

Mikko Tolkkinen

BIODIVERSITY AND ECOSYSTEM FUNCTIONING IN BOREAL STREAMS

*THE EFFECTS OF ANTHROPOGENIC
DISTURBANCES AND NATURALLY STRESSFUL
ENVIRONMENTS*

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MIKKO TOLKKINEN

**BIODIVERSITY AND ECOSYSTEM
FUNCTIONING IN BOREAL
STREAMS**

The effects of anthropogenic disturbances and naturally stressful environments

Academic dissertation to be presented with the assent of the Doctoral Training Committee of Technology and Natural Sciences of the University of Oulu for public defence in the OP auditorium (L10), Linnanmaa, on 2 October 2015, at 12 noon

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Abstract

The effect of biodiversity loss and change on the functioning of ecosystems is one of the key questions in ecological research. For stream ecosystems, compelling evidence indicates that species diversity may enhance ecosystem functions. However, ecosystem functions are often regulated by the same environmental factors that also shape diversity; thus, a major challenge for ecologists is to separate the effects of biodiversity loss on the ecosystem functions from the direct effects of human induced disturbance. In this doctoral thesis, I studied how decomposer communities and ecosystem functions respond to human disturbances (nutrient enrichment, acidification) and a natural stressor (naturally low water pH). I also studied how human disturbances and natural stressors affect the phylogenetic structure of stream fungal communities. I showed that human disturbance had a strong impact on species dominance patterns by reducing species evenness. Species dominance patterns also explained the variation in decomposition rates. Changes in abiotic variables also had a direct effect on leaf decomposition rates. In the naturally acidic sites, human impact (land drainage) further decreased water pH and increased metal concentrations, thereby reducing leaf decomposition rates, whereas high nutrient concentrations enhanced leaf decomposition. Naturally low pH had no effect on decomposition rates. Decomposer community similarity was higher in drainage-impacted sites, but only in naturally acidic, not in circumneutral, streams. Human induced disturbance also modified the phylogenetic similarity of fungal decomposer communities, with communities in disturbed sites consisting of more closely related species when compared to those in circumneutral reference sites. Leaf litter decomposition showed greater temporal variation in human disturbed sites than in reference sites, whereas fungal community variability was similar in disturbed and reference sites. Thus, temporally replicated monitoring may be needed for a reliable assessment of human disturbance in streams. My thesis emphasizes that using both functional and taxonomic measures allows a more comprehensive assessment of biological responses to human disturbance.

Keywords: aquatic fungi, benthic macroinvertebrate, biodiversity, ecosystem function, eutrophication, forest drainage, leaf decomposition, multiple stressor, next-generation sequencing, stream

Tolkkinen, Mikko, Ihmistoiminnan ja luontaisen happamuuden vaikutus biodiversiteettiin ja ekosysteemin toimintaan virtavesissä

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Tiivistelmä

Biodiversiteetin väheneminen ja siitä seuraava ekosysteemin toiminnan heikkeneminen on eräs keskeisimmistä ekologisista kysymyksistä. Ekosysteemin toiminnot ovat kuitenkin monesti yhteydessä ympäristöolosuhteisiin, joten on vaikea erottaa vähentyneen biodiversiteetin ja ympäristöolojen suhteellista merkitystä ekosysteemin toimintoihin. Tässä väitöskirjatyössäni tutkin, kuinka virtavesien hajottajayhteisöt ja ekosysteemin toiminnot (lehtikarikkeen hajotus) muuttuvat valuma-alueen ihmistoimintojen myötä. Tutkin myös, kuinka luontainen stressi (matala pH) vaikuttaa yhteisöihin ja ekosysteemin toimintoihin. Tarkastelen myös akvaattisten sienten fylogeneettistä rakennetta ihmistoiminnan muuttamissa vesiympäristöissä. Osoitan tutkimuksissani, että ihmistoiminnoilla on vaikutuksia hajottajayhteisöiden kokonaisrunsauden jakautumiseen lajien kesken. Muutamien runsaiden lajien dominoimissa yhteisöissä lehtikarikkeen hajoaminen on tehokkaampaa kuin yhteisöissä, joissa lajien runsauserot ovat pienempiä. Myös ympäristöoloilla on vaikutus lehtikarikkeen hajotukseen. Luontaisesti happamissa puroissa metsäojituksen seurauksena lisääntynyt veden metallipitoisuus ja alhainen pH vähentävät hajotuksen määrää. Toisaalta joen korkea ravinnepitoisuus lisää hajotusta. Lehtikarikkeen hajotus vaihtelee enemmän vuosien välillä ihmistoimintojen muuttamissa virtavesissä kuin luonnontilaisissa vesissä. Toisaalta sieniyhteisöt pysyvät koostumukseltaan samankaltaisina vuosien välillä ihmistoiminnan muuttamissa paikoissa ja referenssipaikoissa. Tämä työ osoittaa, että toiminnallisten ja yhteisöihin perustuvien indikaattorien yhteiskäyttö antaa kokonaisvaltaisimman kuvan ihmistoimintojen vaikutuksesta virtavesien ekosysteemeihin.

Asiasanat: akvaattinen sieni, biodiversiteetti, ekosysteemin toiminto, happamuus, joki, lehtikarikkeen hajoaminen, metsäojitus, pohjaeläin, rehevyys

To my family

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Oulu 6.9.2015

Mikko Tolkkinen

List of original articles

This thesis is based on the following publications, which are referred throughout the text by their Roman numerals:

- I Tolkkinen M, Mykrä H, Markkola AM, Aisala H, Vuori KM, Lumme J, Pirttilä AM & Muotka T (2013) Decomposer communities in human-impacted streams: species dominance rather than richness affects leaf decomposition. *Journal of Applied Ecology* 50:1142–1151.
- II Tolkkinen M, Mykrä H, Annala M, Markkola AM, Vuori KM & Muotka T (2015) Multi-stressor impacts on fungal diversity and ecosystem functions in streams: natural vs. anthropogenic stress. *Ecology* 96: 672–683.
- III Tolkkinen M, Mykrä H, Markkola AM & Muotka T Human disturbance increases functional but not structural variability of stream fungal communities. *Functional Ecology*. doi: 10.1111/1365-2435.12469
- IV Mykrä H, Tolkkinen M, Markkola AM, Pirttilä AM & Muotka T. Phylogenetic clustering of fungal communities in anthropogenically altered streams. *Manuscript*.

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1 Introduction

One of the most striking features of our planet is its great variety of life. This astonishing biodiversity is increasingly threatened by human activities, and freshwater ecosystems are among the most threatened ecosystems on the Earth (Sala et al. 2000). The effect of biodiversity loss on the functioning of ecosystems is one of the key questions in ecological research, as the healthy functioning of ecosystems is fundamental to life on Earth. Despite a growing number of studies that consider the biodiversity-ecosystem function (B-EF) relationship, some important aspects are still not well understood (Hooper et al. 2005). Foremost, the majority of previous research has been employed in controlled experiments and the generality of these studies in natural ecosystems remains unclear. Compelling evidence supports the idea that species diversity may enhance ecosystem functions in both terrestrial and aquatic ecosystems (Gessner et al. 2010). However, in natural ecosystems, function is often regulated by the same environmental factors that also shape diversity; thus, a major challenge for ecologists is to separate the effect of biodiversity loss on ecosystem functions from the direct effects of human induced disturbance. Moreover, environmental complexity may modify the force of the B-EF relationship (Langenheder et al. 2010). Considering ecosystem functions and environmental factors together is therefore essential for understanding and predicting the consequences of anthropogenic disturbances on ecosystems (Loreau et al. 2001).

In experimental studies, decomposition rates have generally saturated at a low level of diversity (Jonsson & Malmqvist 2003, Dang et al. 2005), suggesting that addition of species above a critical level may not further increase decomposition rates. Furthermore, most freshwater B-EF studies have focused on species richness, rather than also exploring changes in species relative abundances (Woodward 2009). However, species evenness may respond more rapidly to environmental change than does richness (Hillebrand et al. 2008). Changes to evenness may therefore affect ecosystem functions long before species go extinct, and species dominance rather than richness may be critical in determining the rates of ecosystem functions in real-world ecosystems (Dangles & Malmqvist 2004). Moreover, in natural ecosystems, loss of biodiversity is often non-random with respect to species identity, whereas the experimental studies conducted by most researchers utilise randomly reduced biodiversity (Raffaelli 2004). A few efficient species may have a disproportionately strong effect on ecosystem

functions, suggesting that ecosystem functioning may depend substantially on species identity (Dangles & Malmqvist 2004).

Although ecosystem functioning is a broad term that includes ecosystem properties, ecosystem goods and ecosystem services, researchers most often consider ecosystem function as the cycling of nutrients to biomass production within an ecosystem (Hooper et al. 2005). In stream ecosystems, allochthonous organic material is the most important source of carbon and energy; thus, decomposition of organic material has been considered as a key process in headwater stream ecosystems (Cummins et al. 1973, Vannote et al. 1980). Therefore, since the publication of the seminal paper by Gessner and Chauvet (2002), leaf litter decomposition has been used widely as an indicator of stream ecological integrity. Low pH and associated metals are often related to reduced richness of decomposer communities and depressed decomposition rates (e.g. Baudoin et al. 2008, Simon et al. 2009). By contrast, elevated nutrient concentrations may accelerate leaf decomposition mainly because of increased microbial activity (e.g. Ferreira et al. 2006).

Unlike their responses to human induced disturbances, biological assemblages may have become adapted or acclimated to a natural stressor (e.g. low pH) and are thereby capable of maintaining ecosystem functions in naturally harsh environments. While anthropogenic acidification is among the most detrimental human disturbances to freshwater ecosystems, communities adapted to naturally acidic streams may be as functional as those in circumneutral streams (Dangles et al. 2004, Petrin et al. 2007). Sensitivity to low pH may also vary depending on the source of acidification. Anthropogenic acidification is often linked to atmospheric pollution or discharges from local industry or mining activities (Niyogi et al. 2002). Stream water pH can also be naturally low because of organic acids from peatland-dominated catchments (Mattsson et al. 2007) or because of specific geology (Kwong et al. 2009). While humic substances in peatland streams may buffer against harmful effects of acidification, low pH caused by background geology is often associated with toxic metals (Loukola-Ruskeeniemi et al. 1998). Some studies have examined the ecological effects of low pH arising from humic substances, but little is known about how low pH caused by bedrock geology affects stream communities and ecosystem function.

Streams are often affected simultaneously by several stressors, and species responses to different stressors can be non-additive (Paine et al. 1998). That is, the response may differ from that predicted based on the individual effects of each stressor alone (Townsend et al. 2008). For example, if species tolerances to

natural and anthropogenic stressors are negatively correlated, diversity can be severely reduced as a result of synergistic interactions. Acclimating or adapting to one stressor may also increase organisms' sensitivity to novel stressors, suggesting a potential cost associated with greater tolerance (Kashian et al. 2007). Naturally stressful environments can act as a strong environmental filter that eliminates sensitive specialists, leaving a subset of tolerant species. These tolerant species may be less sensitive to additional stressors, so that the assemblages in naturally harsh environments may be more tolerant to additional stresses when compared with those in more benign environments (Vinebrooke et al. 2004). Although several studies have considered the effect of multiple stressors on stream communities, studies examining the effects of human disturbances in naturally stressed streams are virtually absent.

In highly fluctuating environments, such as streams, the communities and ecosystem process rates may vary among different years, depending on the ambient environment conditions. Although temporal variability is a fundamental property of ecological systems, increased temporal variability may indicate a disturbed ecosystem, even in the absence of any change in community properties or ecosystem process rates (Fraterrigo & Rusak 2008). Studies on functional stability have typically focused on the invariability of species biomass over time (Griffin et al. 2009), while temporal variability in the rates of ecosystem functions has gained much less attention (Dang et al. 2005, Srivastava et al. 2009, Rocha et al. 2011). Species diversity often enhances the temporal stability of ecosystem functions, suggesting that loss of biodiversity may destabilise ecosystems (Hooper et al. 2005). Despite mounting evidence that the temporal stability of ecosystem functions increases with biodiversity (Cardinale et al. 2012), studies assessing the effects of human disturbances and associated reduced biodiversity on temporal variability of ecosystem process rates are largely lacking. However, in their microcosm experiment, Dang et al. (2005) found that variability of leaf decomposition rates was negatively related to fungal richness, suggesting that high biodiversity can maintain stable ecosystem functions.

Anthropogenic disturbances may act as non-random environmental filters, selecting for tolerant species with traits that allow persistence in disturbed environments (Chase & Myers 2011). Closely related species are likely to share similar traits, therefore showing similar tolerance to disturbances. Consequently, disturbances may be reflected in the phylogenetic community structure, with communities in disturbed environments comprising more closely related species than communities in otherwise similar undisturbed environments (Helmus et al.

2010). However, environmental filtering can also cause phylogenetic overdispersion if traits of habitat specialisation are labile and close relatives specialise in different niches (e.g. Cavender - Bares et al. 2004, Fine et al. 2005). Empirical evidence of the factors driving phylogenetic community structure is rather limited, but a few studies have shown that disturbances may indeed create communities dominated by closely related, disturbance-adapted species (Dinnage 2009, Helmus et al. 2010, Brunbjerg et al. 2012, Ding et al. 2012). Similar results have also been reported for aquatic microorganisms, with a clear phylogenetic signal in species ecological niches (e.g. Stegen et al. 2012, Wang et al. 2013). However, ecologists are only beginning to address the effects of environmental stress on the phylogenetic community structure of microorganisms (Goberna et al. 2014).

Despite the growing number of studies of human disturbance on biodiversity and decomposition processes, there is not enough information about spatial and temporal variability of communities and ecosystem processes in stream ecosystems. Limited knowledge on how different biotic and abiotic drivers regulate ecosystem processes hinders the use of functional measurements in stream health assessment (Woodward et al. 2012). Specifically, knowledge of the microbial contribution to ecosystem processes in streams is still rather limited and stream managers lack information on how individual and combined stressors affect microbial communities and microbial decomposition. Although microbial life on Earth, as such, may not be threatened, human induced stressors can affect diversity and community composition of microbes with potentially serious consequences on stream ecosystems.

2 Aims of the study

The overall objective of this thesis was to study the effects of a natural stressor and anthropogenic disturbances on biological communities and ecosystem functions in boreal streams. I also studied the spatial and temporal variation of decomposer communities and ecosystem functions in streams.

First, I studied the individual effects of land use-related anthropogenic disturbance and naturally low pH on macroinvertebrate and aquatic fungal communities and leaf litter decomposition rates. I further assessed the effects of a possible loss of diversity (richness and evenness) and the direct effect of human disturbance on ecosystem functioning (I).

Second, I studied the effect of multiple stressors on fungal communities and ecosystem functions in streams. I assessed how the combined effects of naturally low pH and forest drainage were reflected on stream fungal communities and leaf litter decomposition rates. I specifically assessed whether the combined effects of the stressors are additive or non-additive (synergistic, antagonistic) (II).

Third, I studied how naturally low pH and land use-related anthropogenic disturbances affect the temporal variability of stream fungal communities and leaf decomposition rates (III).

Lastly, I examined how land use-related disturbance and naturally low water pH affect the phylogenetic structure of stream fungal communities. I studied whether closely related fungal species have similar habitat requirements, and therefore co-occur more often than would be expected by chance (IV).

3 Materials and methods

3.1 Study areas

The studies were conducted in first-to-fourth order streams in northern and western Finland between 63° and 66° N and 22° and 27° E. Our data consist of anthropogenically disturbed sites in western Finland (I, III), naturally acidic sites in central Finland (I,II,III,IV) and near-pristine reference sites in northern Finland (I,II,III,IV). A total of 30, 24 and 21 sites were included in papers I, II and III, respectively. The data set in paper IV consists of 30 sites. The dataset for paper II also includes both circumneutral and naturally acidic human-impacted sites with extensive forest drainage in the catchment area. In naturally acidic sites, the catchments are dominated by metamorphosed black shale. Because of black shale, surface waters in these sites have naturally low pH and high metal concentrations in the stream water (Loukola-Ruskeeniemi et al. 1998). The presence of sulphide-rich black shale in the bedrock is associated with acid rock drainage and sulphide weathering. These processes take place essentially throughout the watershed and the tills also have material derived from black shale (Mäkinen et al. 2010). All reference streams had little agriculture (< 5%) in their catchments, no forestry activities near the sampling sites and no obvious signs of human impact in the riparian zone or in the stream channel.

3.2 Field surveys

The subprojects of this thesis were based on several types of data: field surveys of macroinvertebrate (I) and microbial (I,II,III,IV) data, physical and chemical in-stream characteristics (I,II,III,IV) and leaf decomposition experiments in streams (I, II, III).

3.2.1 Leaf decomposition experiments

The leaf decomposition experiments in papers (I) and (II) were conducted in years 2009 and 2010, whereas in paper (III) they were conducted during three consecutive years from 2009 to 2011. Leaf decomposition experiments were conducted to assess the process rates and to extract fungal DNA.

Decomposition experiments were conducted by enclosing six grams of dried alder leaves in mesh bags (15 x 15 cm). We used two different mesh sizes to allow (8 mm, coarse) or exclude (0.2 mm, fine) invertebrates. In papers (II), (III) and (IV), we used only data from the fine mesh bags. The leaves were collected prior to abscission in autumn 2009, 2010 and 2011 and air dried for two weeks. At each site, five bags of coarse (I) and fine mesh (I,II,III,IV) sizes were anchored to the stream bed using house bricks. The experiments started in mid-September every year and the bags were removed after approximately 30 days. Bags were enclosed in zip-lock bags and transferred to a freezer. In the laboratory, litter bags were gently cleaned to remove other material, and invertebrates from the coarse-mesh bags were preserved in ethanol and identified in the laboratory, mostly to species level.

From each fine-mesh bag, a subsample (12.5 cm²) was taken for the extraction of fungal DNA and measurement of ergosterol content. The weight of each subsample was taken into account when calculating the leaf decomposition rate. The remaining leaf material was dried for 48 h at 60 °C and subsamples were ashed for 4 h at 550 °C to convert air dry mass to ash-free dry mass (AFDM). Leaf decomposition rates (*k*) were determined using the negative exponential model. As the water temperature might vary between years, potentially influencing the decomposition process, we corrected the decomposition rates per degree-day in paper (III).

3.3 DNA isolation, sequencing and bioinformatics

Fungal assemblage structure was examined using pyrosequencing. The DNA was extracted from 0.07 g of frozen leaf material using a PowerSoil DNA Isolation kit (MOBIO laboratories, Carlsbad, CA). The internal transcribed region of the ribosomal DNA (rDNA) of fungi was amplified using the primers ITS1F 5'-CTTGGTCATTTAGAGGAAGTAA-3' (Gardes & Bruns 1993) and ITS4 5'-TCCTCCGCTTATTGATATGC-3' (White et al. 1990). The amplicons were sequenced using the GS FLX 454 system (Roche). In paper I, the ITS-sequences were located using the ITS extractor (Nilsson et al. 2010), and false positives were removed. Because of varying lengths of sequences, complete ITS1 sequences were extracted from the reads using a custom script. Reads shorter than 250 nucleotides were discarded, and ITS1 sequences were cut off from their flanking regions. Trimmed and filtered sequences were assembled into contigs with CodonCode Aligner version 3.7.1 (CodonCode Corporation, Dedham, MA)

using end-to-end alignments. The minimum percentage identity was 98%, minimum overlap length was 20 nt and word length was 12 nt. If necessary, the reads were manually edited and reassembled. In paper II, the fungal sequences were analysed using the software tool Pyrotagger (Kunin & Hugenholtz 2010), which trims using the 220 bp sequence length as a threshold and clusters OTUs based on 97% identity using the Uclust algorithm (Edgar 2010). In paper III, fungal sequences were analysed using the Quantitative Insights Into Microbial Ecology (QIIME) pipeline (Caporaso et al. 2010). The sequence library was split by samples and quality filtered based on the quality scores for each sequence. Sequences with quality scores below 25 were removed. Sequences shorter than 100bp or longer than 1000 bp, with more than 2 mismatches in the primer, with ambiguity, or with a maximum homopolymer run exceeding 6 were also removed. Sequences were clustered as OTUs using the Uclust algorithm (Edgar 2010) with 97% sequence similarities. Because sequence numbers varied across samples, the OTU datasets were rarefied to the lowest uniform re-sample size. The OTUs that occurred only at a single site were also removed from statistical analyses. Finally, taxonomy assignment was performed using the BLAST search algorithm against the UNITE database. In paper IV, sequences were aligned using MUSCLE (Edgar 2004), and the aligned sequences were used to construct an approximate maximum-likelihood tree using RAxML v7.0.3 (Stamatakis 2006). Sequence aligning and phylogenetic tree construction were performed using the QIIME software package (Caporaso et al. 2010).

3.4 Statistical methods

Table 1 summarises the statistical methods used in each paper and explains the main objectives of each different method. The goal of paper (I) was to assess the effects of human disturbance on ecosystem functioning and to assess whether decomposer diversity and/or abiotic factors could explain leaf decomposition rates. In paper (I), we also assessed the effect of a natural stressor (naturally low pH) on decomposer communities and leaf decomposition rates.

We used analysis of variance (ANOVA) to examine differences between stream types (circumneutral reference, naturally acidic, anthropogenically disturbed) in species richness, evenness, and abundance of shredders (numerical abundance) and fungi (ergosterol content). Evenness was calculated as $H'/\log(\text{richness})$, where H' is the Shannon diversity. We used two-way ANOVA, with stream type (three levels) and mesh size (two levels) as fixed factors, to

examine variation in leaf decomposition rates (k). Decomposition rates in coarse-mesh bags were corrected by decomposition in fine-mesh bags to obtain the fraction of decomposition attributable to detritivore feeding and physical abrasion (McKie et al. 2006).

Multiple linear regressions were used to examine the relationships between leaf decomposition rate and biological (richness and evenness of shredder taxa/fungal OTUs, ergosterol content, shredder abundance) and environmental variables. For these regressions, macroinvertebrate richness and evenness were calculated using invertebrates from the leaf bags, as these are presumably directly related to leaf decomposition. We screened correlations between water chemistry variables and selected those showing the least inter-correlation ($r < 0.6$) (total phosphorus and pH). We also included three in-stream habitat variables: current velocity, moss cover and substrate size. Current velocity was selected because physical disturbance can affect leaf decomposition (McKie & Malmqvist 2009), and moss cover and substrate size were selected to represent in-stream habitat characteristics known to be important regulators of boreal stream communities (Mykrä et al. 2011).

The most parsimonious multiple linear regression (MLR) model was selected using the Regression with Empirical Variable Selection (REVS) approach (Goodenough et al. 2012). REVS is based on a 'branch-and-bound' all-subsets regression approach and it quantifies the amount of empirical support for each variable in a dataset. A series of models is created: the first model contains the variable with the most empirical support, the second model contains the first variable and the next most-supported, and so on. The best model is selected based on Akaike's Information Criterion (AIC). This approach has been shown to be superior to full, stepwise and all-subsets models for most types of ecological data (Goodenough et al. 2012). This method was used in papers (I), (II) and (III).

We examined the independent influence of each variable significantly related to decomposition in MLR by constructing partial regression plots by regressing the independent variable of interest (e.g. shredder richness) against another independent variable (e.g. current velocity). Residuals of this regression were then plotted against residuals of the regression of the dependent variable (leaf decomposition) and the other independent variable (e.g. current velocity). This approach partials out the effect of other variables on the relationship and allows examination of the independent effect of an explanatory variable (Moya-Laraño & Corcobado 2008). Residuals were only used for graphical purposes, not for

statistical tests, because of the inflated type II error rate. Partial regression plots were used in papers (I), (II) and (III).

In paper (II), we assessed the combined effect of anthropogenic and natural stressors on stream fungal communities and leaf decomposition rates. Because of high collinearity among environmental variables, we reduced the dimensionality of the data using principal component analysis (PCA).

In paper (II), we used two-way ANOVA to test the effects of geology (naturally low pH vs. circumneutral streams) and disturbance (drained vs. non-drained streams) on biological response variables: species richness and evenness of fungal assemblages, fungal biomass (ergosterol content) and breakdown rates (k) of alder leaves. In case of a significant interaction between geology and forestry, we used simple effect tests to compare the means of one factor separately at each level of the other factor (and vice versa).

Relationships between leaf decomposition rates and biological (fungal OTU richness, evenness and ergosterol content) and environmental variables (PCA components) were examined using multiple linear regressions (MLR).

We used nonmetric multidimensional scaling (NMDS) with the Bray Curtis coefficient on log-transformed ($\ln(x + 1)$) abundance data to summarise the variability of fungal assemblage structure between the stream groups. A two way nonparametric Permutational Multivariate Analysis of Variance (PERMANOVA, (Anderson 2001) and Permutational Analysis of Multivariate Dispersions (PERMDISP, (Anderson 2006) were used for further examination of the differences in assemblage structure among the stream groups. PERMANOVA examines differences in assemblage structure among *a priori* defined site groups and partitions dissimilarities for the sources of variation in a multivariate data set, yielding tests of significance of the dissimilarities using a permutation based F-test (Anderson 2001). We ran PERMANOVA using the Bray-Curtis similarity coefficient of log-transformed abundance data with 999 permutations. PERMDISP uses the ANOVA F-statistic to compare among-group differences in the distance from individual observations to their group centroid. The significance of among-group differences is tested through permutation of least-squares residuals. We used the Bray-Curtis similarity coefficient with 999 permutations to test the null hypothesis of no differences in the dispersion (i.e. β -diversity) between low-pH vs. circumneutral sites and impacted vs. reference sites. We could not test for the interaction, because two-way approaches are not available in PERMDISP. In case of a significant difference, Tukey's HSD test was conducted to examine pairwise differences in β -diversity between site groups.

Finally, we used SIMPER (Similarity Percentage Analysis) to assess which fungal taxa contributed most to differences in assemblage structure among the site groups.

In paper (III), we assessed temporal variability of fungal communities and leaf decomposition rates in different stream types (circumneutral reference, naturally acidic and anthropogenically disturbed). As a measurement of temporal variability, we calculated coefficient of variation ($CV = \text{standard deviation}/\text{mean}$) of mean leaf decomposition rates across the three years for each site. We then assessed fungal community stability by calculating the mean among-year dissimilarity of community composition for each site based on the Bray-Curtis coefficient. We also calculated the mean among-year Euclidean distances of water chemistry (pH, Tot-P, Iron) and physical variables (current velocity, water depth) for each site.

To examine the general influence of human disturbance we first used repeated measures ANOVA (rmANOVA) to examine differences between stream types (circumneutral reference, naturally acidic and anthropogenically disturbed) and study years in decomposition rates, fungal OTU richness, evenness and leaf ergosterol content. In the case of a significant year*stream group interaction, we used simple effect tests.

We examined the differences in temporal variability of decomposition rates, fungal communities and environmental conditions among the stream types using ANOVA on the coefficient of variation (CV) of leaf decomposition rates, among-year dissimilarities of community structure and among-year distances of chemical and physical environmental conditions as response variables.

Multiple linear regression (MLR) was used to examine whether stability of leaf decomposition rates (CV) was related to biological (mean richness, mean evenness, CV richness, CV evenness and Bray-Curtis dissimilarity of community composition) and environmental variables (mean Euclidean distance of water chemistry and water physical properties). We also constructed an MLR model to study whether community stability (Bray-Curtis dissimilarity) was related to biological (mean richness and mean evenness) and environmental variables (mean Euclidean distance of water chemistry and water physical properties).

Finally, we compared the consistency of OTU dominance rankings between consecutive years across the ten most dominant taxa for each stream type. The aim of this approach was to detect whether the same species remained dominant in each stream type through time. We further assessed the dominance structure of

fungus communities by constructing rank abundance curves for each stream type, separately for each study year.

In paper (IV), we tested the relationship between OTU niche differences and phylogenetic distances using the Mantel test. Distances between OTU environmental optima were used as a measure of segregation of species ecological niches. We measured the environmental optima (i.e. niche position) for each OTU using OMI (outlying mean index) analysis (Dolédec et al. 2000). This method measures the marginality of a species' habitat position along environmental gradients. The OMI measures the distance between the mean habitat conditions used by a species (species centroid) and the mean habitat conditions of the sampling area (origin of niche hyperspace). OMI analysis describes both unimodal and linear responses of species to environmental gradients (Dolédec et al. 2000). We calculated the OTU habitat positions using log-transformed sequence data and total phosphorus, conductivity, pH, water colour and iron concentrations as environmental variables. OMI values were calculated for OTUs that occurred in at least three sites. We further evaluated the phylogenetic signal in OTU niche differences across a range of phylogenetic depths using Mantel correlograms (Diniz - Filho et al. 2010, Wang et al. 2013). Phylogenetic distances were partitioned into classes (0.02 units) and, within each class, between-OTU phylogenetic distances were correlated against their environmental optimum distances using Pearson correlations. Significance of correlations was tested using 999 permutations.

To test for phylogenetic clustering of fungus communities, we examined whether there was a significant correlation existed between the phylogenetic distances of pairs of OTUs and their degree of co-occurrence (see Cavender - Bares et al. 2004). Pairwise values of co-occurrence were calculated using Schoener's index (C_{ij}) (Schoener 1970). The significance of the correlation between phylogenetic distance and co-occurrence was tested against a null model with a swap algorithm that maintains both taxa richness and frequencies.

We tested the effect of anthropogenic disturbance on phylogenetic diversity using the phylogenetic species variability (PSV) of the fungus OTUs (Helmus et al. 2007). PSV yields values close to 0 if species are closely related ('phylogenetic clustering') and close to 1 if species are phylogenetically remote ('phylogenetic over-dispersion') (Helmus et al. 2007). Because PSV is independent of species richness, it is highly suitable for studying the effects of disturbance on phylogenetic diversity (Helmus et al. 2007). As another measure of phylogenetic diversity, we used phylogenetic species evenness (PSE) (Helmus

et al. 2007). While PSV is based on binary species data, PSE also accounts for species abundances and may therefore be more sensitive than PSV to changes in phylogenetic community structure. We constructed null models to test whether communities in each of our site groups represented non-random draws from the regional species pool, thereby indicating a phylogenetic signal in community structure (Helmus et al. 2010). The null model for PSV was generated using the quasiswap algorithm (Miklós & Podani 2004), which maintains both original taxon frequencies and number of taxa observed at a site. For PSE, we used the quasiswap-count algorithm that also randomises species abundances. PSV and PSE were calculated based on 999 randomly generated assemblages and the observed means for each site group were compared to 95% confidence intervals derived from randomly generated values of PSV and PSE. We assumed that any species could potentially colonise all streams in the study area; therefore, we used the total set of OTUs observed across all samples as the estimate of the regional species pool in the null model analyses.

We examined differences among stream groups in PSV and PSE using ANOVA. To compare phylogenetic variation to taxonomic variation of fungal communities, we also used ANOVA to test for differences in OTU richness and evenness among the stream groups. Because of the wide variation in stream size and catchment geological characteristics, we used a two-factor nested design where stream status (i.e., reference or disturbed) was nested within stream size. There were thus two groups (reference vs. disturbed sites) nested within the large streams, and three groups (circumneutral reference sites, naturally acidic reference sites, and naturally acidic disturbed sites) nested within the small streams. Both factors were considered fixed. In case of a significant stream status effect within the stream-size effect, contrasts were applied to compare reference and disturbed sites within each size category.

We employed indicator species analysis (IndVal) (Dufrêne & Legendre 1997) to identify significant indicator OTUs for the reference vs. disturbed sites in each stream-size category. IndVal combines information of species abundances and occurrences in each group; good indicators are always present at each site within a group and never in other groups (Dufrêne & Legendre 1997). Indicator values range from zero to the maximum of 100 that pertains to maximum indication. The significance of indicator values was tested using Monte Carlo permutation tests with 1000 permutations.

For classification, the ITS sequences of the indicator OTUs, along with sequences of the best BLAST hits, were aligned using ClustalW, and a

phylogenetic analysis was performed by the Maximum Likelihood method in Molecular Evolutionary Genetics Analysis (MEGA) program version 6.06 (Tamura et al. 2013). The confidence of specific clades from the resulting topology was tested by bootstrap analysis with 1000 replicates. All positions containing gaps and missing data were eliminated from the dataset (Complete-Deletion Option).

Table 1. Statistical analysis and objectives of each analysis

| Paper | Method | Objective |
|------------|---|---|
| I | Two-way ANOVA | To test for differences among stream groups and mesh sizes in leaf decomposition rates (<i>k</i>) |
| I, II, III | Multiple linear regression (MLR) | To associate leaf decomposition rates to key biological and environmental variables |
| I, II, III | Partial regression plots | To examine the independent influence of each variable significantly related to decomposition in MLR |
| II | Principal component analysis (PCA) | To identify the main environmental gradients |
| II | Nonmetric Multidimensional Scaling (NMDS) | To summarise the variability of fungal assemblage structure between the stream groups |
| II | A two way nonparametric Permutational Multivariate Analysis of Variance (PERMANOVA) | To examine differences in assemblage structure among the stream groups |
| II | Permutational Analysis of Multivariate Dispersions (PERMDISP) | To examine differences in β -diversity between the stream groups |
| II | Similarity Percentage Analysis (SIMPER) | To assess which fungal taxa contributed most to differences in assemblage structure among the site groups. |
| III | repeated measures ANOVA | To examine differences between stream groups and study years in decomposition rates (<i>k</i>), fungal OTU richness, evenness and leaf ergosterol content |
| III | One-way ANOVA | To test differences among stream groups in coefficient of variation (CV) of leaf decomposition rates, among-year dissimilarities of community structure, and among-year distances of chemical and |

| Paper | Method | Objective |
|-------|-------------------------------------|---|
| | | physical environmental conditions |
| IV | Mantel test | To test relationship between OTUs' niche differences and phylogenetic distances |
| IV | Outlying mean index analysis (OMI) | to measure environmental optima for each OTU |
| IV | Mantel correlogram | To evaluate the phylogenetic signal in OTUs niche differences across a range of phylogenetic depths |
| IV | Null model | To test for a phylogenetic signal in community structure (PSV, PSE). To test correlation between phylogenetic distance and co-occurrence of OTUs |
| IV | Two-factor nested ANOVA | To examine differences among stream groups in PSV and PSE |
| IV | Indicator species analysis (IndVal) | To identify significant indicator OTUs for each stream group |

4 Results and discussion

4.1 The influence of a natural and an anthropogenic stressor on stream decomposer communities and ecosystem functioning (I)

Human activities have reduced the biodiversity of stream communities, with potentially negative consequences on ecosystem functions. A major challenge to research on biodiversity-ecosystem function is to separate the effects of diversity loss on ecosystem functions from the direct effects of human disturbance.

We found that anthropogenic disturbance had a strong effect on decomposer communities, as well as on leaf decomposition rates. Species richness of leaf-shredding macroinvertebrates and aquatic fungi was unrelated to decomposition, whereas fungal evenness was negatively correlated with decomposition, suggesting that human-induced changes in dominance, rather than richness, influence ecosystem functions. Despite a lower shredder diversity in naturally acidic than in circumneutral streams, decomposition rates did not differ among these two stream types, indicating redundancy among species and possible adaptation to naturally low pH (Petrin et al. 2007).

Elevated nutrient concentration often increases leaf decomposition rates, mainly by stimulating fungal activity (Gulis & Suberkropp 2003, Dang et al. 2005). Leaf decomposition rate was indeed faster in anthropogenically impacted streams, where nutrient concentrations were high. Nutrient enrichment can also decrease species evenness by homogenising resources, which may favour competitively superior species (Hillebrand et al. 2008). Moreover, high dominance could have led to a reduction in interspecific competition, thus allowing higher decomposition rates. Accordingly, fungal evenness was significantly lower in the human impacted streams than in the reference sites. Moreover, fungal OTU evenness was the only biological predictor of microbial decomposition rates.

In contrast to microbial decomposition, shredder evenness showed no relationship to decomposition rates, despite the higher degree of dominance in the impacted streams. This result is contrary to the findings of Dangles and Malmqvist (2004), who reported faster leaf decomposition rates in their study streams when shredder dominance was high. In their study, a positive dominance-decomposition relationship was found regardless of the identity of the dominant species. In our study, the abundance of *Asellus aquaticus* was positively related to

decomposition rates of the coarse-mesh bags, which could partly explain the enhanced breakdown rates in anthropogenically impacted streams. Indeed, *Asellus* is a highly tolerant genus, and it often has a disproportionate effect on leaf decomposition rates (Bergfur et al. 2007, Woodward et al. 2012). High abundances of leaf-shredding stoneflies have been related to high leaf litter standing stocks in acidic streams (Woodward 2009). Increased shredder abundance may compensate for the negative effects of reduced species diversity on leaf decomposition rates in these streams (Petrin et al. 2007). In our study, shredder abundances were also higher in the naturally acidic than in the circumneutral streams, albeit not significantly so.

As expected, richness of leaf-shredding invertebrates was lowest in the anthropogenically altered streams. Fungal richness, by contrast, was not affected by human disturbance and it was unrelated to decomposition rates. In contrast to the findings of most experimental studies, fungal diversity was very high in our study. This may partly explain the lack of a relationship between richness and decomposition. In experimental studies, the decomposition rate saturates at a low level of richness, suggesting that even large reductions in fungal richness may not affect decomposition in natural ecosystems (Bell et al. 2009). Experiments also often oversimplify fungal communities by having even inoculum mixtures, while natural microbial assemblages (as in our study) usually comprise a few dominants and a large number of rare species (e.g. Sogin et al. 2006). Species dominance and the identity of the dominant taxa may therefore be more appropriate measures of human disturbance than species richness, particularly for hyperdiverse communities, such as aquatic fungi.

Our results suggest that using both functional and taxonomic measures will, in most cases, allow the most comprehensive assessment of biological responses to anthropogenic stressors. Furthermore, instead of focusing solely on diversity, more emphasis should be placed on changes in dominance patterns and identification of key species that have disproportionate effects on ecosystem processes. Our study suggests that controlling excessive nutrient inputs is critical for improving the ecological functioning of stream ecosystems, especially as the direct control of biodiversity may not be feasible.

4.2 The combined effect of a natural and an anthropogenic stressor to decomposer communities and ecosystem function (II).

In addition to anthropogenic stresses, biological assemblages can face stressors originating from natural sources. Anthropogenic and natural stressors can act simultaneously and species responses to different stressors may differ from those predicted based on the individual effects of each stressor alone (Townsend et al. 2008). In particular, acclimation or adaptation to one stressor may increase an organism's sensitivity to novel stressors, suggesting a potential cost associated with greater tolerance (Kashian et al. 2007). A naturally stressful environment can also act as a strong environmental filter that eliminates sensitive specialists, leaving a subset of generalists able to tolerate the harsh conditions (Chase & Myers 2011).

The effect of forest drainage on microbial leaf decomposition was more pronounced in the naturally acidic than in circumneutral streams, indicating that the decomposition process can be sensitive to additional disturbances in naturally stressed systems. Although such a synergistic response was not observed for fungal diversity, evenness or fungal biomass (ergosterol), the turnover of fungal assemblage composition was significantly lower in the naturally acidic sites affected by drainage than in the corresponding reference (non-drained) sites, implying that human disturbance may simplify fungal assemblages in naturally stressful conditions.

In our study, forest drainage appeared to increase siltation (as indicated by a higher proportion of fine particles) in the circumneutral streams, yet the decomposition rates were similar in both drained and reference streams. In the naturally acidic sites, drainage was associated with increased metal concentrations and decreased water pH, whereas no siltation was evident. A number of studies have shown that fungal assemblages respond more to changes in water chemistry than to changes in the physical habitat (e.g. Niyogi et al. 2003, Clivot et al. 2013). Lack of a strong effect of drainage on water chemistry could thus explain why fungal assemblages and decomposition rates in the circumneutral streams showed no response to drainage. The fact that decomposition rates were related to water acidity and heavy metal content and, to lesser degree, fungal OTU evenness, strongly suggests that the among-region variation in water chemistry was the main determinant of leaf decomposition rates.

The few studies that have assessed the consequences of human disturbance on the compositional turnover of freshwater assemblages have shown that anthropogenic disturbances tend to homogenise assemblages (Olden & Poff 2004, Passy & Blanchet 2007, Donohue et al. 2009), and act as non-random filters, selecting species with traits that allow persistence in the disturbed environment (Chase & Myers 2011). Assemblage turnover was very low in our naturally acidic drained sites and, although local OTU richness was unaffected by drainage, regional richness was reduced, suggesting that low pH, together with increased metal concentrations, acted as a strong environmental filter that selected a limited subset of tolerant taxa from the regional taxon pool. Although tolerant to naturally low pH, these taxa were clearly less effective decomposers. However, fungal biomass tended to be higher in the naturally acidic than in circumneutral sites, suggesting that some taxa may have benefited from low pH. Despite their high biomass, fungal assemblages in the naturally acidic drained sites were nevertheless functionally the least effective (i.e. had the slowest decomposition rate), suggesting that low pH and associated metals affected fungal metabolism rather than biomass.

The strong effect of catchment geology on biological assemblages shows that locally derived chemical and biological baselines are needed for assessing the effects of human activities in naturally harsh environments (Schmidt et al. 2012) and that conservation planning needs to give special attention to these vulnerable, naturally stressful ecosystems. The decreased compositional turnover of fungal assemblages in anthropogenically impacted streams has important implications for stream management. Because of the reduced regional species pool, potential for compensation of local extinctions may be low and fungal assemblages might therefore respond weakly to restoration (Olden & Poff 2004), with consequent effects on leaf decomposition rates.

4.3 The effect of an anthropogenic and a natural stressor on the stability of the fungal community and ecosystem functioning (III)

Although temporal variability is a fundamental property of ecological communities, increased temporal variability may also indicate a disturbance that may go undetected when using average values of community properties or process rates (Fraterrigo & Rusak 2008). We found that fungal communities in disturbed sites were less diverse and more dominated than communities in

reference sites, yet communities at these sites were as stable as those at the reference sites. However, fungal communities in anthropogenically disturbed sites were unable to sustain stable leaf decomposition rates, thus indicating a negative influence of anthropogenic stress on functional stability. In our study, the among-year changes in water chemistry were larger in the impacted sites, being positively related to the temporal variability in leaf decomposition rate. This high variability was not surprising, because these streams are affected by both elevated nutrients and episodic acidification (Saarinen et al. 2010). However, the mean decomposition rates did not differ in the impacted sites, suggesting that decomposition rates in different years were lower or higher in the impacted sites, depending on the levels of nutrients or the pH.

Anthropogenic stress often has a negative influence on decomposer communities (Simon et al. 2009) and species loss has been a key factor when considering the effect of anthropogenic activities on ecosystem stability (Tilman 1999). In contrast to decomposition rates, communities in anthropogenically disturbed sites were as stable as in the reference or naturally acidic sites, despite the reduction in fungal OTU richness in the anthropogenically disturbed sites. However, land use and other anthropogenic disturbances may also influence species relative abundances (Chapin et al. 2000). Related to this, we found that fungal OTU evenness was also lower in the anthropogenically disturbed sites. However, although the dominant OTUs were temporally stable in anthropogenically disturbed streams, they were incapable of maintaining stable ecosystem processes. One explanation is that insurance mechanisms are lacking in highly dominated communities, where ecosystem functions depend strongly on the metabolic performance of the dominant species; consequently, ecosystem process rates may vary without marked changes in their abundance (Schimel et al. 2007). As a result, dominated communities can provide a certain level of ecosystem functions in a stable environment, but in stressed and variable environments, such as human impacted streams, their functional stability is decreased by abiotic fluctuations (Wittebolle et al. 2009, Wright et al. 2009).

In contrast to anthropogenically disturbed sites, decomposition rates were more stable in streams with naturally low pH, indicating more stable ecosystem functions in naturally harsh environments. However, in 2011, the decomposition rates in naturally acidic sites were lower than in 2009 or 2010. This may be explained by the fact that the water pH in 2011 was low in this region. This result partly disagrees with the notion that communities in naturally stressful environments can become adapted or acclimated to a prevailing stressor, for

example, low pH (Dangles et al. 2004, Petrin et al. 2007, Tolkkinen et al. 2013). However, environmental conditions and decomposition rates, overall, were relatively stable in these streams, further suggesting a primary role of disturbance frequency as a determinant of the responses of ecosystems to stress.

Our results showed that leaf decomposition rates are temporally fairly constant in the reference streams, supporting the use of leaf decomposition, and particularly its microbial component, as a measure of ecosystem health in stream bioassessment (Dale & Beyeler 2001). Using both functional and taxonomic measures will in most cases allow a more comprehensive assessment of the biological responses to environmental stressors in streams. Nevertheless, although the process rates and diversity of fungal communities may be regulated in part by the same factors, the process rates seem to be more sensitive to environmental variability. Our results highlight the need to measure not only the mean process rates but also the temporal variability of process rates, as responses to anthropogenic stress may be overlooked by looking solely at mean rates (Fraterrigo & Rusak 2008).

4.4 The effect of natural and anthropogenic stressors on the phylogenetic structure of stream fungal communities (IV)

Recent studies have shown that disturbances can create communities dominated by closely related, disturbance-adapted species (Dinnage 2009, Helmus et al. 2010, Brunbjerg et al. 2012, Ding et al. 2012). We found increased phylogenetic relatedness among fungal OTUs in our anthropogenically disturbed streams, suggesting that disturbances act as a strong selective filter shaping the phylogenetic structure of fungal communities. Furthermore, naturally acidic disturbed sites were also characterised by a number of closely related OTUs. Given that no decrease occurred in OTU richness, it is likely that some fungal taxa were replaced by better-adapted, tolerant taxa.

The phylogenetic distances of the fungal OTUs were positively correlated with their niche differences, indicating a phylogenetic signal in the environmental tolerance of aquatic fungi. Co-occurrence was also negatively related to phylogenetic distance, further suggesting that closely related OTUs were ecologically more similar than were more distantly related OTUs. However, partitioning of the relationship between phylogeny and niche differences using Mantel's correlograms revealed a more complex pattern. The relationship was positive across short phylogenetic distances, negative across intermediate

distances and non-significant across large distances. Thus, the evidence supported niche conservatism only at short phylogenetic distances, while niches converged at intermediate distances, indicating convergently evolved physiological adaptations to the same environmental filter (Losos 2008).

Naturally acidic disturbed sites were characterised by a number of closely related OTUs. Given that many of these indicator OTUs were either very abundant in (or even unique to) these streams, they were not a nested subset of OTUs occurring in reference sites. This suggests that these taxa may not be generally tolerant to environmental stress, but are instead adapted to low pH and may even benefit from competitive release in these environments. These were uncultured OTUs, the majority of them being closely related to *Varicosporium elodeae*, a species reported to be tolerant of low pH and elevated metal concentrations (Azevedo et al. 2009, Niyogi et al. 2009, Ferreira et al. 2012). In larger streams, land-use disturbance affected water quality in two ways: by reducing stream pH and elevating nutrient concentrations. This complicated chemical response may explain the wider phylogenetic distances among indicator OTUs in these streams when compared to naturally acidic streams, where drainage-induced changes to water quality were more consistent.

The high number of indicator OTUs of the genus *Varicosporium* in naturally acidic disturbed sites indicates intraspecific variation in the genetic structure of these OTUs. Duarte et al. (2014) also reported a high degree of intraspecific variation among aquatic fungi along a eutrophication gradient, suggesting that increased intraspecific genetic variation in response to environmental degradation may be common in aquatic fungi. This intraspecific variation could result from rapid genetic changes or small-scale evolutionary processes associated with inherited increases in tolerance to adverse environmental conditions (Medina et al. 2007, Vallino et al. 2011). Genetically inherited tolerance in functionally important species (that is, species important for a given ecosystem process) could buffer ecosystem functions against environmental disruptions. Fernandez et al. (2011), for example, showed that fungal richness had a positive effect on leaf decomposition rates under metal stress only when experimental cultures contained a functional type isolated from a metal-polluted stream. Stress-induced micro-evolution can modify the functional traits of species, leading to altered species performance and a novel state of ecosystem processes in disturbed environments (Medina et al. 2007).

Both PSV and PSE were lower in disturbed sites, indicating that human disturbances decreased the phylogenetic diversity of fungal communities. The

null model analysis showed, however, that the patterns for PSV were random in all but the naturally acidic disturbed sites. Individual large reference streams and small circumneutral reference streams even indicated phylogenetic over-dispersion. Unlike PSV, PSE showed that fungal communities in our study were clustered in both types of disturbed sites, as well as in naturally acidic reference sites. However, PSE also indicated that communities in both large reference sites and circumneutral small reference sites were mainly over-dispersed. Our results thus indicate that fungal communities in near pristine streams may be weakly environmentally structured, with interspecific competition and niche partitioning being the main drivers of fungal community structure. Phylogenetic clustering of communities in naturally acidic reference streams, however, showed that low water pH and associated metals may constitute a major environmental filter also in undisturbed environments.

Fungal communities at disturbed sites were phylogenetically less diverse and more dominated than those in undisturbed streams, while OTU richness and evenness were much weaker indicators of human disturbances. Phylogenetic community structure thus allowed inference about the effects of anthropogenic disturbances on fungal communities, independently of taxonomic structure. At disturbed sites, sensitive fungal species were replaced by a homogenous group of closely related, stress-tolerant taxa. Such a replacement of sensitive taxa by more stress-tolerant taxa likely explains the weak responses of fungal OTU richness to anthropogenic stress, but because these tolerant taxa were phylogenetically closely related, the effect of disturbance was detected as reduced phylogenetic diversity. Our results thus suggest that the phylogenetic structure of fungal communities may be more sensitive to anthropogenic disturbance than its taxonomic structure and that the phylogenetic approach could provide a more effective tool for monitoring ecosystem integrity (Pienkowski et al. 1998, Helmus et al. 2010). The contrasting patterns of phylogenetic clustering and over-dispersion in disturbed and natural environments, however, suggest that different mechanisms may affect the coexistence of fungi in different environmental settings. Using novel sequencing methods can be also more cost effective than traditional methods (e.g. invertebrate kick-net samples).

5 Conclusions

Understanding the effect of human disturbance on ecosystem functioning is one of the most important assignments of ecological research, as the healthy functioning of ecosystems is essential for life on Earth. To date, there is still a lack of information on how biotic (e.g. biodiversity) and abiotic drivers affect stream ecosystem functioning. The microbial component, or the 'rare biosphere' (Sogin et al. 2006), of stream ecosystems has received especially little attention, although microorganisms are the most diverse and abundant group of organisms in all ecosystems (Fierer & Jackson 2006).

The main conclusion of this thesis is that biodiversity is indeed an important part of stream ecosystem functioning. Although species richness did not affect ecosystem functioning, species dominance patterns explained variation in decomposition rates. This finding supports previous research and theory that human disturbance may have more effect on species relative abundances than on species richness *per se*, with potentially wide-ranging consequences on ecosystem functioning (Hillebrand et al. 2008). Microbial dominance patterns, in particular, should not be overlooked. Moreover, our results suggest that, in some cases, the phylogenetic structure of microbial communities may be more sensitive to anthropogenic disturbance than is the taxonomic structure, and that the phylogenetic approach could provide an effective tool for monitoring stream ecosystem integrity (Pienkowski et al. 1998, Helmus et al. 2010). Because of the tight connection between abiotic variables, community composition, and ecosystem functioning, one of the most difficult challenges is testing the direct effect of microbial composition on ecosystem functioning. Therefore, future work should also include experimental studies to quantify the direct causal links of different stressors on stream ecosystem functions and, in particular, to unravel the relationship between microbial composition and ecosystem functioning.

This thesis shows that the effects of human disturbance on stream microbial communities and ecosystem functioning are highly context-dependent. In our studies, changes in water chemistry affected stream decomposer communities and ecosystem functioning more than did changes in hydromorphological conditions. As a result, stream management aiming to enhance ecosystem functioning should focus more on catchment than riffle-scale restoration. Also natural abiotic conditions influenced how the stream ecosystem responded to human disturbance. Therefore, stream management, and particularly conservation planning, should give special attention to vulnerable, naturally stressful ecosystems.

Our results showed that leaf decomposition rates are temporally fairly constant in reference streams, supporting the use of leaf decomposition as a measure of ecosystem health in stream bioassessment (Dale & Beyeler 2001, Gessner & Chauvet 2002). However, in this thesis, we used a single sampling event to measuring decomposition rates. Fungal communities readily change over the course of decomposition and, therefore, repeated sampling would be needed to monitor successional changes in fungal communities during the process

Although leaf decomposition rates and diversity of fungal communities may be regulated by partly the same factors, decomposition appeared to be more sensitive to environmental variability. Decomposition rates varied more in human-disturbed streams, suggesting that temporally replicated monitoring is needed for a reliable assessment of human disturbance in streams with highly fluctuating environmental conditions. Although the dominant taxa in our disturbed sites were temporally stable, they could not maintain stable ecosystem functions. Decomposition rates therefore varied temporally, with no change in the relative abundances of dominant taxa, suggesting that variability was driven by changes in the metabolic activity of the dominant taxa rather than by changes in their abundances. Our results thus emphasise that the use of both functional and taxonomic measures will, in most cases, allow a more comprehensive assessment of biological responses to human disturbance.

The molecular method we used does not allow distinguishing between metabolically active and inactive structures of microbial cells, and we therefore cannot evaluate whether human disturbance reduces the active component of microbes. Hence, future studies should focus more on the active parts of microbes, by studying microbial RNA, in order to distinguish the role of active microbes on stream ecosystem functioning (Lennon & Jones 2011). The use of molecular methods in future should aim to provide a more comprehensive view of the occurrence and ecological roles of aquatic hyphomycetes outside their preferred habitat (e.g. as endophytes in leaves) (Chauvet et al. 2015). The use of traditional morphology-based methods in parallel with novel sequencing methods could give a more reliable assessment of fungal community composition, as a high number for fungal OTU richness can be related to intraspecific variations.

This thesis provides novel information about how anthropogenic disturbance and a natural stressor interact to affect stream decomposer communities and ecosystem functions. The thesis has major implications for understanding the role of microbial biodiversity on stream ecosystem functioning. We showed that the use of new high-throughput sequencing methods can allow researchers to unravel

spatial and temporal variations in microbial biodiversity, allowing the discovery of numerous bacterial and fungal taxa that have gone undetected via traditional methods (Fuhrman 2009, Bärlocher 2010). This is an important contribution, as policy makers are starting to recognise the possible adverse consequences of reduced biodiversity on ecosystem functioning, ecosystem services and human wellbeing (Naeem et al. 2009).

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- I Tolkkinen M, Mykrä H, Markkola AM, Aisala H, Vuori KM, Lumme J, Pirttilä AM & Muotka T (2013) Decomposer communities in human-impacted streams: species dominance rather than richness affects leaf decomposition. *Journal of Applied Ecology* 50:1142–1151.
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- III Tolkkinen M, Mykrä H, Markkola AM & Muotka T Human disturbance increases functional but not structural variability of stream fungal communities. *Functional Ecology*. *Accepted*
- IV Mykrä H, Tolkkinen M, Markkola AM, Pirttilä AM & Muotka T. Phylogenetic clustering of fungal communities in anthropogenically altered streams. *Manuscript*.

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