Dynamics of yeast populations recovered from decaying leaves in a nonpolluted stream: a 2-year study on the effects of leaf litter type and decomposition time

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Abstract

Here we report on the results of a survey of the yeast populations occurring on submerged leaves (alder, eucalyptus and oak) in a natural mountain stream, during different phases of their decomposition and through two consecutive years. Leaf litter mass loss, total yeast counts, Shannon–Weiner index (H'), yeast community structure and physiologic abilities were analyzed to evaluate the dynamics of yeast communities during decay. Seventy-two yeast taxa were recorded, and in all litter types, species of basidiomycetous affinity predominated over ascomycetous ones. Discriminant analysis of presence/absence data (yeast species) showed significant differences both among substrate types ($P < 0.0026$) and with decomposition time ($P < 0.0001$). Carbon and nitrogen source utilization by yeast strains also varied with the substrate ($P < 0.0001$) and decomposition time ($P < 0.0001$). Further conclusions were that: (1) all litter types have in common ubiquitous yeast species, such as *Cryptococcus albidus*, *Debaryomyces hansenii* and *Rhodotorula glutinis*, among the common 20 yeast species; (2) only a few species were dominant, and most species were rare, being recorded once or twice throughout decomposition; and (3) the order of yeast appearance, and their substrate assimilation patterns, strongly suggest a succession phenomenon. Finally, explanations for the distribution patterns and variations in yeast communities are discussed.

Introduction

Leaf decomposition is the major source of carbon and energy for microbial growth in freshwater mountain streams (Fisher & Likens, 1973) and is a complex event, involving both abiotic and biotic processes (Vannote *et al.*, 1980). Whereas macroinvertebrates help to break down plant matter, heterotrophic microorganisms – filamentous fungi, yeasts and bacteria – are involved in the degradation of macromolecules such as cellulose, hemicelluloses, pectin, proteins and lignin, thus providing regeneration of metabolites. In general, fungi (including yeasts) are more significant as leaf-decaying agents than are bacteria, but the relevance of these two groups depends mainly on the duration of the decomposition process and the nutrient levels. Nevertheless, it is widely accepted that fungi are primary colonizers, whereas bacteria are secondary colonizers (Dilly *et al.*, 2001). The phyllosphere, in particular, is a common niche for yeasts (Phaff & Starmer, 1987; Lachance & Starmer, 1998; Inácio *et al.*, 2002). In these habitats, yeasts and yeast-like fungi dominate the leaf surface, especially during the growing season. Several researchers have performed significant studies on the microbial communities in leaves of terrestrial plants, thereby providing a considerable pool of knowledge on aerial and phylloplane yeasts (Chand-Goyal & Spotts, 1996) as well on decaying fruits (Morais *et al.*, 1995).

In contrast to phylloplane microbiology, information on the microbiology of submerged leaves is scarce and especially focused on bacterial and mycelial fungi (Gessner & Chauvet, 1994; Chauvet *et al.*, 1997; Pascoal *et al.*, 2005). However, yeasts have also been isolated from decaying leaves of the salt marsh macrophyte *Spartina alterniflora* (Buchan *et al.*, 2002). More recently, unexpectedly high yeast populations have been reported in submerged decaying leaf litters,

either by culture-dependent methods (Sampaio *et al.*, 2004) or using molecular techniques (Nikolcheva & Bärlocher, 2004). In fact, freshwater yeast populations have been almost ignored, despite the fact that they are common in aquatic systems (Hagler & Ahearn, 1987; Gadanho & Sampaio, 2004), where their distribution mainly depends on the properties and type of water (fresh water or seawater), pollution level, and season (Hagler & Mendonça-Hagler, 1981; Sláviková & Vadkertiová, 1997; Almeida, 2005).

In this article, the results of long-term studies (171 days) carried out in two consecutive years, on the saprophytic yeasts that colonize autochthonous and exotic leaf litters submerged in a nonpolluted mountain stream are presented. The main aims were to: (1) identify the cultivable yeast populations present in decaying leaves; (2) study the structure (species composition) of the yeast communities during decomposition in an aquatic system; (3) study the metabolic profile of yeast communities; and (4) evaluate the relationships between leaf litter types and yeast populations.

Materials and methods

Study area

The study site is located in the River Olo, a second-order stream in the Alvão Natural Park, in northeastern Portugal. The watercourse length is 40 km, and there is a catchment basin area of 143.8 km^2 with low human impact. However, in the lower reaches of this basin, forestation with exotic species such as pine (*Pinus pinaster* Ait. and *Pinus sylvestris*, L.) and eucalyptus (*Eucalyptus globulus* Labill.) has taken place. In the 2 years of study, the minima and maxima for several physical–chemical parameters of water quality were: pH 5.2–5.5, conductivity 18.5 –22.8 $\mu S\,cm^{-1}$, temperature 9.1–12.0 °C, dissolved oxygen 10.4 –12.9 $mg\,L^{-1}$, chemical oxygen demand 1.0–2.0 $mg\,L^{-1}$, nitrate 0.06–0.10 $mg\,L^{-1}$, nitrite 0.007– 0.0082 $mg\,L^{-1}$, ammonium 0.003 – 0.004 $mg\,L^{-1}$, total nitrogen 3.9–4.98 $mg\,L^{-1}$, and orthophosphate 0.04–0.05 $mg\,L^{-1}$. Alder [*Alnus glutinosa* (L.) Gaertn.], willow (*Salix atrocinerea* Brot.), ash (*Fraxinus angustifolia* Vahl.) and oaks (*Quercus pyrenaica* Willd and *Quercus robur* L.) characterize the riparian vegetation.

Litterbags

Healthy and senescent leaves of adult trees from the species *Al. glutinosa*, *E. globulus* and *Q. robur* were collected, air dried at 40 °C (48 h), to kill the phylloplane populations, and stored until use. Dehydrated leaves were weighed (4.0 ± 0.1 g per litterbag), and placed in 5-mm mesh bags (20×15 cm). The litterbags were attached to nylon ropes, and firmly anchored to the streambed, at about 1.5 m from the water surface. After 1, 7, 14, 28, 56, 112 and 171 days of immersion (from the end of January to June), seven bags of each leaf species were collected from the water, sealed in a sterile plastic bag, and transported to the laboratory in a cool box.

Agar plate counts of colonizing yeasts

Three leaf litterbags of each species before stream incubation (day 0) and those collected at the sampling periods mentioned above were aseptically transferred to Erlenmeyer flasks containing 100 mL of sterilized 0.1% (w/v) peptone water. Flasks were then submitted to a three-step process: shaking at 100 r.p.m. (Certomat H, B. Braun, Germany) for 30 min, sonication in an ultrasonic bath (Sonorex Super 10P) at 35 kHz for 3 min, and vortexing. To determine the viable counts of yeasts, aliquots of 100 μL were spread onto plates containing Wort agar (Difco, Detroit), acidified with lactic acid to pH 3.5 (Wort-Lac agar), to prevent bacterial growth, and the dilution plate-count technique was used. For each dilution, we took three replicates. The plates were incubated at 23 ± 2 °C for 5 days, after which colonies were counted and expressed as CFUs per milliliter of undiluted peptone water. In order to express the results as CFUs per foliar residual dry weight (DW), leaves were oven dried (104 °C) to a constant weight.

Yeast identification

The different yeast colonies were isolated and maintained on YMA (0.3% malt extract, 0.3% yeast extract, 1.0% glucose, 0.5% peptone, and 2.0% agar) at 4 °C. Yeast identification was performed with the standard morphologic and physiologic tests according to Barnett *et al.* (2000). Yeast isolates that could not be properly identified were studied by sequence analysis of the D1/D2 domains of the 26S rRNA gene. Total DNA was extracted using the protocol of Sampaio *et al.* (2001) and the modifications introduced by Gadanho *et al.* (2003) after culture growth on MYP agar (0.7% malt extract, 0.05% yeast extract, 0.25% soytone, and 1.5% agar). DNA was amplified using primers ITS5 (5′-GGA AGT AAA AGT CGT AAC AAG G-3′) and LR6 (5′-CGC CAG TTC TGC TTA CC-3′). Cycle sequencing of the 600–650-bp region at the 5′-end of the 26S rRNA gene D1/D2 domain employed forward primer NL1 (5′-GCA TAT CAA TAA GCG GAG GAA AAG) and reverse primer NL4 (5′-GGT CCG TGT TTC AAG ACG G). The amplified DNA was sequenced in an Amersham Pharmacia ALF express II automated sequencing system, using standard protocols. The sequences obtained were compared with those deposited at the GenBank database (National Center for Biotechnology Information, NCBI) and identified them using the Basic Local Alignment Search Tool (BLAST), also available from the NCBI.

In order to analyze the metabolic profiles of the isolated yeast strains, 46 carbon and nitrogen sources traditionally utilized on yeast identification were used (Barnett *et al.*, 2000).

Data analysis and statistics

In order to determine whether yeast densities varied with leaf litter species and time of decomposition, the data were normalized by log transformation [log $(x+1)$] before two-way factorial ANOVA was performed (STATVIEW 4.53). The data was normalized after performing the Shapiro–Wilks W normality test (STATISTICA, 1999). The test was repeated after normalization, to ensure that the transformation satisfied the normal distribution.

The species richness (S), the frequency of occurrence of each species and the number of yeasts per sample were recorded and used to calculate the Shannon–Wiener diversity index (H'), using PRIMER 5.2.2 (Clarke & Gorley, 2001).

For each litter species and at each incubation period, the number of positive records of a species per total number of samples was scored as a single record, regardless of the abundance of a given species, giving the percentage frequency of isolation, which, in the data matrix, substitutes the value 1 (Parente & Ricciardi, 2002). Other multivariate data, such as those generated by carbon and nitrogen utilization by each yeast strain, received the same treatment as the data for species presence/absence.

To determine which substrates/yeast species (quantitative variable) contributed to the discrimination among the different litter types, and the sampling days (categorical groups), the raw data by the multiple discriminant analysis (MDA) were analyzed. For this purpose, a forward stepwise analysis was computed with the package STATISTICA (1999).

Results

Foliar mass loss and yeast colonization of submerged leaves

Alder leaves presented the highest mass loss values, reaching more than 95.0% by day 112 (Fig. 1a). By contrast, oak and eucalyptus leaves proved to be more recalcitrant than alder. Parallel observations of yeast population densities were conducted during different phases of decomposition over a 171-day period (Fig. 1b). Day 0 was not included, because we did not obtain any fungal growth in all litter types. However, for alder leaves, the last sampling period was day 112. In oak samples, variation of the yeast counts was less pronounced throughout decomposition than for eucalyptus or alder. Alder yeast densities, recorded in the samples of early decomposition stages, exhibited the highest values when compared with the other two litter types. Significant statistically differences ($F=39.612$, $P<0.0001$) were found in the yeast

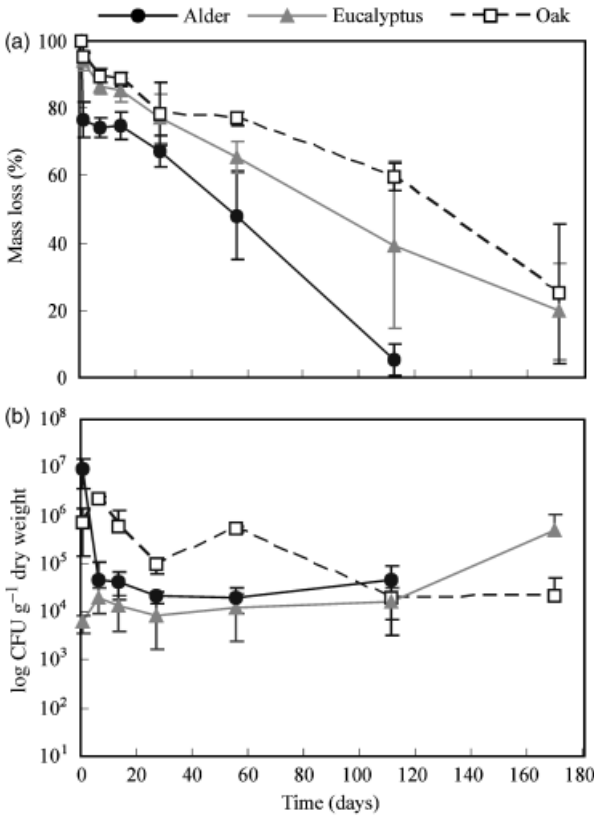

Fig. 1. Temporal dynamics of litter mass loss and yeast density in alder, oak and eucalyptus litters. (a) Dry mass loss in percentage (mean ± SD, eight replicates). (b) Semilogarithmic plot of yeast CFU per unit foliar dry weight (mean ± SD, six replicates).

counts among the three litters (eucalyptus < alder < oak). Moreover, ANOVA analysis also demonstrated significant differences ($F=6.035$, $P<0.0001$) when the period of decomposition was considered and when both factors (litter × time) were combined ($F=11.648$, $P<0.0001$). Taken together, the results clearly show that the yeast colonization densities were different between leaf litter types and throughout the decay period.

Species richness and diversity

In total, 72 yeast species belonging to 26 genera from the three decomposing substrates were recovered. Figure 2 provides a complete list of the species found in each leaf litter type. 'Black yeasts' were also observed in the studied plant litters, but identification down to the species level was done only for *Aureobasidium pullulans*. Therefore, the remaining 'black yeasts' were not included in this study. Globally, in all samples from the three leaf litters, the results show a clear predominance of basidiomycetous yeast isolates, with values around 78%, 82% and 85% for alder, oak and eucalyptus, respectively. Despite being less abundant,

Incubation Days

Taxon	1	7	14	28	56	112
Aureobasidium pullulans	+	+	+			
Bullera pyricola	+					
Candida maltosa	+					
Cryptococcus albidus	+	+	+		+	
Cr. laurentii	+	+	+	+		
Cr. podzolicus	+					
Rhodotorula foliorum	+	+				
Rh. graminis	+	+				
Rh. hyalophila	+	+				
Rhodosporidium shaerocarpum	+					
Tremella foliacea	+	+				
Trichosporon mucoides	+					
Cr. aerius		+			+	
Cr. amylolentus		+				
Cr. luteolus		+				
Cr. macerans		+	+ (*)		+ (*)	
Cr. skinneri		+				
Fitobasidium floriforme		+				
Leucosporidium scottii		+				
Rh. aurantiaca		+			+	
Rh. colostri		+	+ (*)	+	+	+
Rh. creatinivora		+				
Rh. glutinis		+			+	+
Willopsis saturnus		+				
B. alba			+			
Cystofilobasidium capitatum			+			
Cy. infirmo-miniatum			+			
Metschnikowia bicuspidata			+		+	
Tr. moniliiforme			+			
C. bertae				+		
C. castrensis				+		
C. sake				+		
C. valdiviana				+		
C. vartiovaarae				+		
Cr. fuscescens				+		
Cr. humicolus				+		
Pichia anomala				+		
Debaryomyces occidentalis				+		
C. haemulonii					+	
D. hansenii					+	
Fibulobasidium inconspicuum					+	
Tr. cutaneum					+	
Wickerhamiella domercqiae					+	
Lipomyces tetrasporus					+	
Rhodosporidium toruloides						+

Alder

Taxon	1	7	14	28	56	112	171
Aureobasidium pullulans	+	+	+				
Bullera alba	+						
Candida castrensis	+						
C. ishiwadae	+						
C. variovaarae	+						
Cryptococcus albidus	+	+	+	+	+	+	
Cr. amylolentus	+						
Cr. laurentii	+	+				+	
Cr. podzolicus	+						
Cr. macerans	+						
Cystofilobasidium bisporidii	+						
Cy. capitatum	+						
Cy. infirmo-miniatum	+						
Debaryomyces hansenii	+						
Leucosporidium scottii	+						
Rhodotorula aurantiaca	+						
Rh. foliorum	+						
Rh. fragaria	+						
Rh. glutinis	+						
Tr. cutaneum	+						
Xanthophyllomyces dendrorhous	+	+	+				
Bullera nirakii		+					
Cr. albidosimilis		+					
Rh. colostri		+ (*)	+ (*)	+	+ (*)	+	
Bullera penniseticola			+				
Candida etchellsii			+		+	+	
C. valdiviana			+		+		
Filobasidium floriforme			+				
Fibulobasidium inconspicuum			+				
Fellomyces polyborus			+		+		
Rhodosporidium shaerocarpum			+	+ (*)			
Sporidiobolus salmonicolor			+				
Sterigmatosporidium polymorphum			+				
Tr. moniliiforme			+				
Kluyveromyces marxianus					+		
Rh. crocea					+		
Tremella foliacea					+		+
Cr. ferigula						+	
Cr. terreus						+	+
Cystofilobasidium sp.						+	
P. anomala						+	+
Willopsis saturnus						+	+
Citeromyces matritensis							+
Rh. minuta							+

Oak

Eucalyptus

Fig. 2. Isolated yeast species colonizing alder, oak and eucalyptus leaf litters at different stages of degradation by their order of appearance. Asterisk indicates molecular identification for at least one isolate.

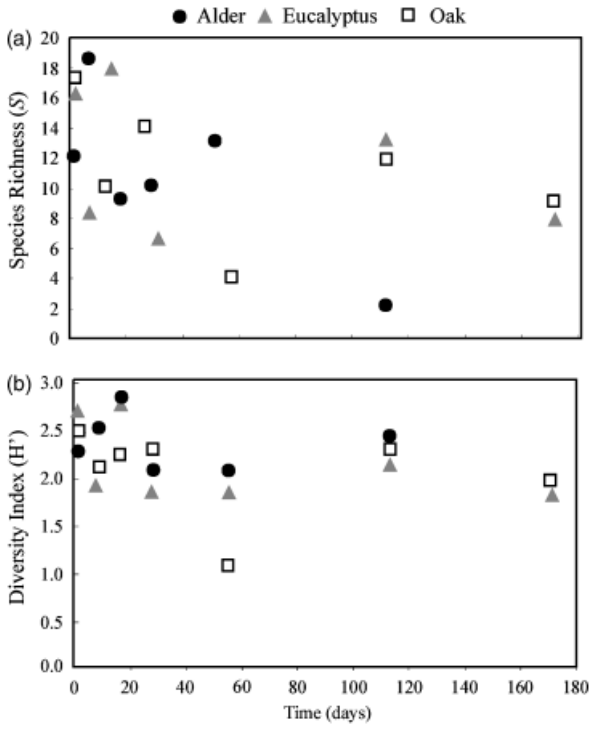

Fig. 3. Temporal dynamics of species richness and diversity in alder, oak and eucalyptus litters. (a) Number of yeast species (*S*) isolated by culture-dependent techniques. (b) Shannon–Wiener diversity index (*H*′).

ascomycetous yeast species presented higher diversity than basidiomycetous yeasts. In the case of alder leaves, yeast species of ascomycetous affinity such as *Au. pullulans*, several species of *Candida*, *Metschnikowia bicuspidata*, and *Pichia anomala*, were dominant at the early decomposition stages (days 1–7), whereas *Debaryomyces hansenii*, *Debaryomyces occidentalis*, *Lipomyces tetrasporus*, *Williopsis saturnus* and *Wickerhamiella domercqiae* dominated at the intermediate stages (days 28 and 56). In addition, the colonization pattern of yeast species was analyzed with regard to their order of appearance during the decomposition of alder, oak and eucalyptus leaves (Fig. 2). Only a few species showed a persistent presence (three or more sampling periods), namely: *Cryptococcus laurentii*, *Cr. albidus*, *Rhodotorula colostri* and *Rh. glutinis* for alder litter; *Cr. albidus*, *Cr. laurentii*, *Rh. colostri* and *Sporobolomyces roseus* for oak litter; and *Cr. laurentii*, *Cystofilobasidium capitatum*, *Leucosporidium scottii*, *Trichosporon cutaneum*, *Rh. colostri* and *Cr. albidus* for eucalyptus litter. It is important to note that all these species are of basidiomycetous affinity.

Concerning yeast species richness (*S*) with duration of decomposition, the highest values were obtained for days 1 and 7 (Figs 2 and 3a). This is not surprising, as the yeasts present in the water stream colonize the intact leaves. After the initial colonization, and in all the leaf litter types, *S* declined and recovered again, but never reached the initial values. Shannon–Wiener diversity (*H*′) revealed a similar pattern to *S* values (Fig. 3b), with the highest values being reached in alder, followed by eucalyptus and oak litters. In fact, the lowest *H*′ values at all sampling periods were found in oak leaf litter.

Yeast community analysis according to duration of decomposition and among leaf litters

The MDA of the yeast species frequency (litter as the discriminant factor) showed distinct yeast communities among the litter types (Wilks $\lambda = 0.00024$, $F = 7.445$, $P < 0.0026$). The first root function, explaining 98.6% of the total discriminant power, revealed that eucalyptus samples were clearly separated from the remaining litters (Fig. 4a), and the yeast species responsible for this separation were *Xanthophyllomyces dendrorhous*, *Cryptococcus terreus*, *Le. scottii*, *Fellomyces polyborus*, *Tremella foliacea* and *Pic. anomala*. Species such as *Sporo. roseus*, *Wil. saturnus*, *Sterigmatosporidium polymorphum*, *Rh. glutinis*, *Rhodotorula minuta*, *Rhodotorula crocea*, *Cystofilobasidium bisporidii*, *Cystofilobasidium infirmo-miniatum*, *Sporidiobolus salmonicolor*, and *Filobasidium floriforme* correlated with the second root, which is responsible for the distinction between alder and *Cystofilobasidium* oak yeast communities.

The MDA using time as the discriminant factor (Fig. 4b) showed that yeast communities changed during decomposition (Wilks $\lambda = 0.00001$, $F = 5.800$, $P < 0.0001$). The first root function contributed 72.4% of the total discriminant power, exhibiting a temporal gradient. The species correlated with the first root and responsible for the discrimination between decomposition periods were *Cryptococcus amylolentus*, *Cy. bisporidii*, *D. hansenii*, *Rhodotorula graminis* and *X. dendrorhous*.

Yeast assimilation profiles according to duration of decomposition and among leaf litters

For the purpose of finding whether both yeast species colonization pattern and assimilating profiles were influenced by substrate modifications, we investigated the assimilation profiles of 44 carbon and nitrogen sources. MDA was applied to the data generated by each yeast strain in order to determine which nutritional variables contributed most to discrimination between the different litter species (three groups) and decomposition time (seven sampling periods). This MDA (litter factor) clearly showed a litter-type gradient (Fig. 4c). The model is based on 25 variables (Wilks $\lambda = 0.4647$, $F = 3.287$, $P < 0.0001$) and roots 1 and 2 account for 53.0% and 47.0% of the discriminant power, respectively. For root 1, the variables that made the most important contribution to discriminant power were lactose,

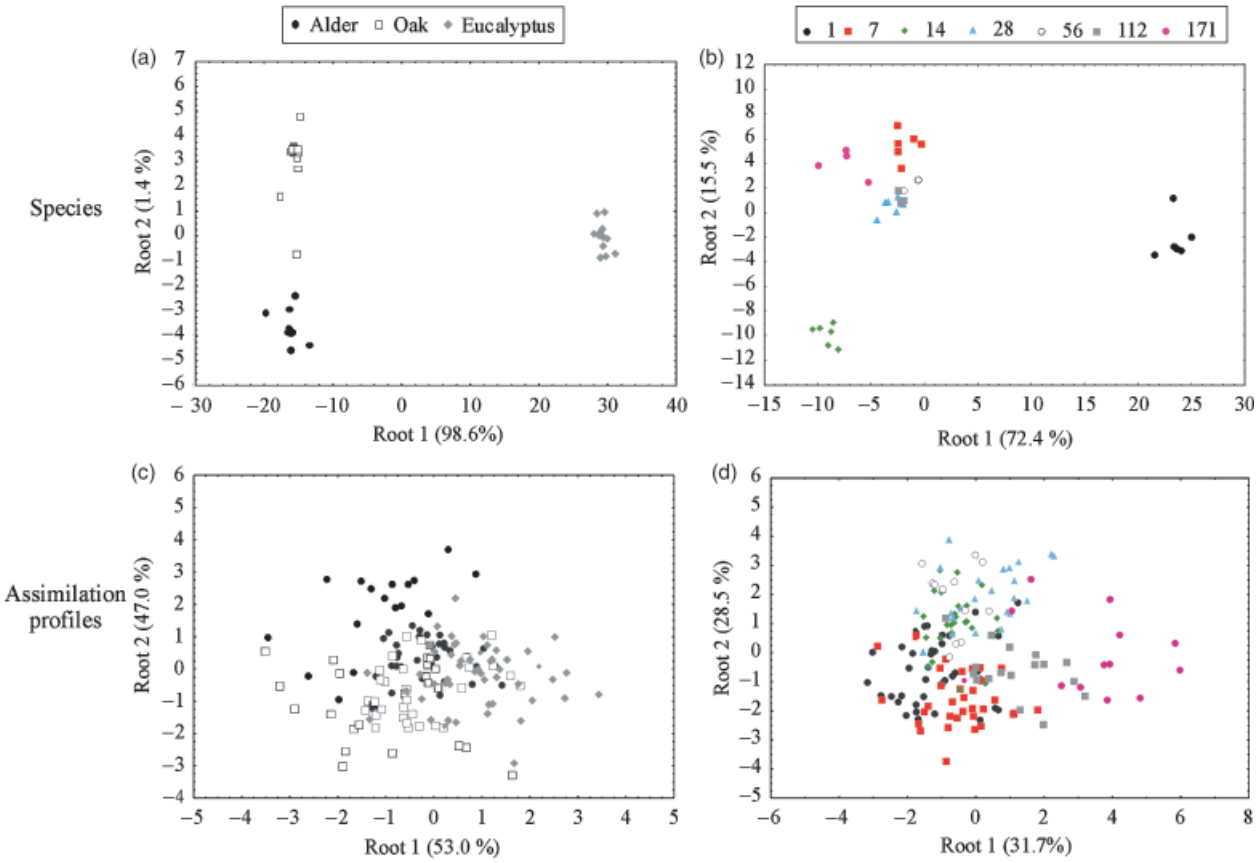

Fig. 4. Diagrams for the first two roots obtained by MDA of yeast species composition (a, b) and on yeast strain profile for the utilization of carbon and nitrogen sources (c, d). (a, c) Data labeled by litter type. (b, d) Data labeled by decomposition time.

galactitol, L-sorbose, salicin, cellobiose, urea hydrolysis, L-rhamnose and D-glucuronate. For root 2, D-arabinose, D-ribose, galactose, maltose, arbutin, trehalose, xylitol, erythritol, citrate, cadaverine and creatine were the most important. The assimilation abilities of yeast strains isolated from alder leaf were scattered between the first and second roots. In contrast, in the other two litters (oak and eucalyptus), yeast strains were more concentrated along the second discriminant function.

As shown in Fig. 4d, the MDA (time factor) revealed a temporal gradient related to nutrient utilization along the first discriminant function (day 1, days 7–171), and along the second root from day 7 to 171. Of the 44 initial variables, 39 contributed 60.2% of the discriminant power (Wilks $\lambda = 0.0266$, $F = 3.052$, $P < 0.0001$). Root 1 accounted for 31.7% of all discriminant power. Sucrose, L-lysine, nitrite, nitrate and cadaverine were the variables that made the most important contribution. Root 2 contributed 28.5% to the discriminant power, and included the contribution of variables such as ethanol, methanol, and cellobiose.

Discussion

In this study, yeast community composition during leaf litter decay was assessed by culture-dependent methods, which are widely used for the enumeration of microbial communities on leaves (Jacques & Morris, 1995; Müller *et al.*, 2003). Furthermore, a recent study of yeast diversity in an estuary (Gadanho & Sampaio, 2004) revealed a better performance of culture-dependent methods over molecular techniques (temperature gradient gel electrophoresis). Eucalyptus and oak leaves exhibited a lower mass loss than alder leaf litter, as previously observed (Sampaio *et al.*, 2001). In oak, the most recalcitrant and stable substrate, yeast densities reached the highest values. Nevertheless, the numbers of yeast species isolated from the three litter types were identical: 40, 45 and 48 for eucalyptus, alder and oak, respectively. Eucalyptus and oak, both considered to be substrates of low nutritional quality (Pozo, 1993; Bärlocher *et al.*, 1995; Canhoto & Graça, 1999), proved to be equally appropriate for yeast colonization as alder, a substrate of high nutritional quality (Gessner & Chauvet, 1994). Similar

results, although for distinct microbial communities such as aquatic hyphomycetes, were found for alder and eucalyptus (Chauvet *et al*., 1997) and for red maple and rhododendron (Gulis & Suberkropp, 2003).

During litter decay, both species richness (S) and Shannon's diversity index (H') declined. Apparently, the number of species is related to the substrate complexity (Foster & Fogleman, 1993). In the present study, both values were higher at the beginning of the experiment. According to Sampaio *et al*. (2001), a mass of leaves initially rich in soluble sugars was rapidly colonized by yeasts, which resulted in high diversity values. Similar results were found in other yeast ecological successions, such as corn ensilage, fermented sugar cane and agave, in decaying fruits, and during decomposition of *Q. rotundifolia*, *Q. robur*, *Acer rubrum* and *Tilia cordata* leaves (Middelhoven & van Baalen, 1988; Lachance, 1995; Morais *et al*., 1995; Schwan *et al*., 2001; Sadaka & Ponge, 2003; Nikolcheva *et al*., 2005; Cai *et al*., 2006). The appearance of new yeast populations at the end of the experiment provides indirect evidence of a new niche space, reinforcing the previous assumption (Connell & Slatyer, 1977; Walker & Chapin, 1987). Moreover, the presence of both primary and secondary colonizer yeast species at the later colonization stages supports a model based on tolerance and/or competition among species (Frankland, 1998; Sharma *et al*., 1998; Jackson, 2003).

Our results show a predominance of basidiomycetous yeasts over ascomycetous yeasts, even though the composition of yeast communities was distinct among litter types. Three groups of yeasts were isolated: those common to all litters, those shared by two types of litter, and those that appeared only in one particular leaf litter. In the first group of yeasts, we find generalist species that are frequently isolated from aquatic environments, decomposing organic matter, phylloplane and soils such as *Au. pullulans*, *Cy. capitatum*, *Cy. infirmo-miniatum*, *Pic. anomala*, *Rh. aurantiaca*, *Rh. colostri*, *Wil. saturnus* and *Le. scottii*, and the generalist species *D. hansenii*. Species belonging to the complexes of *Cr. albidus* and *Rhodot. glutinis*, both with worldwide distribution, and to the genus *Trichosporon*, were also found (Phaff & Starmer, 1987; Kurtzman & Fell, 1998; Polyakova *et al*., 2001; Libkind *et al*., 2003; Glushakova & Chernov, 2004). For the group of yeasts shared by two types of litter, the pairs alder–eucalyptus, oak–eucalyptus and alder–oak share three, eight and nine yeast species, respectively. Finally, there are yeast species isolated from a particular leaf type: 12, 11 and nine species in alder, oak and eucalyptus, respectively. *Rhodotorula* and *Rhodosporidium* species exhibited some preference for alder and oak, whereas *Cryptococcus ferigula* and *X. dendrorhous* preferred eucalyptus leaves. These results corroborate the discriminant analysis, which shows differences among litter types based on yeast frequency data.

In general, ascomycetous yeasts were isolated in the first or in the later decomposition stages, a result also observed by other authors (Kuter, 1986; Crawford *et al*., 1990). The presence of *M. bicuspidata* and *Wic. domercqiae* suggests an invertebrate connection (Kurtzman & Fell, 1998; Cáceres *et al*., 2006).

The MDA showed that the capacity of the yeast strains to assimilate carbon and nitrogen sources varied among the litter species, and throughout decomposition. The differences in the assimilation abilities of the yeast strains among the three litters are possibly related to distinct chemical foliar compositions among them and throughout decomposition. It is known that alder leaves have higher nutritional quality than oak and eucalyptus, because they have lower levels of molecules such as phenols, condensed tannins, essential oils and lignin (Bärlocher *et al*., 1995; Sampaio *et al*., 2001). The fact that some basidiomycetous yeasts are capable of metabolizing recalcitrant aromatic compounds (Sampaio, 1999; Middelhoven *et al*., 2001) may explain why they appeared more frequently in eucalyptus and oak leaves.

The clear differentiation of the assimilation capacities of yeast strains throughout decomposition, which was supported by discriminant analysis, suggests that the yeast community responds to habitat modifications. Also, community parameters such as S and H' dynamics, which were higher at the beginning of this experiment, could indicate that competition for nutrients and space were initially important, as pointed out by Walker & Chapin (1987). With the aging of the biofilm, the competition for nutrients decreases, as new resources (metabolic products released from other organisms) and habitats become available (Jackson, 2003). For instance, D-glucosamine could be formed as a result of peptidoglycan and quitin hydrolysis (Kuter, 1986; Amelung *et al*., 2001). In the final decomposition phase, yeasts that have broader assimilation abilities predominate, and this may explain why unusual substances such as cadaverine and ethylamine (biogenic amines produced by microbial decarboxylation) are used. In fact, utilization of amines as a sole source of nitrogen by yeasts has been recognized for a long time (Middelhoven *et al*., 1986).

Conclusions

These results, in both qualitative and quantitative terms, clearly support the proposition that yeasts, mostly basidiomycetous, are present in an aquatic saprophyte-type ecosystem. Yeast colonization differs among decomposing leaf litter types, and throughout the decay period. The yeast populations respond to specific substrate changes that occur during leaf litter decomposition. The order of species appearance, the substrate assimilation patterns and the

changes in species richness and diversity strongly suggest a succession phenomenon.

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