; Table 2), with values being significantly higher in invaded streams than in native or conifer streams (Tukey test, $p < 0.001$ for both comparisons); significant differences were also found among streams within each stream type (nested ANOVA, $p < 0.001$) (Table 2). Conductivity was low across streams (Table 1), but significant differences were found among stream types (nested ANOVA, $p < 0.001$; Table 2), with significantly higher conductivity in invaded streams followed by conifer streams and lastly by native streams (Tukey test, INV × CON and INV × NAT, $p < 0.001$; CON × NAT, $p = 0.005$);

significant differences were found among streams within each stream type (nested ANOVA, $p < 0.001$). pH was circumneutral across streams (Table 1), but significantly differed among stream types (nested ANOVA, $p = 0.002$; Table 2), with significantly higher values in invaded streams than in native or conifer streams (Tukey test, $p = 0.005$ for both comparisons); no significant differences were found among streams within each stream type (nested ANOVA, $p = 0.258$) (Table 2). Temperature, pH and conductivity data were not homoscedastic (Bartlett test, $p < 0.004$), neither after data transformation, and thus these results need to be interpreted with caution.

Table 1. Physical and chemical characteristics (mean ± SD) of the three stream types (3 streams per type). For each variable, stream types with the same letter do not significantly differ (nested ANOVA followed by Tukey test, $p > 0.050$).

Stream characteristics	Native	Conifer	Invaded	Methods
Temperature (°C)	9.57 ± 0.98 a	10.37 ± 1.22 a	13.19 ± 2.20 b	Thermometer
Conductivity (µS/cm)	50.9 ± 31.0 a	59.0 ± 8.3 b	112.7 ± 43.2 c	Probe (CyberScan 600, Eutech instruments, Nijkerk, The Netherlands)
pH	6.5 ± 0.4 a	6.5 ± 0.4 a	7.4 ± 0.3 b	idem
PO_4^{3-} (µg/L)	78.90 ± 98.84 a	83.10 ± 10.82 ab	178.37 ± 24.74 b	Automated Analyzer (San++, Skalar Analytical B.V., Breda, The Netherlands)
NH_4^+ (µg/L)	12.83 ± 2.36 a	14.02 ± 1.35 a	11.27 ± 1.12 a	Automated Analyzer (San++, Skalar Analytical B.V., Breda, The Netherlands)
NO_3^- (µg/L)	15.89 ± 17.70 a	204.08 ± 180.01 a	383.75 ± 322.59 a	Automated Analyzer (San++, Skalar Analytical B.V., Breda, The Netherlands)

Phosphorus concentrations were relatively high (Table 1) and significantly differed among stream types (nested ANOVA, $p = 0.017$; Table 2), with significantly higher values in invaded than native streams (Tukey test, $p = 0.015$). Ammonium and nitrate did not significantly differ among stream types (nested ANOVA, $p = 0.316$ and 0.208, respectively; Table 2). No significant differences were found among streams within each stream type for any of the nutrients (nested ANOVA, $p > 0.147$; Table 2).

Table 2. Summary table for nested ANOVAs performed on water variables of the study streams.

Source of variation	df	MS	F	p
Temperature				
Intercept	1	8580.500	5336.869	< 0.001
Forest	2	88.621	55.120	< 0.001
Stream(Forest)	6	12.468	7.755	< 0.001
Error	63	1.608		
Conductivity				
Intercept	1	396602	5632	< 0.001
Forest	2	27092	385	< 0.001
Stream(Forest)	6	7004	99	< 0.001
Error	63	70		
pH				
Intercept	1	3314.201	3239.899	< 0.001
Forest	2	7.315	7.151	0.002
Stream(Forest)	6	1.358	1.327	0.258
Error	63	1.023		
PO_4^{3-}*				
Intercept	1	130.857	424.932	< 0.001
Forest	2	1.398	4.539	0.017
Stream(Forest)	6	0.527	1.711	0.147
Error	36	0.308		
NH_4^+				
Intercept	1	7265.065	301.830	< 0.001
Forest	2	28.667	1.191	0.316
Stream(Forest)	6	14.431	0.600	0.729
Error	36	24.070		
NO_3^-*				
Intercept	1	8.813	1.122	0.297
Forest	2	12.900	1.642	0.208
Stream(Forest)	6	3.925	0.500	0.804
Error	36	7.855		

*, Log(x)-transformed

Litter species

The broadleaf species did not significantly differ in initial nutrient concentrations, but *P. undulatum* had higher polyphenol concentration and specific leaf area and lower lignin concentration and toughness than *I. perado* (Table 3; Table 4). *C. japonica* needles were the substrate of poorest nutritional quality with lower phosphorus and nitrogen concentrations and higher lignin concentration than the broadleaf species (Table 3; Table 4).

Table 3. Initial chemical and physical characteristics (mean ± SE) of the three litter species used in the decomposition experiment. Litter species with the same letter do not significantly differ (1-way ANOVA followed by Tukey test, $p > 0.050$).

Litter variables	*Ilex perado*	*Pittosporum undulatum*	*Cryptomeria japonica*	Methods
Phosphorus (% DM)	0.054 ± 0.003 ab	0.082 ± 0.011 b	0.031 ± 0.003 a	Spectrophotometrically after basic digestion with sodium persulphate and sodium hydroxide (APHA, 1995)
Nitrogen (% DM)	1.08 ± 0.04 b	1.02 ± 0.08 b	0.34 ± 0.02 a	IRMS auto analyzer (IRMS Thermo Delta V advantage with a Flash EA, 1112 series)
Carbon (% DM)	49.4 ± 0.1 b	48.1 ± 0.2 c	51.3 ± 0.2 a	idem
Lignin (% DM)	28.9 ± 0.5 b	25.4 ± 0.6 c	44.2 ± 0.3 a	Goering & van Soest method (Goering & van Soest, 1970)
Polyphenols (% DM)	4.6 ± 0.3 b	10.0 ± 0.3 c	7.7 ± 0.2 a	Folin-Ciocalteu's method (Graça et al., 2005)
Toughness (g)*	387 ± 12 a	141 ± 8 b	–	The mass needed to penetrate the leaf mesophyll using a penetrometer (Graça et al., 2005)
Specific leaf area (mm^2/mg)#	9.1 ± 0.4 a	10.5 ± 0.4 b	–	Ratio between discs area (12 mm diameter) and dry mass (105ºC, 48h)

*Toughness was not determined for *C. japonica* because the needles were too thin to be used in the penetrometer; #Specific leaf area was not determined for *C. japonica* because leaf discs could not be extracted.

Table 4. Summary table for one-way ANOVAs performed on initial chemical and physical characteristics of the three litter species used in the decomposition experiment.

Source of variation	df	MS	F	p
Phosphorus				
Intercept	1	0.028	205	< 0.001
Litter species	2	0.002	15	0.005
Error	6	< 0.001		
Nitrogen				
Intercept	1	5.98	646	< 0.001
Litter species	2	0.51	55	< 0.001
Error	6	0.01		
Carbon				
Intercept	1	22135.26	324341	< 0.001
Litter species	2	7.66	112	< 0.001
Error	6	0.07		
Lignin				
Intercept	1	9704.08	12831	< 0.001
Litter species	2	299.93	397	< 0.001
Error	6	0.76		
Polyphenols				
Intercept	1	501.32	2091	< 0.001
Litter species	2	22.35	93	< 0.001
Error	6	0.24		
Toughness				
Intercept	1	701349	1447	< 0.001
Litter species	1	153132	316	< 0.001
Error	8	485		
Specific leaf area				
Intercept	1	951.84	1095	< 0.001
Litter species	1	4.94	6	0.044
Error	8	0.87		

Litter decomposition

Litter mass remaining decreased exponentially over time, with 3 – 64% of initial AFDM remaining for *P. undulatum* leaves, 45 – 88% for *I. perado* leaves and 54 – 91% for *C. japonica* needles after 55 days (Fig. 6). However, only *P. undulatum* leaves lost substantial mass during the initial leaching phase (22 – 29% of initial AFDM in the first six days of incubation, compared with a maximum of 4% for *I. perado* leaves and 3% for *C. japonica* needles) (Fig. 6). This translated into decomposition rates of 0.0110 – 0.0544 d^{-1} for *P. undulatum* leaves, 0.0025 – 0.0139 d^{-1} for *I.*

perado leaves and 0.0018 – 0.0110 d^{-1} for *C. japonica* needles, and of 0.0048 – 0.0544 d^{-1} for invaded streams, 0.0018 – 0.0158 d^{-1} for native streams and 0.0022 – 0.0156 d^{-1} for conifer streams (Table 5).

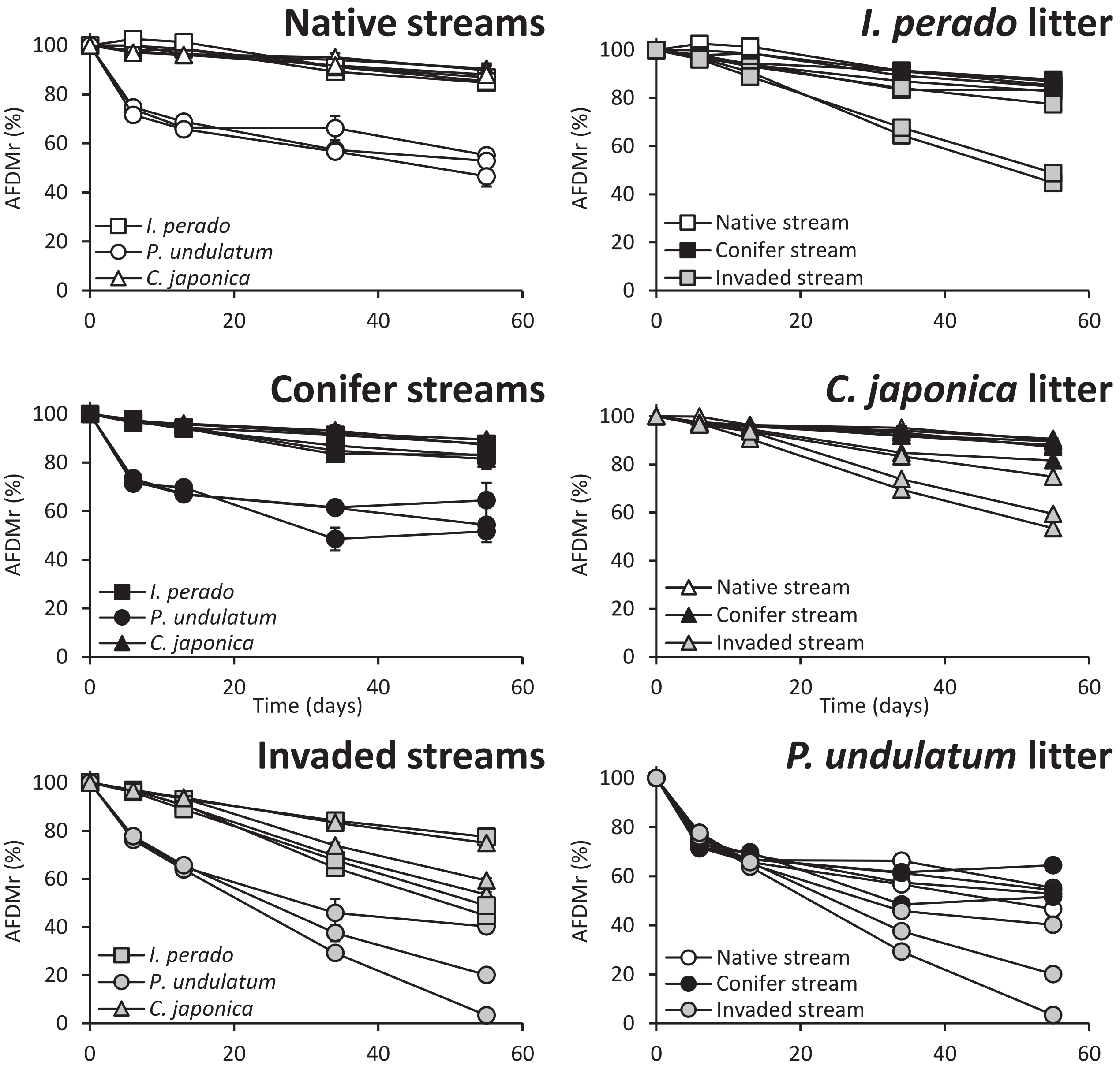

Fig. 6. Ash free dry mass remaining (AFDMr) of *I. perado*, *C. japonica* and *P. undulatum* litter incubated in streams flowing through native vegetation (Native streams), conifer plantations (Conifer streams) and forests invaded by exotic species (Invaded streams) (three streams per type) over 55 days in winter 2014/2015. Values are means ± SE; SE bars are smaller than symbols in most cases.

Table 5. Decomposition rates (*k*) of *I. perado*, *C. japonica* and *P. undulatum* litter incubated in streams flowing through native vegetation (Native stream), conifer plantations (Conifer stream) and forests invaded by exotic species (Invaded stream) (three streams per type) over 55 days in winter 2014/2015, SE and coefficients of variation (R^2) of the regressions ($p < 0.006$ for all).

Stream	Litter species	*k* (d^{-1})	SE	R^2
Native streams				
NAT1	*I. perado*	0.0030	0.0002	0.96
	C. japonica	0.0019	0.0002	0.91
	P. undulatum	0.0123	0.0017	0.83
NAT2	*I. perado*	0.0027	0.0005	0.74
	C. japonica	0.0024	0.0002	0.96
	P. undulatum	0.0158	0.0017	0.89
NAT3	*I. perado*	0.0025	0.0003	0.86
	C. japonica	0.0018	0.0002	0.84
	P. undulatum	0.0138	0.0015	0.89
Conifer streams				
CON1	*I. perado*	0.0026	0.0003	0.84
	C. japonica	0.0022	0.0001	0.96
	P. undulatum	0.0110	0.0020	0.74
CON2	*I. perado*	0.0039	0.0003	0.93
	C. japonica	0.0041	0.0004	0.92
	P. undulatum	0.0156	0.0020	0.84
CON3	*I. perado*	0.0037	0.0002	0.98
	C. japonica	0.0024	0.0002	0.90
	P. undulatum	0.0131	0.0017	0.85
Invaded streams				
INV1	*I. perado*	0.0048	0.0002	0.97
	C. japonica	0.0075	0.0001	1.00
	P. undulatum	0.0193	0.0017	0.92
INV2	*I. perado*	0.0139	0.0007	0.97
	C. japonica	0.0110	0.0003	0.99
	P. undulatum	0.0544	0.0039	0.95
INV3	*I. perado*	0.0124	0.0006	0.97
	C. japonica	0.0091	0.0003	0.99
	P. undulatum	0.0294	0.0008	0.99

Litter decomposition significantly differed among litter species, stream types and streams (ANCOVA, $p < 0.001$) but the significant interactions stream type × species ($p = 0.001$), stream type × stream ($p = 0.001$) and species × stream ($p = 0.045$) suggest that effects of one factor are dependent on another factor (Table 6).

Table 6. Summary table for ANCOVA performed on ln(AFDMr) of the three litter species incubated in stream flowing through native, conifer and invaded streams (three streams per type) over 55 days in winter 2014/2015.

Source of variation	df	MS	F	p
Intercept	1	0.374	5.429	0.020
Time	1	9.031	130.999	< 0.001
Stream type	2	2.422	35.124	< 0.001
Litter species	2	8.580	124.456	< 0.001
Stream	2	0.555	8.053	< 0.001
Stream type × Litter species	4	0.342	4.961	0.001
Stream type × Stream	4	0.334	4.851	0.001
Litter species × Stream	4	0.170	2.469	0.045
Stream type × Litter species × Stream	8	0.121	1.751	0.087
Error	294	0.069		

In fact, decomposition was significantly faster for *P. undulatum* leaves in invaded streams than in native and conifer streams (Tukey test, $p < 0.001$ for both comparisons), with no significant differences between the latter two stream types ($p = 1.000$) (Table 7). Decomposition of *I. perado* leaves was significantly faster in invaded streams than in native streams (Tukey test, $p = 0.035$), but no significant differences were found in litter decomposition between invaded and conifer streams or between native and conifer streams ($p = 0.154$ and $p = 1.000$, respectively) (Table 7). Decomposition of *C. japonica* did not significantly differ among stream types (Tukey test, $p > 0.267$) (Table 7).

Also, decomposition of *P. undulatum* leaves was significantly faster than that of *I. perado* leaves or *C. japonica* needles in native, conifer and invaded streams (Tukey test, $p < 0.001$ for both comparisons in all stream types), while no significant differences were found between *I. perado* leaves and *C. japonica* needles in all stream types ($p > 0.997$) (Table 7).

Table 7. Decomposition rates (*k*) of *I. perado*, *C. japonica* and *P. undulatum* litter incubated in streams flowing through native vegetation (Native stream), conifer plantations (Conifer stream) and forests invaded by exotic species (Invaded stream) (mean of 3 streams per type) over 55 days in

winter 2014/2015. Treatments with the same letter do not significantly differ (ANCOVA followed by Tukey unequal HSD test, $p > 0.050$).

Litter species	k (d^{-1})	SE	Tukey unequal HSD test
Native stream			
I. perado	0.0027	0.0001	a
C. japonica	0.0020	0.0002	a
P. undulatum	0.0140	0.0010	c
Conifer streams			
I. perado	0.0034	0.0004	ab
C. japonica	0.0029	0.0006	ab
P. undulatum	0.0132	0.0013	c
Invaded streams			
I. perado	0.0104	0.0028	b
C. japonica	0.0085	0.0017	ab
P. undulatum	0.0344	0.0104	d

Spore production by aquatic hyphomycetes

Sporulation rates generally increased until a peak was attained by day 13 – 34, while in the conifer stream it increased over the incubation period (3-way ANOVA, $p < 0.001$; Table 8) (Fig. 7). Sporulation rates significantly differed among litter species (3-way ANOVA, $p < 0.001$; Table 8) with values being significantly higher for *P. undulatum* leaves (maximum values: 184 – 9583 conidia mg^{-1} AFDM d^{-1}) than for *I. perado* leaves (69 – 5547 conidia mg^{-1} AFDM d^{-1}) or *C. japonica* needles (3 – 1459 conidia mg^{-1} AFDM d^{-1}) in all streams (Tukey test, $p \leq 0.001$) (Fig. 7), while no significant differences in sporulation rates were found between *I. perado* leaves and *C. japonica* needles (Tukey test, $p = 0.492$).

Sporulation rates also significantly differed among streams (3-way ANOVA, $p < 0.001$; Table 8), with values being significantly higher in the invaded stream (maximum values: 1459 – 9583 conidia mg^{-1} AFDM d^{-1}) than in the native (3 – 237 conidia mg^{-1} AFDM d^{-1}) or conifer stream (70 – 184 conidia mg^{-1} AFDM d^{-1}) (Tukey test, $p < 0.001$ for both comparisons), while no significant differences were found between the latter two stream types (Tukey test, $p = 0.795$) (Fig. 7).

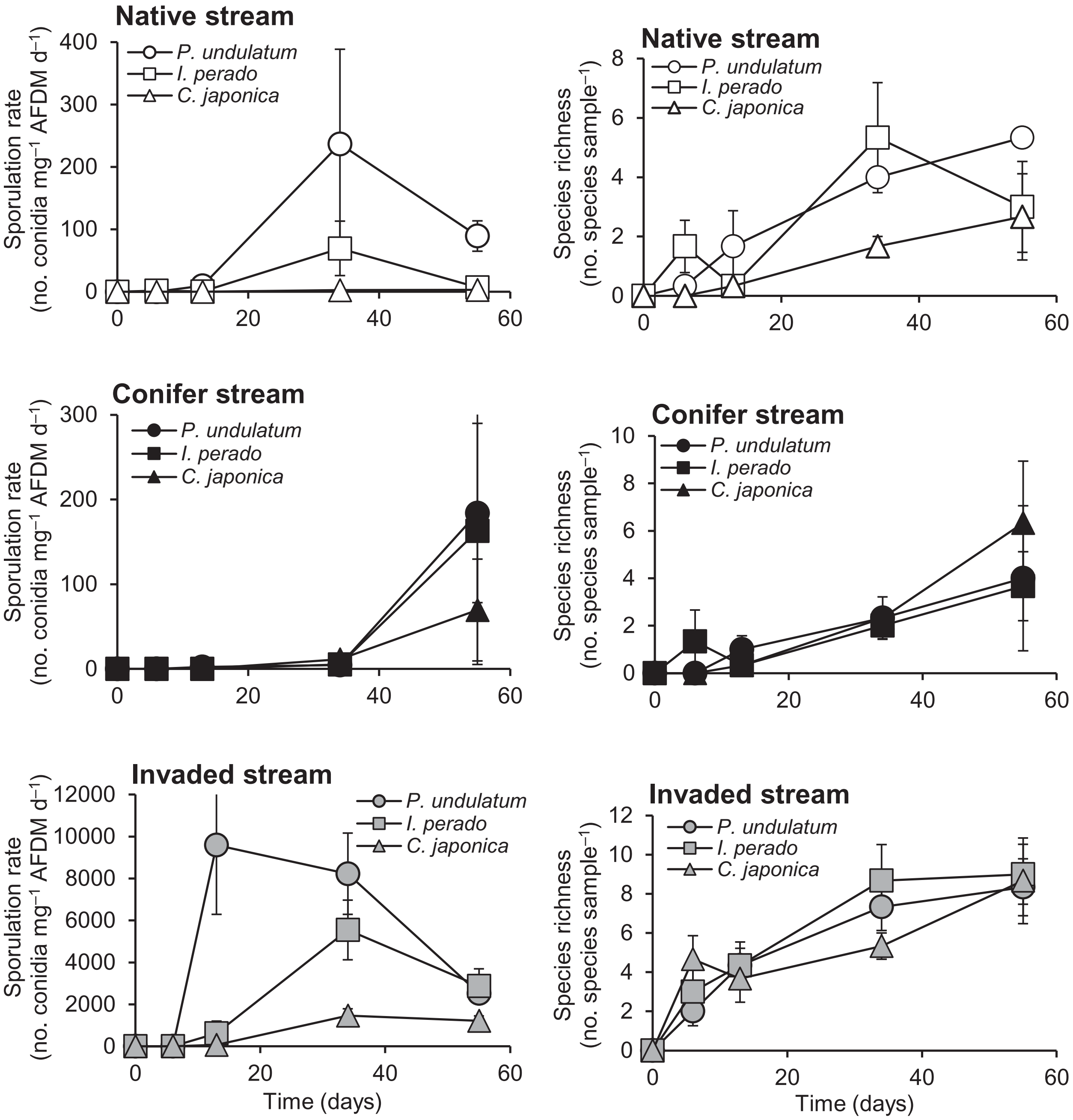

Fig. 7. Sporulation rates and species richness of aquatic hyphomycetes associated with *I. perado*, *C. japonica* and *P. undulatum* litter incubated in streams flowing through native vegetation (Native stream), conifer plantations (Conifer stream) and forests invaded by exotic species (Invaded stream) (one stream per type) over 55 days in winter 2014/2015. Values are means ± SE; SE bars are smaller than symbols in most cases.

Aquatic hyphomycete communities

Species richness generally increased over the incubation period (3-way ANOVA, $p < 0.001$; Table 8), with a maximum of nine species per sample being found on *I. perado* leaves on day 34 and day 55 and on *C. japonica* needles on day 55 in the invaded stream (Fig. 6). No significant differences in species richness per sample were found among litter species (3-way ANOVA, $p < 0.275$; Table 8). Species richness significantly differed among streams (3-way ANOVA, $p < 0.001$; Table 8), with significantly higher values in the invaded stream (maximum values: 8 – 9 species sample^{-1}) than in the native (3 – 5 species sample^{-1}; Tukey test, $p < 0.001$) or conifer stream (4 – 6 species sample^{-1}; Tukey test, $p < 0.001$) for all litter species, while no significant differences were found between native and conifer streams (Tukey test, $p = 0.775$) (Fig. 6).

Table 8. Summary table for 3-way ANOVAs performed on aquatic hyphomycetes sporulation rates and species richness (both log(x+1)-transformed) associated with three litter species incubated in three streams differing in riparian vegetation (one stream per type) over 55 days in winter 2014/2015.

Source of Variation	df	MS	F	p
Sporulation rate				
Intercept	1	198.990	508.308	< 0.001
Time	2	9.182	23.454	< 0.001
Litter species	2	5.051	12.902	< 0.001
Stream	2	45.395	115.958	< 0.001
Stream × Litter species	4	0.884	2.258	0.075
Stream × Time	4	0.91	2.326	0.068
Litter species × Time	4	0.384	0.981	0.426
Stream × Litter species × Time	8	0.832	2.124	0.049
Error	54	0.391		
Species Richness				
Intercept	1	27.444	568.873	< 0.001
Time	2	1.227	25.438	< 0.001
Litter species	2	0.064	1.324	0.275
Stream	2	1.457	30.193	< 0.001
Stream × Litter species	4	0.075	1.564	0.197
Stream × Time	4	0.05	1.046	0.392
Litter × Time	4	0.032	0.659	0.623

Stream × Litter species × Time	8	0.024	0.494	0.855
Error	54	0.048		

Thirty-eight species of aquatic hyphomycetes were found associated with decomposing litter in this study (Appendix 1). Fungal communities in the conifer stream were dominated by *Heliscus lugdunensis* Sacc. & Therry (45 – 66% relative abundance) while those in the invaded stream were dominated by *Lunulospora curvula* Ingold (30 – 55%) (Fig. 8). In the native stream, *Tetracladium maxilliforme* (Rostrup) Ingold (25%) and *Tricladium chaetocladium* Ingold (19%) contributed the most to spore production in *I. perado* leaves, *T. chaetocladium* dominated spore production in *C. japonica* needles (44%) and *Articulospora tetracladia* Ingold dominated spore production in *P. undulatum* leaves (41%) (Fig. 8). Fungal communities were thus primarily structured by stream type (ANOSIM, global R = 0.54, p = 0.001), with communities in the three streams being clearly separated in the bi-dimensional space (R > 0.50, p = 0.001 for pair-wise comparisons) (Fig. 9). Litter species had a secondary role in structuring fungal communities (ANOSIM, global R = 0.16, p = 0.001), with significant differences being found only between *P. undulatum* and *I. perado* (R = 0.14, p = 0.006) or *C. japonica* (R = 0.25, p = 0.001), but not between *I. perado* and *C. japonica* (R = 0.08, p = 0.056) (Fig. 9).

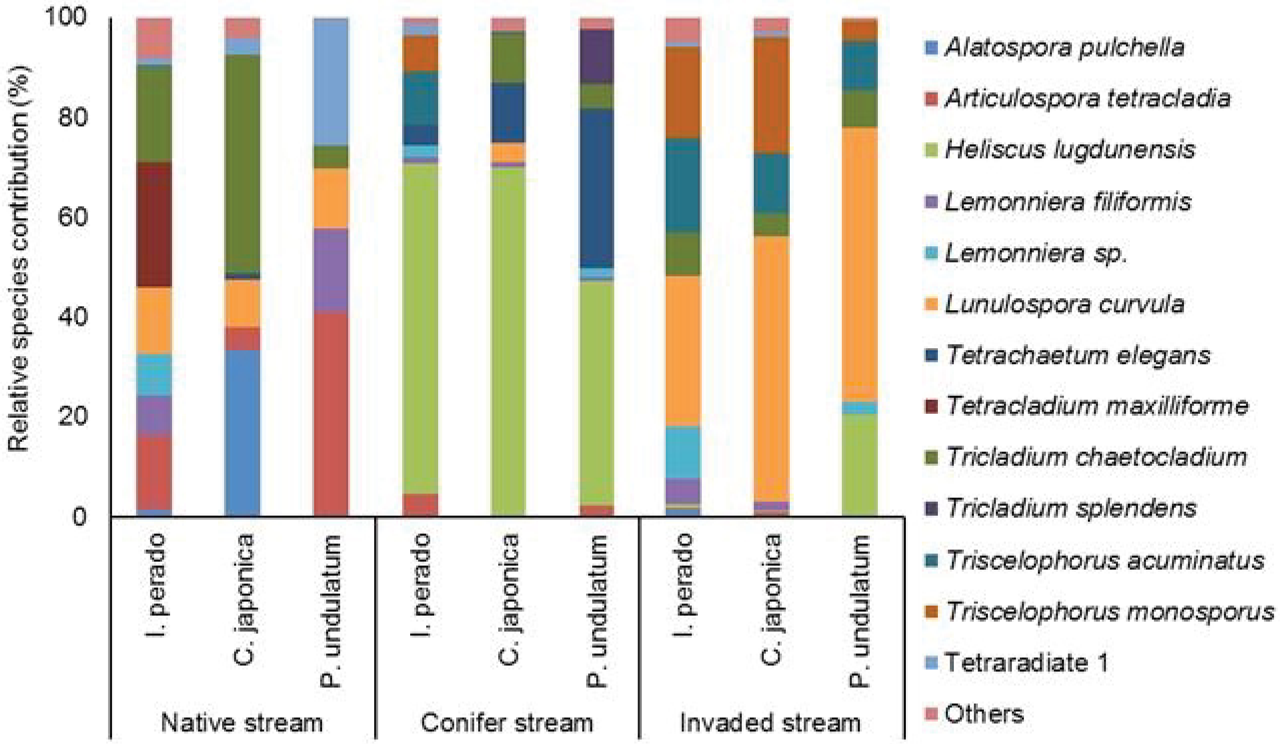

Fig. 8. Relative abundance (based on spore production) of aquatic hyphomycete species associated with *I. perado*, *C. japonica* and *P. undulatum* litter incubated in streams flowing through native vegetation (Native stream), conifer plantations (Conifer stream) and forests invaded by exotic species (Invaded stream) (one stream per type) over 55 days in winter 2014/2015.

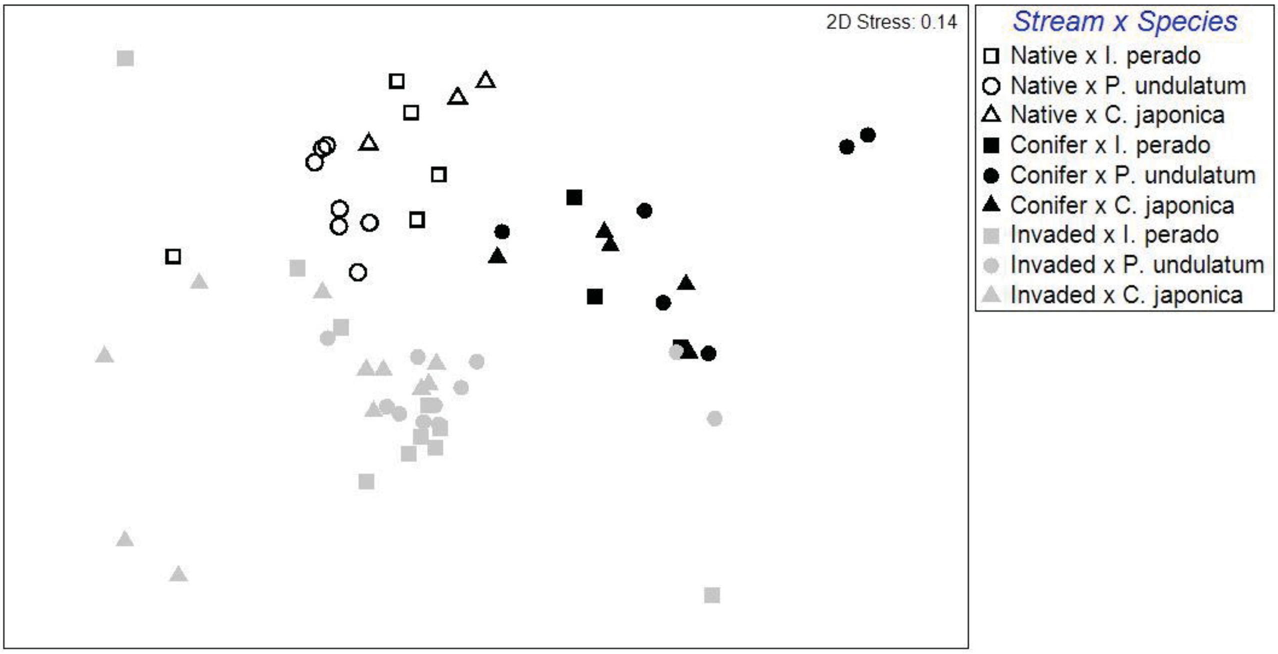

Fig. 9. NMDS of aquatic hyphomycete communities (based on spore production) associated with *I. perado*, *C. japonica* and *P. undulatum* litter incubated in streams flowing through native vegetation (Native stream), conifer plantations (Conifer stream) and forests invaded by exotic species (Invaded stream) (one stream per type) over 55 days in winter 2014/2015.

Discussion

This study shows that forest changes in São Miguel island may impact stream ecosystem functioning and fungal decomposers, but the effects strongly depend on the type of forest change and on litter identity.

Conifer plantations affect aquatic hyphomycete community structure but not reproductive activity and litter decomposition

Decomposition rates of *P. undulatum* and *I. perado* leaves and *C. japonica* needles, and species richness and reproductive activity of litter associated aquatic hyphomycetes, did not significantly differ between conifer and native streams, suggesting no effect of the replacement of native forests by conifer plantations on the heterotrophic pathway. The absence of an effect of conifer plantations on litter decomposition was observed for the three litter species used, despite the fact that they markedly differed in their physical and chemical characteristics, which suggests that this response is independent of litter characteristics. This can be partially explained by the lack of significant differences in water variables between stream types. This result contrasts with that of Martínez et al. (2013) who found an inhibition of the decomposition of alder (*Alnus glutinosa* Gaertn.) leaves, but not pine (*Pinus radiata* D. Don) needles, in streams draining pine plantations compared with native deciduous streams. In turn, Whiles & Wallace (1997) found a stimulation of decomposition rates of pine and red maple (*Acer rubrum* L.) litter in pine streams compared to hardwood streams. Nevertheless, other studies found no differences in decomposition rates between streams with native riparian vegetation and streams flowing through conifer plantations, despite the significant differences found in terms of shredder community structure between stream types (Riipinen et al., 2010; Hisabae et al., 2011). These different effects of forest change on decomposition rates among studies may be due to the differences in the identity of the litter used or to differences in the type of conifer plantation (Ferreira et al., 2016a).

However, aquatic hyphomycete community structure differed between native and conifer streams with some fungal species present only in one stream type and marked differences in community evenness with communities in the conifer stream being clearly dominated by *H. lugdunensis* while those in the native stream are more even. This result concords with other studies that found differences in community structure between native streams and streams with changed riparian vegetation (Bärlocher and Graça 2002; Ferreira et al., 2006a). The fact that these changes in decomposer community structure were not translated into differences in litter decomposition rates between stream types suggests some degree of functional redundancy among decomposer communities (Bärlocher and Graça 2002; Ferreira et al., 2006a).

Invasion of native forests by exotic plant species may affect aquatic hyphomycete communities and litter decomposition

Decomposition rates of *P. undulatum* and *I. perado* leaves, but not of *C. japonica* needles, were significant higher in invaded than native streams. Differences between stream types may have been due to changes in forest composition. However, differences between stream types in our study may also be due to differences in water temperature, nutrients and pH, known as moderating factors of ecosystem functioning, which were higher in invaded than native streams.

Differences in the response of litter decomposition to stream type among litter species may be due to differences in litter characteristics. Conifer needles are considered as a poor quality resource for microbial decomposers due to its toughness, low nitrogen concentration and elevated levels of lignin and polyphenols (Sedell et al., 1975; Bärlocher and Oertli, 1978; Quinn et al., 2000a, b; Girisha et al., 2003). Litter quality was reported in Kennedy and Hobbie (2004) as being the main factor that influenced decomposition rates, where saltcedar (*Tamarix ramosissima* Ledeb) invasion of riparian areas altered litter decomposition rates through its influence on litter quality rather than by altering the environment for decomposition.

The stimulation of litter decomposition rates was observed in studies addressing the invasion of native deciduous forests by *Fallopia japonica* (Houtt.) Dcne (Lecerf et al., 2007) and the invasion of eucalypt native forests by *Salix babylonica* SB (Schulze and Walker, 1997). In contrast, studies addressing the invasion of native deciduous forests by *T. ramossisima* (Kennedy and Hobbie, 2004), *Rhododendron ponticum* L. (Hladyz et al., 2011) or *Prunus padus* L. (Roon et al., 2014) found an inhibition of litter decomposition rates in invaded streams. However, because these studies have addressed the invasion of riparian areas by different species, comparisons among studies are difficult.

Similarly to decomposition rates, sporulation rates and species richness were also higher in the invaded than in the native stream. This may be due to higher water temperature and nutrients concentrations in the invaded stream. Higher water temperature and nutrient availability have been shown to stimulate fungal activity (Chauvet and Suberkropp 1998; Gulis and Suberkropp, 2003; Gulis et al., 2006; Ferreira and Chauvet, 2011). The influence of higher water temperature was also detected on fungal communities as the dominant species in invaded streams was *Lunulospora curvula*, followed by *Triscelophorus monosporus*, which are two species known as being ‘warm water’ species. More favourable conditions (e.g. higher nutrient availability) in invaded streams may also explain the higher species richness compared with native streams.

Litter identity moderates de effects of forest change

Decomposition rates, aquatic hyphomycete sporulation rates and species richness were higher for *P. undulatum* leaves than for *I. perado* leaves and *C. japonica* needles, with no significant differences between the latter two. This can be explained by differences in litter characteristics. Substrates that are softer and have higher specific leaf area, lower lignin concentration and higher nutrients concentrations (as in the case of *P. undulatum*) are more attractive to aquatic hyphomycetes that colonize these substrates first and decompose them faster than more recalcitrant substrates (e.g. *I. perado* and *C. japonica*). Previous studies have shown that leaf toughness was the primary determinant of decomposition rates, which were negatively correlated with leaf strength (Li et al., 2009) and lignin is known as a strong inhibitor of litter decomposition (Lecerf and Chauvet, 2008b; Schindler and Gessner, 2009; Frainer et al., 2015), also in São Miguel streams (Ferreira et al., 2016b). The strong leaching phase observed only for *P. undulatum* leaves also contributed to explain the higher decomposition rates of this species.

Conclusions

In summary, this study contributes to fill the knowledge gap about the effects of forest change on stream ecosystem functioning. Conifer plantations do not seem to strongly affect microbial decomposer activity and litter decomposition in Azorean streams, likely because the aquatic communities are already adapted to low quality litter and are highly redundant, and there is no significant change in water characteristics in conifer streams. However, changes in aquatic hyphomycete community structure in conifer streams may have unpredictable effects in case of additional environmental change. Thus, maintenance of a buffer of native riparian forest in conifer plantation may be recommended. Litter decomposition was stimulated in invaded streams compared with native streams, which could result from forest change or be due to their higher water temperature and nutrient availability in invaded streams. Since invasion of native forests by exotic species occurs mostly at lower elevations, where it is not possible to find native streams, it is difficult to evaluate the extent to which invasion is affecting stream ecosystem functioning.

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Appendix 1. Relative abundance (%; based on spore production) of aquatic hyphomycete species associated with *I. perado*, *C. japonica* and *P. undulatum* litter incubated in streams flowing through native vegetation (Native stream), conifer plantations (Conifer stream) and forests invaded by exotic species (Invaded stream) (one stream per type) over 55 days in winter 2014/2015.

Stream type	Native stream			Conifer stream			Invaded stream		
Litter species	*I. perado*	*C. japonica*	*P. undulatum*	*I. perado*	*C. japonica*	*P. undulatum*	*I. perado*	*C. japonica*	*P. undulatum*
Alatospora acuminata Ingold						0.1	1.2		0.2
Alatospora pulchella Marvanová	1.5	33.3					1.6	0.1	
Anguillospora longissima (Sacc. & P. Syd.) Ingold	0.1						0.4		
Articulospora tetracladia Ingold	15.0	4.8	41.2	4.6	0.1	2.2	0.1	0.8	0.1
Campylospora sp.							< 0.1		
Clavatospora aquatica de Wild.					1.9		0.1	< 0.1	
Clavatospora longibrachiata (Ingold) Marvanová & Sv. Nilsson								0.1	
Flagellospora curvula Ingold					0.5		< 0.1		
Fontanospora eccentrica (Petersen) Dyko			0.2	0.1				0.1	0.1
Fontanospora fusiramosa Marvanová, Peter J. Fisher & Descals							< 0.1		
Heliscella stellata (Ingold & V. J. Cox) Marvanová									0.1
Heliscus lugdunensis Sacc. & Therry				66.0	70.0	45.0	0.9	0.2	20.3
Lemonniera aquatica de Wild.	3.3			1.4			0.3	0.2	0.2
Lemonniera filiformis R.H. Petersen	7.9		16.6	1.4	0.9	0.8	5.0	1.9	
Lemonniera pseudofloscula Dyko	0.1				0.2	2.1	1.3	0.1	< 0.1
Lemonniera sp.	8.3			2.2		1.7	10.6	0.1	2.6
Lemonniera terrestris Tubaki							< 0.1	0.5	
Lunulospora curvula Ingold	13.4	9.5	12.1		4.0	0.1	30.0	53.1	54.7
Mycocentrospora sp.	0.2								
Pleuropedium multiseptatum Marvanová & Descals		4.2							
Sigmoid								1.2	
Stenocladiella neglecta (Marvanová & Descals) Marvanová & Descals	0.3					0.2			< 0.1

Tetrachaetum elegans Ingold		1.2		4.3	12.0	32.0		0.1	
Tetracladium furcatum Descals							< 0.1		
Tetracladium marchalianum de Wild.							1.4	0.1	0.1
Tetracladium maxilliforme (Rostrup) Ingold	25.0								
Tetracladium setigerum (Grove) Ingold	2.9								
Tricellula aquatica Webster	0.2								
Tricladium chaetocladium Ingold	19.0	43.8	4.5		9.8	4.6	8.8	4.6	7.6
Tricladium curvisporum Descals			0.1						
Tricladium patulum Marvanová & Marvan	1.2					0.1			
Tricladium sp.									< 0.1
Tricladium splendens Ingold					0.3	11.1			
Triscelophorus acuminatus Nawawi	0.5			10.7			18.8	12.1	9.8
Triscelophorus monosporus Ingold				7.1			18.2	23.2	3.8
Tetraradiate 1	1.2	3.3	25.3	2.1	0.3		1.2	1.0	0.1
Tetraradiate 2	0.1		0.1					0.6	< 0.1
Tetraradiate 3									< 0.1
Total species richness (no. species treatment^{-1})	18	7	8	10	11	12	21	20	18