Leaf-litter-associated fungi and bacteria along temporal and environmental gradients in boreal streams

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ABSTRACT: Aquatic fungi and bacteria have long been recognized as key drivers in ecosystem processes such as leaf litter decomposition. We examined fungal and bacterial communities on decaying alder *Alnus glutinosa* (L.) Gaertner leaves along a gradient of increasing agricultural land use and associated nutrient enrichment in 9 boreal streams during 4 separate seasons (fall 2003, spring 2005, fall 2005, and spring 2006). Denaturing gradient gel electrophoresis (DGGE) and quantitative polymerase chain reactions (qPCR) showed that agricultural land use had significant effects on both bacterial and fungal communities, and on the ratios of fungi to total microbes associated with decomposing leaf litter. Furthermore, landscape factors and fluvial geomorphology appeared to influence the community composition of fungi and bacteria. Seasonal effects were found for fungal community structure only, indicating a higher temperature sensitivity of fungi compared to bacteria.

KEY WORDS: Sweden · Leaf litter · DGGE · qPCR · Aquatic fungi · Aquatic bacteria · Season · Land use

INTRODUCTION

Running waters are typically heterotrophic, with their main source of energy and carbon consisting of terrestrial subsidies such as leaf litter (Fisher & Likens 1973). Decomposition of leaf litter is regulated by a number of mechanisms, including fragmentation and decomposition mediated by invertebrates and various microorganisms such as aquatic fungi and bacteria (Webster & Benfield 1986, Gessner & Chauvet 1994, Royer & Minshall 2003). The aquatic microbes play a central role in this context, because they increase the nutritional value of the leaf litter available to invertebrates (Suberkropp 1992) and facilitate invertebrate assimilation of decomposing leaf litter through this conditioning effect (Cummins 1973, Arsuffi & Suberkropp 1989). Accordingly, fungi and bacteria are important components of the aquatic food web and play a vital functional role in running waters (Hieber & Gessner 2002).

Microbially mediated decomposition is controlled by both internal and external factors, such as the state of leaf litter that is entering a water body and the temperature of the receiving water (Cummins 1974, Petersen & Cummins 1974, Nikolcheva & Bärlocher 2005). Other factors that influence the decomposition process include the quality of the leaf litter, primarily the amount of lignin and tannins in that material (Gessner & Chauvet 1994), and the nutrient load; i.e. nitrogen and phosphorus concentrations in the water column (Suberkropp & Chauvet 1995, Grattan & Suberkropp 2001). Moreover, the composition of microbial communities is regulated by substrate quality (Gulis 2001, Das et al. 2007, Bärlocher et al. 2008), nutrient concentrations, and physicochemical factors (e.g. light, temperature, pH, and hydrology) (Gao et al. 2005, Fierer et al. 2007, Fernandes et al. 2009). For example, Gulis & Suberkropp (2003b) found that fungal species richness increased with increasing nutrient concentrations, and this was

accompanied by increasing decomposition rates and elevated bacterial numbers at nutrient-enriched sites. The relationship between fungi and bacteria is affected by habitat characteristics as well, illustrated by the finding that the biomass of bacteria exceeds the biomass of fungi on fine particles, whereas the opposite is seen on wood and leaves (Findlay et al. 2002). Fluvial geomorphology, hydrology, chemistry, and the amount and type of organic material present in a catchment are all affected by land use (Hynes 1975, Allan 2004), and thus catchment characteristics can also affect microbial communities in streams (Fischer et al. 2009).

Seasonal variation in fungal community composition has frequently been reported. Most of these differences have been attributed to temperature effects and the characteristics of the available substrate (Gessner et al. 1993, Garnett et al. 2000, Nikolcheva & Bärlocher 2005), although Buesing et al. (2009) found little temporal variation in fungal communities in freshwater marshes. On the other hand, few studies have examined seasonal changes in bacterial communities on leaf litter, which applies particularly to running waters, and evaluations of temporal patterns of bacteria in freshwaters have provided conflicting results. For instance, several investigations have found seasonal variability in freshwater bacterioplankton communities (Yannarell et al. 2003, Wu & Hahn 2006, Lymer et al. 2008), whereas Lindström (1998) observed no seasonal pattern in such communities. Also, Buesing et al. (2009) found weak seasonal variation in bacterial community composition on different substrates in freshwater marshes.

Although fungi and bacteria coexist on decomposing litter, they interact in various ways. Bacteria have been found to suppress fungi (Mille-Lindblom & Tranvik 2003), but it has also been reported that bacteria are suppressed, enhanced, or even unaffected by the presence of fungi (Gulis & Suberkropp 2003a, Mille-Lindblom & Tranvik 2003). Hence, decomposition rates of leaf litter can change if the ratio of fungi to bacteria is altered. Moreover, bacteria and fungi respond differently to environmental factors. For example, temperature can strongly affect fungi (Bärlocher et al. 2008, Dang et al. 2009) but not necessarily bacteria to the same extent (Bergfur & Friberg 2012), which suggests that seasonal temperature changes can have different impacts on these 2 groups of microorganisms.

In the fall of 2003, leaf litter decomposition was studied by incubating leaves of alder *Alnus glutinosa* (L.) Gaertner in 9 boreal streams in south-central Sweden to assess the effects of nutrient enrichment on the decomposition process (Bergfur et al. 2007b). This analysis was subsequently repeated in spring 2005, fall 2005, and spring 2006 (Bergfur 2007). The results of those experiments suggested that nutrient enrichment had little or no effect on the decomposition rates in these streams. In contrast, when Fischer et al. (2009) investigated microbial community composition on the leaves used in the 2003 study only, the results indicated that catchment characteristics had a strong influence on the composition of both fungal and bacterial communities. Although the study in 2003 focused primarily on the effect of invertebrate consumers on decomposition rates, fungal biomass (e.g. ergosterol) was found to have a significant positive effect on decomposition rates as well (Bergfur et al. 2007b). However, microbial community composition was not analysed in detail in that study.

In the present study, we examined fungal and bacterial community composition on the same leaf material collected from 9 boreal streams along an agricultural land-use gradient to determine decomposition rates (Bergfur 2007). It was hypothesised that the season and the degree of agricultural land use would affect microbial community patterns, as assessed by DGGE, and the relative amounts of fungal and bacterial DNA determined by quantitative polymerase chain reaction (qPCR). We hypothesised that microbial communities would differ between sites with different levels of nutrient enrichment, since nutrients are known to affect microbial communities. Moreover, due to the higher sensitivity of fungi to temperature, we hypothesised that there would be stronger seasonal effects on the fungal community composition compared to the bacteria. We used multivariate statistics to examine the influence of environmental factors such as land use, water chemistry, and instream morphology on the community composition of both fungi and bacteria.

MATERIALS AND METHODS

Site description

This research was performed in 9 boreal streams (Fig. 1) in south-central Sweden (Bergfur 2007). The catchment areas vary between 44.8 km² (Sandån) and 843 km² (Sagån) and are located at 4 to 158 m above sea level. Land use was characterized by means of a general overview map (scale 1:100 000), and site characteristics were documented according to standardized protocols (Wilander et al. 2003). For example, the proportion of land use within the catchment (e.g.
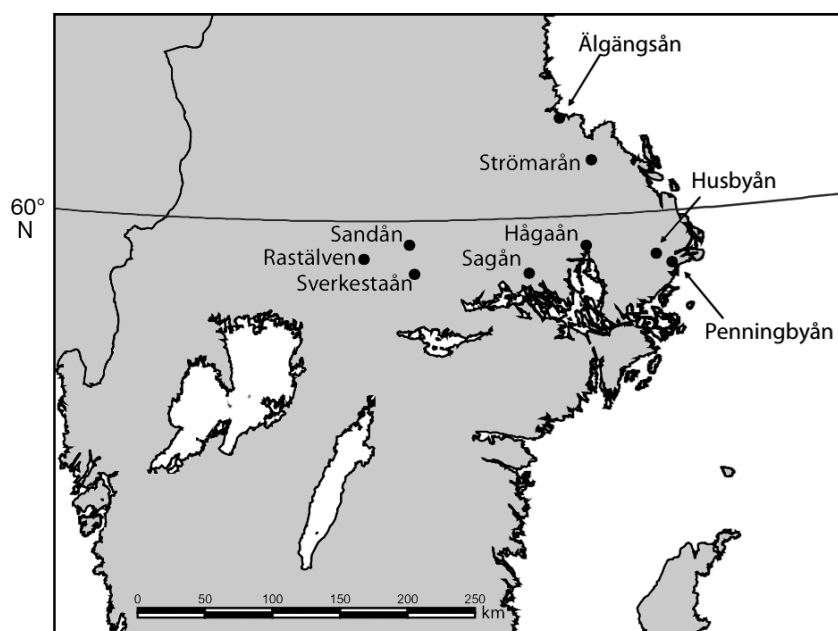
Fig. 1. Locations of the 9 streams studied in south-central Sweden

arable land use, forest and other open land), as well as particle size of the stream bed sediment were estimated (e.g. cobbles, >6 to 20 cm and sand, <0.2 cm) (see Bergfur 2007 for further details). Water chemistry was analyzed at each of 4 sampling periods (i.e. fall 2003, spring 2005, fall 2005, and spring 2006). All analyses of nutrients (different fractions of phosphorus and nitrogen) and other variables (pH, alkalinity, conductivity) were performed according to certified laboratory protocols (Wilander et al. 2003), and the results have been reported by Bergfur (2007). Total phosphorous ranged from 10.9 ± 2.8 (SD) µg l^{-1} in Sandån to 124.5 ± 78.3 µg l^{-1} in Sagån, $NO_2 + NO_3 - N$ ranged from 77.8 ± 71.4 µg l^{-1} in Sandån to 2334.5 ± 1224.1 µg l^{-1} in Sagån (Bergfur 2007).

Leaf litter decomposition

In each sampling period, leaves of alder *Alnus glutinosa* were incubated in mesh bags (5 mm mesh; 4.00 ± 0.02 (SD) g of air-dried leaf material per bag) for 34 d (Bergfur 2007, Bergfur et al. 2007b). Three replicate bags were placed in each stream and incubated for 34 d. The bags were collected, stored at 4°C and within 28 h of collection, invertebrates were rinsed from the leaves (Bergfur et al. 2007b). The leaves were then freeze-dried and stored at −20°C until DNA extraction was performed. During the spring studies, some of the litter bags were lost (only 1 or 2 litter bags were retrieved from some sites).

DNA extractions

DNA was extracted as described by Fischer et al. (2009). Briefly, freeze-dried homogenized leaf litter was placed in a 2 ml bead solution tube, treated for 30 s in a bead beater, then cooled on ice and treated for an additional 30 s in a bead beater. Extracts obtained in this manner were cleaned and isolated on spin filters (DNA purification kit, UltraClean™ Soil DNA Isolation Kit; Mo BIO Labs) as described in the alternative protocol for maximum yields provided by the manufacturer. The DNA extracts from the study by Fischer et al. (2009) had been stored at −20°C until they were analyzed in the present investigation. In order to include as many sites and seasons as possible and to keep the number of samples analysed with DGGE to a manageable number, DNA extracts were pooled (i.e. 3 original replicates were reduced to 1 sample). DNA extracts from each site and sampling occasion were pooled before PCR amplification. Also, due to the loss of litter bags, replicate samples were missing from some of the sites and seasons; therefore, for some sites only 1 sample existed to begin with. The process of pooling reduced the number of samples to 1 per site and season, making the data more balanced and easier to handle in the DGGE analyses. In a previous study, it was found that after 34 d of incubation the microbial communities on alder leaves in replicate litter bags collected in the Sverkestaån stream were very similar, e.g. similar DGGE band patterns were found for both fungi and bacteria, respectively (Fischer et al. 2009). Therefore, the loss of information by pooling was considered negligible.

qPCR

All qPCR reactions were carried out in Lightcycler® 480 Multiwell Plates in a Lightcycler® 480 instrument (Roche Diagnostics). The bacterial quantification was conducted according to the hydrolysis probe assay described by Yu et al. (2005), except for using a Black Hole Quencher1 in the TaqMan™ (BAC516F) probe (Table 1). TaqMan™ probes were labelled with a fluorescent reporter dye (6-carboxyfluorescein) and Black Hole Quencher 1, which were attached to the

Table 1. Primer pairs and probes used in PCR and qPCR (all from Sigma-Aldrich™)

Target	Name	Sequence (5'–3')	Concentration (µM)	Analysis	Reference
Fungi	ITS3F-GC	CGC CCG CCG CGC CCC GCG CCC GGC CCG CCG CCC CCG CCC CGC ATC GAT GAA GAA CGC AGC	10	PCR	Manerkar et al. (2008)
Fungi	ITS4R	TCC TCC GCT TAT TGA TAT GC	10	PCR	Manerkar et al. (2008)
Bacteria	GM5F-GC	CGC CCG CCG CGC GCG GCG GGC GGG GCG GGG GCA CGG GGG GCC TAC GGG AGG CAG CAG	10	PCR	Yu et al. (2005)
Bacteria	DS907R	CCC CGT CAA TTC CTT TGA GTT T	10	PCR	Yu et al. (2005)
Fungi	ITS3F	GCA TCG ATG AAG AAC GCA GC	10	qPCR	Manerkar et al. (2008)
Fungi	ITS4R	TCC TCC GCT TAT TGA TAT GC	10	qPCR	Manerkar et al. (2008)
Bacteria	BAC338F	ACT CCT ACG GGA GGC AG	10	qPCR	Yu et al. (2005)
Bacteria	BAC805R	GAC TAC CAG GGT ATC TAA TCC	10	qPCR	Yu et al. (2005)
TaqMan™ probe	BAC516F	(6FAM)TGC CAG CAG CCG CGG TAA TAC(BHQ1)	4	qPCR	Yu et al. (2005)

5' and 3' ends, respectively, as recommended for use in a LightCycler® 480. Reaction mixtures (total volume 20 µl) contained 10 µl of LightCycler®480 Probes Master (Roche Diagnostics), 1 µl of each primer and corresponding TaqMan™ probe (Table 1), 2 µl of PCR-grade water, and 5 µl of template DNA (diluted 1:100). The protocol used in bacterial qPCR reactions involved initial denaturation at 94°C for 10 min, followed by 29 cycles of denaturation at 94°C for 10 s, annealing at 60°C for 30 s, and extension at 40°C for 10 s as described by Yu et al. (2005), except for the number of cycles.

LightCycler® 480 SYBR Green I Master hot-start reaction mix for PCR (Roche Diagnostics) was used in qPCR reactions on fungal DNA according to the manufacturer's protocol. In short, 10 µl of Master Mix was mixed with 1 µl of each forward and reverse primer (Table 1), 3 µl of PCR-grade water, and 5 µl of DNA template (diluted 1:100) to achieve a final volume of 20 µl. Reactions were carried out according to the protocol outlined by Manerkar et al. (2008), with initial denaturation at 95°C for 2 min and 30 s, followed by 45 cycles of denaturation at 95°C for 15 s, annealing at 55°C for 20 s, extension at 72°C for 30 s, and finally 83°C for 15 s. Additional melting curve analyses were run for 1 cycle with initial denaturation at 95°C for 5 s followed by annealing at 65°C for 1 min, and thereafter 5 acquisitions per °C until the target temperature of 97°C was reached (results not reported here). Negative controls (PCR-grade water) were run in parallel in both bacterial and fungal qPCR analyses. *Escherichia coli* (DSM 10198) and *Penicillium chrysogenum* (DSM 844), purchased from the Deutsche Sammlung von Mikroorganismen

und Zellkulturen, were used to obtain standard curves. Concentrations of extracted DNA for standard curves were measured by Quant-iT (Invitrogen). To overcome the problem of substantial intra- and inter-assay variation among qPCR products (Smith et al. 2006), we used duplicate samples in 2 separate runs in both bacterial and fungal qPCR reactions. Data were analyzed with LightCycler® 480 software 1.5.0 (Roche Diagnostics). Mean DNA concentrations (µg ml^{-1}) were calculated using standard curves for *E. coli* and *P. chrysogenum*. Since no records were available on the amount of leaf litter used in the DNA extractions performed in 2004 (Fischer et al. 2009), it was not possible to estimate DNA concentration (or gene copy number) per gram of these samples. Accordingly, to allow comparative analyses of the complete data set, we calculated the ratio of fungal DNA to the sum of fungal and bacterial DNA in leaf-litter samples, which is hereby designated F/FB.

DGGE

Polymerase chain reactions were performed on a Primus 96 Advanced® Gradient thermocycler (PEQLAB, Biotechnologie). Reaction mixtures were prepared in illustra™ puReTaq Ready-To-Go PCR Beads (GE Healthcare) using 19 µl of RNAse-free water, 2.5 µl of forward primer, 2.5 µl of reverse primer, and 1 µl of DNA template (diluted 1:10) per Taq bead. The primers used are listed in Table 1. PCR reactions were carried out according to Manerkar et al. (2008). PCR products were analyzed by

DGGE using a DCode universal mutation detection system (Bio-Rad Laboratories). PCR products (15 µl aliquots) were loaded on a 6.5% polyacrylamide gel run in 1 × TAE buffer at 60°C and 75 V. Bacterial PCR products were run for 13 h on a gel with a denaturing gradient of 35 to 60%, and fungal PCR products for 11 h on a gel with a gradient of 35 to 55% (Fischer et al. 2009). Hyperladders (Invitrogen) were run simultaneously on all gels to facilitate comparison of the results. The gels were stained with SYBR® Safe DNA gel stain (Invitrogen) in 1 × TAE for 30 min according to the manufacturer's protocol, and images were taken under ultraviolet illumination. Images were analyzed in AlphaVIEW SA 3.2.2 (Cell Biosciences). The hyperladder separated into several distinct bands in the gels, and these bands were used to align the gels. The recorded bands were considered as operational taxonomic units (OTUs) as described by Fischer et al. (2009). By using the hyperladder as a standard, the samples could be compared to each other based on how many OTUs were formed and by how far they had migrated in the gels. Blurred bands were excluded from analysis. A total of 3 gels were compared for fungi and 4 gels were compared for bacteria.

DGGE band patterns can only provide an estimate of differences between samples with respect to the presence or absence of certain phylotypes (Nikolcheva & Bärlocher 2005). Moreover, the number of bands obtained does not necessarily represent the overall microbial richness within a sample. Amplification of bacterial DNA could potentially amplify DNA from mitochondria and chloroplasts within the leaf litter matrix due to the evolutionary link between mitochondria, chloroplasts and bacteria (Rastogi et al. 2010). However, fingerprinting methods such as DGGE provide an image of the pattern of changes within and among microbial communities, provided that the same materials and methods (e.g. primers, extraction kits, etc.) are used (Bent et al. 2007, Lindström et al. 2007). This was the case in the present study, suggesting that the observed patterns provide useful insights at least for comparative purposes regarding changes among the dominating taxa.

Statistical analyses

Principal component analysis (PCA) was performed on water chemistry and land use data to identify the main gradients, and the sites were grouped into 3 categories as previously described (Bergfur 2007). The Sagån, Husbyån, and Hågaån streams were considered to be of similar habitat type and are referred to as habitat type 1, with a large proportion of arable land in the catchment area and high nutrient loads. The Penningbyån, Strömmarån, and Älgängsån streams were moderately affected by nutrients and arable land use, and are referred to as habitat type 2. The Sverkestaån, Rastälven, and Sandån streams were classified as experiencing low nutrient loads with a catchment dominated by forest, and hereafter are referred to as habitat type 3. Bergfur (2007) provides details on stream characteristics, results of PCA analysis, and categorization of the study sites. As mentioned previously, some replicate litter bags were lost; however, by pooling extracts and by grouping sites into categories, we were able to include more sites and seasons in order to investigate the temporal patterns among streams affected by different levels of nutrient loads and catchment land use, even though these bags were lost. Our aim was not to investigate variation within streams, but to investigate variation among streams of different nutrient loads and among different seasons.

Permutational multivariate ANOVA (PERMANOVA) (Anderson 2005) was used to study the effect of habitat type and season on community patterns (e.g. DGGE band patterns). The R-vegan function 'adonis' was used with the Bray-Curtis dissimilarity measure and with habitat type and season as factors (R Development Core Team 2013), and the PERMANOVA was run using 9999 permutations. Canonical correspondence analysis (CCA) was used to examine species–environment relationships for both the fungal and bacterial communities. CCA is a multivariate constrained ordination technique that extracts the major gradients among a combination of explanatory variables (Legendre & Legendre 1998), such as in this dataset. Hence, CCA allowed us to extract the environmental variables that explained a significant amount of the variation within the community composition pattern. Because detrended correspondence analysis (DCA) revealed the length of gradients to be >2 for both fungal and bacterial DGGE band patterns, we chose to use a unimodal ordination method (Legendre & Legendre 1998, Lepš & Šmilauer 2003). Temporal variation was not of primary interest in this analysis, and hence partial CCA (pCCA) was run in which season was entered as covariable (coded as a dummy variable) to reduce the influence of season (ter Braak & Verdonschot 1995). Forward selection using 9999 Monte Carlo permutations under a reduced model was run to identify the environmental variables that best described the gradients in microbial community composition. Variables were added

until further additions did not contribute significantly to the explanatory power of the model (ter Braak & Verdonschot 1995). Bonferroni correction was applied as described by Lepš & Šmilauer (2003). All ordinations were performed using Canoco for Windows v.4.5 (ter Braak & Smilauer 2002). All stream characteristics used in PCA and pCCA, except for pH, were $\log_{10}(x + 1)$ transformed to approximate normal distribution. In addition, although longitude and latitude were used as in the PCA, these variable were removed from the pCCA since they were correlated with other land use variables (Bergfur 2007, Bergfur et al. 2007a,b).

A 2-way ANOVA was performed on F/FB ratios using season and the 3 categories of habitat type as independent factors. This analysis was followed by Tukey's HSD post hoc test when effects were significant (α = 0.05). ANOVA were carried out using JMP 9 (SAS Institute).

Fig. 2. Mean (±SE) ratio of fungal DNA to fungal plus bacterial DNA (F/FB) on decaying leaf litter. White bars: streams experiencing high nutrient loads; grey bars: moderate nutrient loads; black bars: low nutrient loads

RESULTS

A total of 46 bands were identified in the fungal DGGE gels. The number of fungal OTUs was highest in the Sverkestaån in fall 2005 (n = 20) and lowest in the Älgängsån in spring 2005 (n = 6). Forty bands were identified in the bacterial DGGE gels. The highest number of bacterial OTUs identified in a single sample was 15, which was noted for the Älgängsån in spring 2006; the corresponding lowest number was 6, which was recorded for the Husbyån in fall 2005. Habitat type had a significant effect on the F/FB ratio ($F_{2,30}$ = 16.4, p < 0.0001), and was found to be significantly highest in habitat type 3 (streams with low nutrient load and dominated by forested catchments). Habitat types 1 and 2 did not differ significantly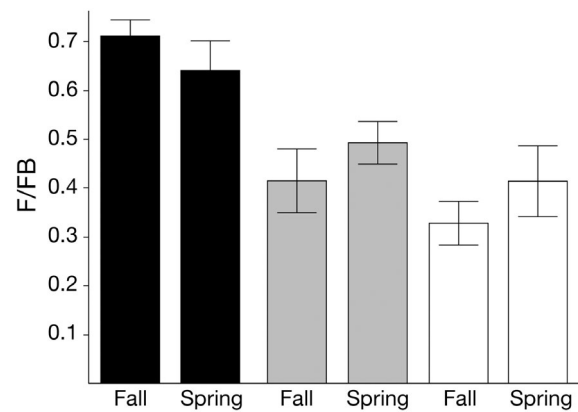 from each other (Fig. 2). The 2-way ANOVA showed no significant effect of season, nor were any significant interaction effects found on the F/FB ratio. PERMANOVA on the DGGE band patterns showed that habitat type had a significant effect on both bacterial and fungal community composition (Table 2, p = 0.0001), and season had a significant impact on fungal community composition (p = 0.0001).

Six catchment variables were found to be significant in the pCCA of fungal community composition (i.e. DGGE band patterns): degree of open land, percent sand in stream sediments, percent cobble, percent fine gravel, proportion of arable land in the riparian zone, and open water in the catchment (Fig. 3a). All these variables describe catchment and morphological characteristics of the 9 sites, indicating that they were primary determinants of leaf-litter-associated fungal community composition in the streams, whereas water chemistry was not a significant explanatory factor in that context. The pCCA

Table 2. Results from PERMANOVA analysis on fungal and bacterial DGGE band patterns. Habitat type and season (e.g. fall and spring) were used as factors. PERMANOVA was run using 9999 permutations; α = 0.05

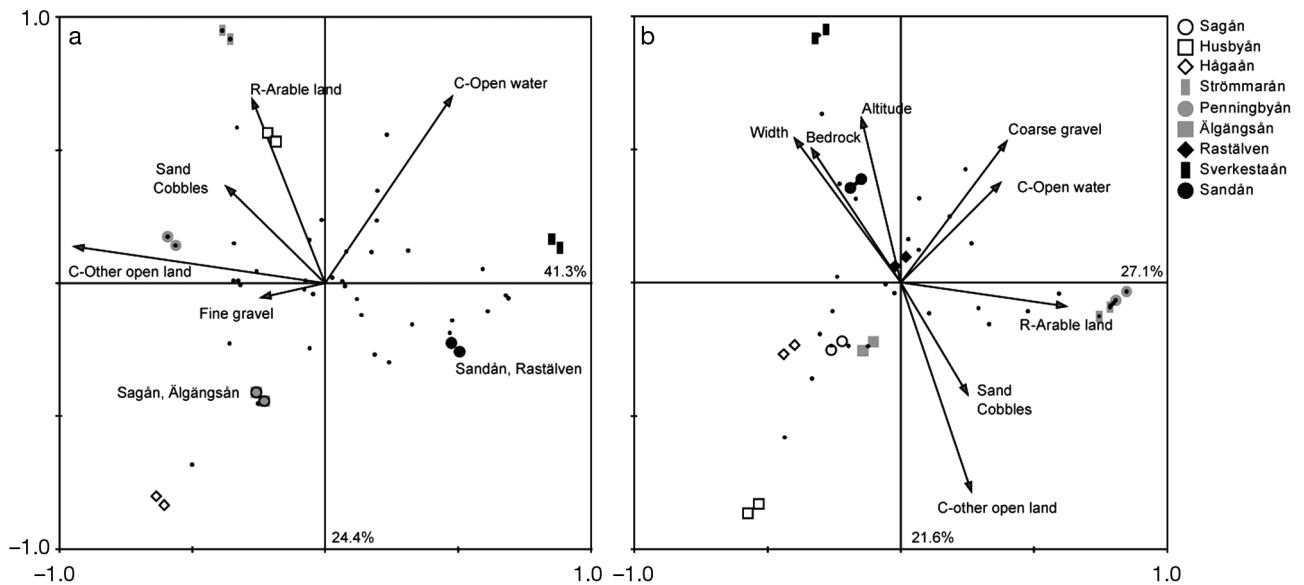|		df	SS	MS	F-value	R^2	p-value
Fungi	Habitat type	2	1.16	0.58	6.83	0.26	0.0001
	Season	1	0.48	0.48	5.63	0.11	0.0001
	Habitat type × Season	2	0.27	0.14	1.60	0.06	0.09
	Residuals	30	2.55	0.09		0.57	
	Total	35	4.46			1.00	
Bacteria	Habitat type	2	1.59	0.79	9.87	0.38	0.0001
	Season	1	0.14	0.14	1.74	0.03	0.12
	Habitat type × Season	2	0.09	0.04	0.55	0.02	0.86
	Residuals	30	241	0.08		0.57	
	Total	35	4.22			1.00	

Fig. 3. Partial canonical correspondence analysis (pCCA) triplots of (a) fungal DGGE band patterns (total inertia 2.30; Eigenvalue axis 1: 0.237, axis 2: 0.140) and (b) bacterial DGGE band patterns (total inertia 2.94; Eigenvalues axis 1: 0.352, axis 2: 0.281). Habitat type 1: Sagån, Husbyån, and Hågaån; habitat type 2: Strömmarån, Älgängsån, and Penningbyån; habitat type 3: Sverkestaån, Rastälven, and Sandån. Each dot represents associated DGGE bands (i.e. operational taxonomic units). C: catchment; R: riparian. Stream names are given in the graphs to indicate where symbols are overlapping

of bacterial DGGE band patterns identified 9 environmental variables that explained a significant amount of variation in bacterial community composition: percent open land in the catchment, percent sand and cobbles in stream sediment, arable land in the riparian zone, open water, coarse gravel, altitude, bedrock and width (Fig. 3b). Similar to the pCCA results regarding fungal community composition, no water chemistry variables explained the community composition of bacteria.

DISCUSSION

In this study, habitat type had a significant effect on F/FB ratios. PERMANOVA on community composition showed that habitat type had a significant effect on both bacterial and fungal community composition, whereas season only affected the fungal community.

When the effect of season was reduced in our pCCA, both fungal and bacterial communities were largely influenced by land use and substrate grain size. Notably, our results showing that water chemistry did not have a significant effect on microbial community composition contrast with previous investigations where water chemistry and temperature were found to be important factors influencing leaf-

litter-associated microbial communities (Duarte et al. 2009, Fernandes et al. 2009).

Our results indicate that the proportion of other water bodies within a catchment is an essential factor controlling both fungal and bacterial communities. In agreement with that observation, Lindström et al. (2006) found that water connectivity was a significant factor influencing bacterial community composition in lakes, and our findings suggest that this may also be true for fungi and bacteria on leaf litter in streams. Substrate is a key factor structuring microbial communities in running waters (Nikolcheva & Bärlocher 2005), and our results also indicate that the substrate plays a notable role in structuring microbial communities associated with leaf litter in streams. In particular, the proportion of sand and cobble had a significant influence on both bacterial and fungal communities, as well as arable land in the riparian zone. Grain size influences hydrological processes and patterns, and it has been shown that hydrology affects biofilm structure and functioning (Romaní et al. 2013).

The pool of microbial species available in the water column is responsible for colonization, and hence determines the composition of microbial communities on leaf litter (Curtis & Sloan 2004, Langenheder et al. 2006, Battin et al. 2007). This microbial reservoir is in turn derived from the surrounding catch-

ment, soils, groundwater, and riparian zones (Lindström et al. 2006, Beier et al. 2008). This information, together with the current results and those reported by Fischer et al. (2009), further illustrate that the surrounding landscape and fluvial geomorphology are important factors influencing fungal and bacterial communities on decaying leaf litter in freshwaters.

We found that F/FB ratios were higher in the habitat type 3 category than in the habitat types 1 and 2 (i.e. streams with higher nutrient loads and degree of arable land use within the catchment). This means that the ratio of bacterial to fungal DNA increased with the proportion of arable land in the catchment. In a similar study by Manerkar et al. (2008), fungal DNA exceeded bacterial DNA during the initial stages of leaf decomposition (Weeks 2 and 3), although relative amounts of fungal and bacterial DNA were similar after 35 d. In the same investigation, fungal DNA and release of fungal conidia exhibited similar temporal patterns, with values increasing during the early stages of decomposition and declining during late stages (Manerkar et al. 2008, their Figs. 3 & 4), suggesting that the amount of fungal DNA covaries with fungal reproduction. Therefore, it is possible that the effect of habitat type on the F/FB ratio in the current study was due to a positive effect of nutrient enrichment on sporulation (Pascoal & Cássio 2004, Ferreira et al. 2006), which reduced the amount of fungal DNA after 34 d of incubation.

Season (e.g. fall and spring) was found to have a significant effect on fungal community structure but not on bacterial community structure, although no significant effect of season was found on F/FB ratios. Other studies have also described seasonal dynamics in aquatic fungi. Gessner et al. (1993) described seasonal dynamics in aquatic hyphomycete communities on decaying alder leaves. In other freshwater environments, such as freshwater marshes, little to no effect of season has been found on fungal or bacterial communities (Buesing et al. 2009). Garnett et al. (2000) noted that fungal species richness was greater in fall than in summer, which these authors attributed to temperature effects. Moreover, in a study on fungal succession patterns by Gessner et al. (1993), when water temperatures did not exceed a threshold of 16 to 18°C, there was very little to no species replacement. Bärlocher et al. (2008) and Suberkropp (1984) also reported significant effects of temperature on stream fungi. Unfortunately, temperature data for our investigation are incomplete because temperature data loggers were lost (Bergfur 2007). Nevertheless, in the streams for which such data are available, the temperature exceeded 16°C

only in the Husbyån, the Rastälven, and the Penningbyån streams in spring 2006. The maximum temperature, which was recorded in the Husbyån, was 18.2°C. Therefore, it is possible that temperature thresholds with substantial effects on boreal fungal community composition were reached in these streams.

As mentioned above, several studies on seasonal patterns of bacterial community composition have provided contradictory results. We found no seasonal effect on bacterial community composition. Similarly, in a study by Yannarell et al. (2003) on bacterial communities in freshwater lakes, spring and fall samples were stable and similar to each other, whereas summer communities underwent drastic changes. A previous study found that in stream bed sediment samples there were no clear differences in bacterial gene copy number between summer and fall (Baxter et al. 2013).To our knowledge, there are few studies on running water and leaf-litter-associated bacteria, especially regarding the temporal patterns in bacterial community structure. However, previous experiments on stream derived litter-associated fungal and bacterial communities have shown that temperature has a stronger effect on fungi than on bacteria (Bergfur & Friberg 2012).

One possible source of error in this study is that several gels were used, and the loading order of the samples in the wells was not randomized. For example, in the fungal DGGE, the samples from the different habitat types were loaded onto 3 different gels, whereas the bacterial samples were not loaded in any particular order in the gels. However, the samples from each site were always loaded in wells next to each other. By casting several gels for comparison, there is a risk of error due to irregularities introduced during the casting process. However, despite this possible source of error, season was found to be a factor affecting fungi but not bacteria, even though the loading order was not based on season. Moreover, habitat type affected both fungi and bacteria even though loading order differed between the 2 groups. Hence, our results, together with those of Fischer et al. (2009) indicate that the microbial communities within streams are controlled by the characteristics of the surrounding landscape as well as in-stream characteristics. Few studies have focused on the bacterial community on decaying leaves within streams, with more focus on aquatic fungi. Hence, our study adds to a growing field in aquatic ecology examining the microbial communities within running waters — an area where there is still a fundamental lack of knowledge.

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