

Mari Tolkkinen

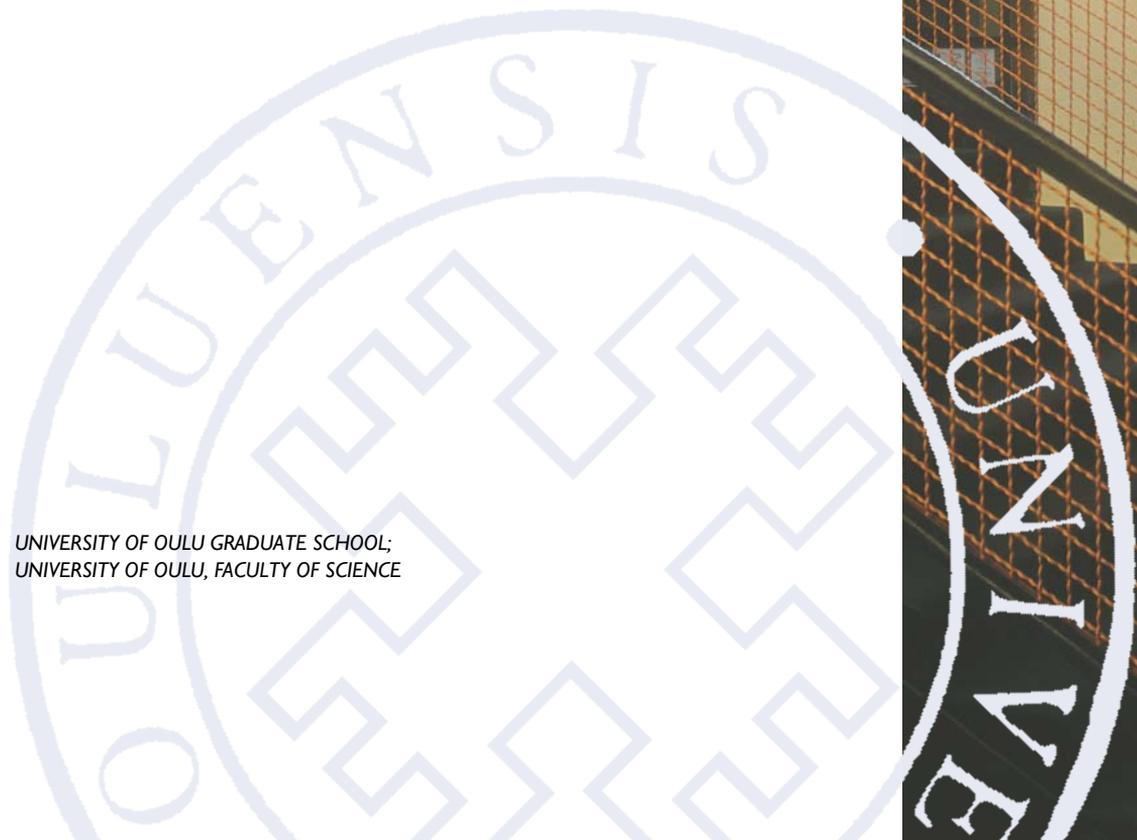
MULTI-STRESSOR EFFECTS IN BOREAL STREAMS

*DISENTANGLING THE ROLES OF NATURAL AND
LAND USE DISTURBANCE TO STREAM
COMMUNITIES*

UNIVERSITY OF OULU GRADUATE SCHOOL;
UNIVERSITY OF OULU, FACULTY OF SCIENCE

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MARI TOLKKINEN

**MULTI-STRESSOR EFFECTS
IN BOREAL STREAMS**

Disentangling the roles of natural and land use
disturbance to stream communities

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Abstract

As human activities are increasingly affecting natural communities, many communities are impacted by multiple stressors and their interactions. Understanding how natural and anthropogenic stressors act individually and in concert is essential for managing and conserving natural ecosystems efficiently. In this thesis I studied how geology-related natural acidity, land drainage and their interaction affect biological communities and leaf decomposition in boreal headwater streams. I further assessed the concordance of communities along natural and anthropogenic stressor gradients. As model organisms, I used benthic diatoms, bryophytes, invertebrates and leaf-decomposing fungi.

I showed that geology-related natural acidity constitutes a strong environmental filter for stream communities, reducing species richness and changing community composition. Community concordance was also generally strongest along the natural acidity gradient. However, natural acidity mostly did not homogenize communities nor did it affect leaf decomposition by fungi. Effects of peatland drainage differed between the two stream types, being mainly sedimentation in the circumneutral streams and increasing metal concentrations in the acid streams. Overall, changes in community composition were better able than pure species richness to track single stressor impacts. Furthermore, fungal assemblages were more homogeneous and decomposition rates slower in drained acidic sites than in any other stream type. Thus the drainage-induced shift in water chemistry in the acidic streams seems to constitute an even stronger environmental filter than sedimentation. Conservation planning needs to give special attention to these vulnerable, naturally stressful ecosystems.

Keywords: aquatic fungi, biodiversity, bryophyte, concordance, diatom, ecosystem function, invertebrate, leaf decomposition, natural acidity, peatland drainage, stream community, stressor interaction

Tolkkinen, Mari, Luonnollisen stressin ja ihmistoiminnan vaikutukset virtavesien eliöyhteisöihin.

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Tiivistelmä

Ihmistoiminnan vaikuttaessa yhä enemmän luonnon elinympäristöihin eliöyhteisöihin kohdistuu usein samanaikaisesti monenlaisia paineita. Ekosysteemien tehokas hoitaminen ja suojele edellyttävät tarkkaa tietoa siitä, miten luonnollinen stressi ja ihmistoiminta yhdessä ja erikseen vaikuttavat ekosysteemeihin. Väitöskirjassani tutkin, kuinka geologiasta johtuva luonnollinen happamuus ja metsäojitus vaikuttavat boreaalisten purojen eliöyhteisöihin ja lehtikarikkeen hajotukseen. Tarkastelin myös eliöryhmien yhdenmukaisuutta ihmistoimintagradienilla ja luonnollisella stressigradienilla. Malliorganismeinani olivat piilevät, vesisammalet, pohjaeläimet ja hajottajasienet.

Väitöskirjassani osoitan, että geologiasta johtuva puroveden happamuus toimii merkittävänä ympäristösuodattimena purojen eliöyhteisöille vähentäen lajirunsausta ja muokaten lajistoa. Myös eliöryhmien lajistovaihtelu oli yhdenmukaisinta luonnollisella happamuusgradientilla. Toisaalta luonnollinen happamuus ei vaikuttanut merkittävästi lehtikarikkeen hajotukseen tai purojen väliseen sieniyhteisöjen monimuotoisuuteen. Metsäojituksen fysikaalis-kemiallinen vaikutus erosi purotyypeittäin: pH-neutraaleissa puroissa ojitus pääosin lisäsi pohjan hiekoittumista, kun taas happamissa puroissa veden metallipitoisuudet kasvoivat entisestään. Yleisesti ottaen sekä luonnollisen happamuuden että metsäojituksen vaikutukset näkyivät parhaiten muutoksina eliöyhteisöjen lajikoostumuksessa. Lisäksi ojitetuissa happamissa puroissa hajottajasieniyhteisöjen lajistot olivat keskenään samankaltaisempia ja lehtikarikkeen hajotus hitaampaa kuin muissa purotyypeissä. Metsäojituksen aikaansaama muutos luonnollisesti happamien purojen vesikemialla näyttää siis olevan jopa merkittävämpi ympäristösuodatin kuin pohjan hiekoittuminen. Luonnollisesti stressattujen elinympäristöjen herkkyys maankäytön muutoksille tulisikin huomioida ympäristön hoidon suunnittelussa nykyistä paremmin.

Asiasanat: akvaattinen sieni, biodiversiteetti, boreaalinen latvapuro, ekosysteemin toiminta, happamuus, lehtikarikkeen hajoaminen, metsäojitus, piilevät, pohjaeläimet, vesisammalet, yhteisörakenne, ympäristösuodatin

*Look deep into nature, and then you will understand
everything better. –Albert Einstein*

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be-born baby for staying in the tummy until I have made it through the defense...
please do!

Oulu, January 2016

Mari Tolkinen

Abbreviations

ANOVA	analyses of variance
BLAST	basic local alignment search tool
DOC	dissolved organic carbon
FOREGS	Forum of European Geological Surveys
HPLS	high-performance liquid chromatography
ITS	internal transcribed spacer
rDNA	ribosomal DNA
OTU	operational taxonomic unit
PERMDISP	permutational analysis of multivariate homogeneity of group dispersions
SIMPER	similarity percentages

List of original articles

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

- I Annala M, Mykrä H, Tolkkinen M, Kauppila T & Muotka T (2014) Are biological communities in naturally unproductive streams resistant to additional anthropogenic stressors? *Ecological Applications* 24: 1887–1897.
- II Tolkkinen M, Mykrä H, Annala M, Markkola A. M., Vuori K. M. & 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 J, Mykrä H, Virtanen R, Tolkkinen M, Kauppila T, Paasivirta L & Muotka T (2016) Land use impacts on benthic community composition and concordance in streams across a natural stress gradient. *Ecological Indicators* 62: 14–21.

Author contributions

Paper	I	II	III
Original idea and study design	TM, HM, TK, MJT	TM, HM, MT, MJT	TM, HM, MJT
Field work	MJT, MT	MT, MJT	MJT, MT, RV
Species identification and laboratory work	MJT	MT, AMM	MJT, RV
Data analyses	MJT, HM	MT	MJT
Manuscript preparation	MJT, TM, HM	MT, TM, HM, MJT	MT, TM, HM, MJT MJT, TM
Manuscript revision and finalizing	MJT, HM, MT, TK, TM	MT, TM, HM, MJT, AMM, KMV	MJT, TM, HM, MT, RV, TK, LP

MJT = Mari J. Tolkkinen/Annala, HM = Heikki Mykrä, TM = Timo Muotka, MT = Mikko Tolkkinen, TK = Tommi Kauppila, RV = Risto Virtanen, LP = Lauri Paasivirta, AMM = Annamari Markkola, KMV = Kari-Matti Vuori

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1 Introduction

Almost all natural communities are exposed to some kind of stressor, not only because of pervasive human activities, but also because of the strong natural variation of environmental conditions. Studies on the biological responses to environmental stress have focused traditionally on single-stressor responses even though most ecosystems are subjected simultaneously to multiple stressors of both natural and anthropogenic origin. Furthermore, biotic responses to multiple stressors are often non-additive (Paine *et al.* 1998), that is, responses to interacting stressors may be less than or greater than the sum of the responses to individual stressors. Thus failing to reconcile stressor interactions may result in a gross underestimation of the ecological impacts of several, simultaneously operating stressors (Kashian *et al.* 2007).

Community response to anthropogenic disturbance in naturally stressful habitats may differ from that in more benign habitats (Paine *et al.* 1998, Christensen *et al.* 2006). For example, if species' tolerances to natural and anthropogenic stressors are negatively correlated, diversity can be severely reduced as a result of synergistic interactions (Vinebrooke *et al.* 2004). Synergism refers to a situation where the combined effect of two stressors exceed their potential individual effects. Synergistic interactions between a natural and an anthropogenic stressor may occur because acclimating or adapting to a natural stressor may increase organisms' sensitivity to novel stressors, suggesting a potential cost associated with greater tolerance (Kashian *et al.* 2007). Increased tolerance requires more energy to repair the damages caused by a stressor, potentially compromising the ability of organisms to withstand additional environmental perturbation (Congdon *et al.* 2001). It is equally possible, however, that a naturally stressful environment acts as a strong environmental filter that eliminates sensitive species, leaving only a subset of species able to tolerate the harsh conditions (Chase and Myers 2011). Such generally tolerant species may be less sensitive to novel stressors, and assemblages in naturally harsh conditions may thus be more tolerant to additional stress than those in more benign environments (Vinebrooke *et al.* 2004). In such a case, the combined effect of multiple stressors would be antagonistic, that is, the response to interacting stressors would be less than their individual effects suggest. Initial environmental conditions and evolutionary processes may thus determine the response of communities to current stressors.

Distinguishing environmental stress from natural variation of environmental factors can be challenging (Bijlsma & Loeschcke 2005). In this thesis I refer to “a

stressor” as an environmental factor that exceeds organisms’ common environmental tolerance, potentially decreasing the survival of organisms and causing changes in biological communities. Acidity-basicity condition is one example of such a naturally varying environmental factor and, especially in most extreme cases, may constitute a strong environmental stressor for stream organisms. In acidic streams, species sensitive to low pH are typically absent, and communities may be less diverse than those in circumneutral streams (Baudoin *et al.* 2008, Schmidt *et al.* 2012). The situation where only a subset of species in the regional species pool is able to persist in an acidic environment (Ledger and Hildrew 2005) may induce biotic homogenization and, consequently, impair ecosystem functions. However, naturally acidic streams may also support communities as functional and diverse as those in circumneutral streams (Dangles *et al.* 2004b, Petrin *et al.* 2007a and b, Hogsden and Harding 2012).

The autumnal input of riparian leaf litter is the primary source of energy and carbon in small woodland streams, and microbes, together with leaf-shredding invertebrates, are the main consumers of the leaf material. Low water pH and increased metal concentrations may have detrimental effects on microbial diversity and stream ecosystem functioning. The diversity of aquatic fungi generally declines with decreasing pH (Wood-Eggenschwiler and Bärlocher 1983, Baudoin *et al.* 2008) and leaf decomposition is typically reduced in acidic streams (Chamier 1987, Dangles *et al.* 2004a, Simon *et al.* 2009). However, through evolutionary time, assemblages may have become adapted or acclimated to naturally low pH, being capable of maintaining ecosystem functions in naturally acidic streams (Dangles *et al.* 2004a, Petrin *et al.* 2007b). Indeed, some fungal taxa can tolerate fairly low pH, and fungal diversity in naturally acid streams can be high (Bärlocher 1986, Thompson and Bärlocher 1989). Naturally high metal concentrations and low pH may be less harmful to biota than human-induced degradation, indicating adaptation or acclimation to naturally harsh habitats (Collier *et al.* 1990, Petrin *et al.* 2008, Schmidt *et al.* 2012).

In this thesis I studied land use impacts on naturally acid headwater stream biodiversity. The issue is timely as running waters have been seriously degraded by intensified land use during the past few decades (Dudgeon *et al.* 2006). Headwater streams are particularly vulnerable to anthropogenic disturbances because of their intimate contact with the riparian zone (Richardson and Danehy 2007). Human activity in the riparian area affects streams by altering energy pathways, sediment runoff, stream temperature and water chemistry, hence potentially leading to changes in community composition and decline in local species richness (α

diversity) and among-stream turnover (β diversity) of the stream biota (Passy and Blanchet 2007, Richardson and Danehy 2007, Donohue *et al.* 2009, Suurkuukka *et al.* 2014). Such community changes may further result in altered ecosystem functions (Covich *et al.* 2004, Carlisle and Clements 2005, Tolkkinen *et al.* 2013) and functional homogenization of communities (Clavel *et al.* 2011). If communities in naturally acid and circumneutral streams differ in their sensitivity to land use impacts, differences in the initial background acidity need to be considered in land use and environmental management protocols.

2 Aims of the thesis

The overall objective of this thesis was to study the responses of boreal headwater stream communities and ecosystem functions to land use disturbance and, particularly, whether the responses to land use differ among naturally harsh and more benign streams. We approached the study question from multiple points of view to obtain a holistic picture on land use disturbance effects on stream biota. Moreover, we assessed several ecologically important taxonomic groups to compare the responses of ecologically widely distinct organisms as they may differ in their sensitivity to a particular stressor. The specific study questions of each subproject aim to benefit basic research as well as freshwater management.

All subprojects employ the same study design (Fig. 1), which allows the comparison of individual and combined effects of forest drainage (ditched vs. near-pristine reference streams) and catchment geology (naturally acidic vs. circumneutral streams) on stream communities.

First, we studied if and how the local taxonomic richness (α diversity) and spatial turnover (β diversity) of fungi, diatoms, bryophytes and invertebrates (I, II) differ due to forest drainage in naturally acid and circumneutral streams. We expected the most sensitive species to be present only in the circumneutral non-drained streams. We predicted α and β diversity to be highest in circumneutral non-drained streams, because sensitive species are likely absent from the acid sites, reducing richness and homogenizing their communities. The lowest α and β diversity was expected in acid streams additionally stressed by drainage due to potentially synergistic effect of the two stressors (“cost-of-tolerance” hypothesis, Kashian *et al.* 2007). Alternatively, communities in naturally acidic streams may consist of the most tolerant taxa that are largely unaffected by further stressors (Grime *et al.* 2008, Damschen *et al.* 2012), and therefore diversity in these streams could remain unaffected by drainage. In that case responses to multiple stressors would be antagonistic. Finally, if communities show simple additive responses, drainage ditching would affect communities in acid streams as much as those in circumneutral streams, and α and β diversity would be directly related to stress level.

Second, we assessed how the community composition of fungi, diatoms, bryophytes and invertebrates is affected by forest drainage in naturally acid and circumneutral streams (II, III), because changes in community composition may occur independent of shifts in species richness. We also aimed to relate changes in community composition to major environmental gradients. We expected naturally acid stream assemblages to differ from those in circumneutral streams because

acid-sensitive species should be absent or scarce in the acid streams while the abundance of tolerant species may increase due, for example, to competitive release.

Third, we assessed how the rate of microbial decomposition, one of the key ecosystem processes of headwater streams, differs due to forest drainage in naturally acid and circumneutral streams (II). Decreased consumer diversity does not necessarily translate to impaired ecosystem functioning if the remaining assemblage members are able to sustain the key functions (McKie and Malmqvist 2009, Tolkkinen *et al.* 2013). Based on this hypothesis ('redundancy hypothesis'), accompanied by the adaptation/acclimation hypothesis (Dangles *et al.* 2004a, Petrin *et al.* 2007b), we predicted that the decomposition rates in naturally acid and circumneutral reference streams should not differ. Further, we hypothesized that because of species' positive co-tolerances to forest drainage and natural acidity (Vinebrooke *et al.* 2004), fungal leaf decomposition should be less responsive to additional disturbance in naturally acidic than in circumneutral sites.

Finally, we aimed to determine the rate of covariation among biological groups (i.e. concordance) along natural and anthropogenic stressor gradients (III). Concordance between a pair of communities is expected if they respond similarly to major environmental gradients. We expected the communities to show strong concordance along all stressor gradients, but especially so along the eminently strong natural gradient from circumneutral to highly acidic streams.

3 Materials and methods

3.1 Study area

Our study streams are located in two adjacent drainage basins in northern Finland (64°–66°N, 26°–29°E). All the streams are order 1-to-2 headwater sites and they run through peatlands and mixed riparian forests. The two basins differ in terms of bedrock geology: the Iijoki basin is mostly dominated by gneiss–granites, whereas the Oulujoki basin is characterized (in addition to gneiss–granites) by metamorphosed black shales, causing naturally increased metal concentrations and decreased pH of surface waters (Loukola-Ruskeeniemi *et al.* 1998). Therefore the streams among the two drainage basins differ in terms of water pH and metal concentrations, whereas the study sites in both regions were selected to be as closely similar as possible in regard to in-stream and riparian characteristics. Thus, as these two regions differ markedly based only on geology, we were able to compare the responses of stream communities to forestry activities in areas with contrasting background geology, one with naturally stressed conditions (combination of low pH and high metal concentrations), the other a reference (circumneutral pH, low metals).

Forestry, particularly peatland drainage, is the major form of land use in both study areas. Drainage ditching is practiced in Finland and other European countries to channel surplus water to streams to enhance forest growth. The drainage of peatlands for silvicultural purposes in Finland reached a peak in the 1960s to 1980s. As a consequence, 5.5 million ha of the forest area of Finland have been drained, constituting about 50% of the total peatland area of the country (Peltomaa 2007). Drainage has severe repercussions on streams: it adds surplus sediments into stream channels, reduces water quality and modifies catchment hydrology (Vuori *et al.* 1998), thus potentially acting as an environmental filter precluding the occurrence of some species. Disturbed sites were selected based on the percentage of forest ditches in the near-stream catchment (a 1 km long, 100 m wide upstream riparian buffer). Half of the study sites were drained (>27% of the buffer drained), the other half being reference sites (<15% drained) in both areas. In papers I and II, we sampled 12 study sites in each stream group (altogether 48 streams, Fig. 1), and in paper III six sites per stream group (altogether 24 streams).

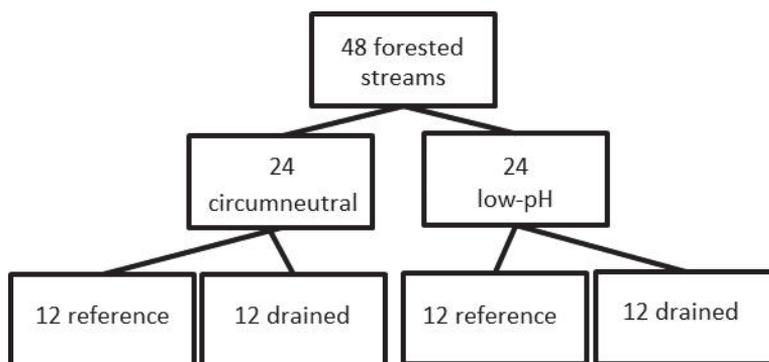


Fig. 1. A schematic presentation of the study design of subprojects I and III. The same set of streams were used in subproject II, but it comprised only half of the sites in each stream group. 'Reference' refers to non-drained streams.

3.2 Biological data

We sampled benthic diatoms, bryophytes and invertebrates from all the 48 study streams for papers I and III, and identified fungal OTUs (Operational Taxonomic Units) from 24 streams for paper II (Fig. 1).

Invertebrates (I, III) were sampled in October 2010. In each site, we collected a 2-minute kick-net (net mesh size 0.3 mm) sample and preserved the invertebrates and associated material in 80% ethanol. Such a sample covers ca 1.3 m² of the stream bed, capturing about 75% of all species present in a riffle, mainly missing species that occur only sporadically in streams (Mykrä *et al.* 2006). In the laboratory, all invertebrates were separated from other material and identified using a light microscope, mainly to species level. True flies, oligochaetes, and aquatic mites were identified to a coarser taxonomic level, except non-biting midges which were identified to species level in paper III.

Bryophytes (I, III) were sampled in either June 2008 or 2010. Ten 50 × 50 cm quadrats were established at each site and all aquatic and semiaquatic bryophyte species were identified and their proportional abundance estimated for each quadrat. In addition, we surveyed carefully the whole study section to record all less abundant species that did not occur in our regular samples; these species were given a relative abundance of 0.01% per site.

Diatoms (I, III) were sampled in October 2010. We selected randomly five stones (diameter 5–25 cm) in each riffle and scraped diatoms off the stones with a toothbrush. The subsamples were pooled into a composite sample for each site

(Kelly *et al.* 1998). Acid combustion was used to clean frustules of organic matter. Cleaned diatoms were mounted on Naphrax (Brunel Microscopes, Chippenham, UK) and a subsample of 500 frustules per site was identified to the lowest possible level, usually species, using phase-contrast light microscopy (magnification 1000×).

Fungal OTUs (II) were identified using pyrosequencing. Fungal DNA was extracted from 0.07 g of frozen leaf material (from decomposition experiment, September 2010) using PowerSoil DNA Isolation Kit (MOBIO Laboratories, Carlsbad, California, USA). The rDNA coding regions were amplified using the fungal ITS primers 50-CTTGGTCATTTAGAGGAAGTAA-03 and 50-TCCCTCCGCTTATTGATATGC-03 (Gardes and Bruns 1993). The amplicons were sequenced using the GS FLX 454 system (Roche, Basel, Switzerland). Sequencing tags were analyzed using the software tool Pyrotagger (Kunin and Hugenholtz 2010), which clusters OTUs based on 97% identity. The OTUs were annotated using BLAST searches of NCBI (National Center for Biotechnology Information, USA), GenBank's non-redundant nucleotide database. OTU naming was based on the best BLAST hits. For data analyses, the OTU data were rarefied to the lowest shared sample size.

Fungal biomass (II) was estimated from freeze-dried, pulverized, leaf samples using a modified ergosterol assay (Nylund and Wallander 1992). Ergosterol extracts were quantified with high-performance liquid chromatography (HPLC) using a reverse-phase C18 column (Agilent 1100 Series HPLC, Agilent Technologies, Waldbronn, Germany) equipped with a pre-cartridge and methanol as the eluant (1.0 mL/min, column temperature 30 °C). Commercial ergosterol was used as standard. Results are expressed as ergosterol concentration in the litter ($\mu\text{g/g}$ litter dry mass).

3.3 Leaf decomposition experiment

In paper II, leaf decomposition experiments were conducted in 24 streams (six reference streams and six impacted by drainage in each of the two basins). We collected alder (*Alnus incana*) leaves just prior to abscission and air dried them for two weeks. Then we enclosed six grams of dried leaves in 15 × 15 cm mesh bags. We used the mesh size of 0.2 mm to prevent invertebrates from entering the bags. Five individually tagged bags were placed onto the stream bed at each site in mid-September 2010 and anchored using house bricks. The bags were removed from the stream after 30 days, sealed in zip-lock bags and transferred to a laboratory

freezer. In the laboratory, litter bags were gently cleaned to remove any accumulated sediments. Leaf material was dried for 48 h at 60 °C and subsamples were ashed for 48 h at 550 °C to convert dry mass to ash-free dry mass. Leaf breakdown rates were determined using the negative exponential decay model (see paper III).

3.4 Environmental data

Environmental measurements for all three papers were conducted in September 2010. Stream slope was measured for a distance of 15–20 m, depending on channel steepness, using a clinometer. Water depth and current velocity were measured at 30 random points along 4–8 cross-sectional transects at each site. The percentage cover of particle size classes was visually estimated in 10 randomly placed 50×50 cm quadrats at each study site, using a modified Wentworth scale. These estimates were subsequently used to calculate a mean particle size, the percentage cover of fine sediments and substratum diversity for each site. Stream width was measured at five cross-channel transects. Canopy cover of the riparian vegetation was estimated using a densiometer at 20 points in cross-channel transects covering the whole study section. Finally, as a surrogate for the quality of riparian leaf litter input, we estimated the ratio of deciduous/coniferous riparian trees on both sides of the stream in a 50-m section directly upstream of a site.

We collected water samples in October 2010 using a protocol based on the Forum of European Geological Surveys (FOREGS) field manual and the internal protocols of the Geological Survey of Finland (Salminen *et al.* 1998). The samples were collected in plastic bottles and stored in a cool box. Samples for the metal analyses were filtered onto 0.45 µm glass-fiber filters in the field and fixed with 0.5 mL of 65% nitric acid. Water samples were taken to the laboratory where they were analyzed for metals, sulphate, total phosphorus, electrical conductivity, alkalinity, pH, and dissolved organic carbon using Finnish National Standards (National Board of Waters 1981).

3.5 Statistical analyses

All analyses were performed in R (R Core Team 2014) with package *vegan* (Oksanen *et al.* 2015), except the indicator species analyses in paper III which were conducted with package *indicspecies* (De Cáceres and Legendre 2010) and

SIMPER analysis in paper II which was performed with the PAST software (Hammer *et al.* 2001).

3.5.1 Analyses in paper I

We first used principal components analysis (PCA) to summarize the environmental data, separately for the physical habitat (both in-stream and riparian) variables and water chemistry variables. We then used two-way analysis of variance (2-way ANOVA) to test for differences in species richness (α diversity) and abundance among treatment combinations, with geology (low-pH vs. circumneutral sites) and drainage ditching (drained vs. non-drained streams) as the main effects. In the case of a significant interaction, we used simple effect tests to compare the means of one factor separately at each level of the other factor (and vice versa).

To test for differences in β diversity among the stream groups, we first tested whether β diversity in each group was a result of stochastic or deterministic (niche-based) processes. We used Jaccard dissimilarity based on presence-absence data as our measure of β diversity. Because α diversity varied considerably among sites and treatments, we used a null model approach to test whether the observed within-group (i.e., stream type) β diversity differed from that expected by chance (Chase and Myers 2011). For the among-group (stream type) comparison, we used an approach similar to the probabilistic Raup-Crick metric of similarity (Chase *et al.* 2011). Among-group differences in the probabilities were examined using the test of homogeneity of dispersion (PERMDISP; Anderson *et al.* 2006). We tested the null hypothesis that there were no differences in the dispersion (i.e., β diversity) between low-pH vs. circumneutral sites and drained vs. non-drained sites. In case of a significant overall difference, we used Tukey's HSD test to examine pairwise differences in β diversity.

3.5.2 Analyses in paper II

Because of high collinearity among environmental variables, we reduced the dimensionality of the data using PCA. Only components with eigenvalues exceeding one were included in further analyses.

We used two-way ANOVA to test the effects of geology (naturally low pH vs. circumneutral streams) and drainage (drained vs. non-drained streams) on selected environmental and biological response variables. In case of a significant interaction

between geology and drainage, we used simple effect tests to compare the means of one factor separately at each level of the other factor (and vice versa). Evenness was calculated as $H'/\log(\text{richness})$, where H' is Shannon diversity.

Relationships between leaf decomposition rates and biological (fungal richness, evenness and biomass) and environmental variables (PCA components) were examined using multiple linear regressions. The most parsimonious model was selected using the regression with empirical variable selection (REVS) approach (Goodenough *et al.* 2012).

To examine the independent influence of each biotic variable on decomposition rate, we constructed partial regression plots by regressing the independent variable of interest (e.g., fungal OTU evenness) against another independent variable (e.g., a PCA component). We used the partitioning approach of Moya-Laraño and Corcobado (2008) to examine the independent effect of each explanatory variable on decomposition rate.

Non-metric multidimensional scaling (NMDS) on abundance data was used to summarize the variability of fungal assemblage structure between the stream groups. We also conducted a two-way permutational multivariate analysis of variance (PERMANOVA; Anderson *et al.* 2006) to examine differences in community structure among the site groups, with geology (low-pH vs. circumneutral streams) and drainage ditching (drained vs. non-drained streams) as the main effects. PERMDISP was used to compare among-group differences in dispersion (using Bray-Curtis similarity coefficient) and Tukey's HSD to examine pairwise differences in β diversity.

Finally, we used SIMPER (similarity percentage analysis) to assess which fungal taxa contributed most to differences in assemblage structure among site groups. This analysis separates the contribution of each species to the observed similarity between site groups, allowing one to identify the species that are most important in producing the observed pattern of similarity.

3.5.3 Analyses in paper III

We used NMDS to visualize variability in community structure (abundance data) for each biological group. We explored the relationship between the environmental variables and biological communities by fitting the environmental variables as vectors onto the NMDS ordination space (envfit; Oksanen *et al.* 2015) for each biological group. A two-way nonparametric PERMANOVA was conducted as in paper II to examine differences in assemblage structure among the stream groups.

We conducted a Procrustes rotation to assess the community-environment correlations and community concordance. To take the differing impact of drainage in circumneutral and acid streams into account, we first re-organized our data into three environmental gradients: (i) natural acidity gradient (reference streams: circumneutral and naturally acidic non-drained streams), (ii) sedimentation gradient (reference and disturbed circumneutral streams), and (iii) acidification gradient (drainage-induced acidity: reference and disturbed naturally acidic streams). Thus each stressor gradient composed of 24 streams. We performed Procrustes rotation and Procrustes analysis (Protest) to evaluate the degree of correlation between the environment and the biological communities (Peres-Neto and Jackson 2001). We conducted the correlations only for the set of environmental variables of greatest relevance, that is, water chemistry for the natural acidity gradient and the acidification gradient, and habitat variables for the sedimentation gradient. We performed Procrustes rotation for the corresponding NMDS and PCA ordinations. The non-randomness of each pair of configurations was then tested using Protest. We also assessed the degree of concordance between each pair of communities (diatoms, bryophytes, invertebrates) along each of the three environmental gradients with Procrustes rotation and Protest.

4 Results and discussion

4.1 Effects of natural acidity on stream communities

Much as expected, naturally acid and circumneutral streams differed due mainly to lower pH and higher heavy metal concentrations in the acidic sites. We showed that geology-related natural acidity constitutes a strong environmental filter for lotic communities. From the four biological groups studied, three had richer assemblages in circumneutral compared to acid streams. This coincides with the well-established fact that species sensitive to low pH are typically absent in acid streams (Ledger and Hildrew 2005, Petrin *et al.* 2007b, Schmidt *et al.* 2012). The only exception was the fungal community which showed no response to natural acidity in terms of taxa richness, although also their diversity typically declines with decreasing pH (Wood-Eggenschwiler and Bärlocher 1983, Baudoin *et al.* 2008). Furthermore, fungi were not only the group most tolerant of natural acidity, but as their biomass tended to be higher in the naturally acid compared to circumneutral streams, some taxa seem to have benefited from low pH.

All biological groups had distinct community composition in circumneutral vs. acidic sites. However, natural acidity did not homogenize communities, as evidenced by largely similar beta-diversity values in different stream types. Fungi in naturally acid streams were also as effective decomposers as those in circumneutral streams, thus indicating adaptation or acclimation to the harsh environment (Collier *et al.* 1990, Dangles *et al.* 2004b, Petrin *et al.* 2007a).

The strong deterministic control by water pH and metal concentrations over lotic communities was also evident from community concordances and the community-environment correlations, as both were strongest along the natural acidity gradient. The community-water chemistry correlation was especially strong for invertebrates, coinciding well with their general responsiveness towards water acidity (Petrin *et al.* 2008, Schmidt *et al.* 2012). Compared to invertebrates, diatoms showed a weaker correlation with water chemistry, which was unexpected as diatoms are known to link strongly to water pH not only in anthropogenically acidified (e.g. Telford *et al.* 2006, Andrén and Jarlman 2008) but also in naturally acid freshwaters (Schowe *et al.* 2013, Schowe and Harding 2014).

4.2 Land use effects along the natural acidity gradient

The effect of forest drainage differed among circumneutral and naturally acid streams. In the circumneutral region, drainage mainly increased channel sedimentation, while in the acid region it increased metal concentrations. Such geology-based differences in land use effects undoubtedly contribute to how biological communities respond to changes in land use.

Field studies assessing the effect of multiple simultaneously acting stressors have mostly found antagonistic community-level responses (Crain *et al.* 2008), which our results partly support. However, antagonistic effects mostly applied to α diversity, whereas β diversity, community composition and leaf decomposition responded in an additive or synergistic way. Only invertebrate β diversity and fungal richness remained completely unaffected by the stressors. A direct comparison among our results and previous studies is complicated by the fact that in our studies, the effect of land drainage differed between the drainage basins.

Drainage affected diatom and bryophyte community composition in both stream types, whereas invertebrate communities diverged due to sedimentation. Anthropogenic acidity only affected invertebrates once larval black flies, the only major unidentified invertebrate group, were removed from the data. Correlations between biological communities and the stream environment were consistently stronger along the anthropogenic acidity gradient than the sedimentation gradient. Furthermore, correlation with water chemistry was strongest for invertebrates, implying that pH and metals had indeed a substantial deterministic control over invertebrate assemblages. Thus these three biological groups seem to involve acid-tolerant species that are on the edge of their metal (or pH, or their interaction) tolerance range in our naturally acid streams and cannot cope with further increase of metals (or decline of pH). On the other hand, some extremely acid-tolerant taxa may benefit from the intensified acidity (e.g. some diatoms; DeNicola 2000), contributing to changes in community composition by increasing their abundance in naturally acid human-impacted streams. Overall, changes in community composition were better able to track single stressor impacts than the pure species richness component of diversity, suggesting that subtle biotic changes may go unnoticed if only species richness is used in environmental impact assessment.

Fungal β diversity was the only community variable that showed a synergistic response, with naturally acid drained sites having more homogeneous assemblages than any other stream type. This suggests that anthropogenic disturbance seems to act as a non-random filter for fungi in the acid sites, selecting species with traits

that allow persistence in the disturbed environment (Chase 2010). Other studies have similarly shown that fungal assemblages respond more to changes in water chemistry than in the physical habitat (Niyogi *et al.* 2003, Clivot *et al.* 2013). Thus the fact that the drainage impact differed among circumneutral (sedimentation) and acid (decreased pH and increased metals) streams could explain why fungal β diversity responded to drainage in acid but not in circumneutral sites.

Interestingly, also leaf litter decomposition rate showed a synergistic effect being lowest in acid disturbed streams, thus the hypothesis about positive co-tolerance (Vinebrooke *et al.* 2004) gained no support. Based on stressor effects on fungal richness and species composition, changes in fungal assemblage structure rather than species richness per se had more of an impact on decomposition rate, such that acid-tolerant taxa were less effective decomposers (slower leaf decay rate with decreased pH). The fact that fungal biomass did not explain the inefficiency of fungal decomposition indicates that low pH and associated high metal concentrations affected fungal metabolism. Another explanation could be that pectinases that accelerate the maceration of leaves tend to be less active in acidic streams (Chamier and Dixon 1982). Either way, we noticed that decomposition rates were related to water acidity and metal concentrations (and, to a lesser degree, fungal OTU evenness), suggesting that among-region variation in water chemistry associated with black shale geology was indeed the main determinant of differences in leaf decomposition rates.

Community concordance was stronger along the anthropogenic acidity gradient compared to the sedimentation gradient, as were the community-environment correlations. Thus the drainage-induced shift in water chemistry in the black-shale-dominated region seems to constitute an even stronger environmental filter than sedimentation. This is not really surprising considering the extreme chemical conditions in our low-pH disturbed sites. However, the eminently strong pH range along the natural acidity gradient resulted in an even stronger community concordance than those along either of the human-disturbance gradients.

5 Conclusions and implications for management

We found that the impact of land use differed among the two catchments, increasing channel sedimentation in the circumneutral streams while decreasing pH (and increasing metal concentrations) in the naturally acid streams. This phenomenon shows the pros and cons of field research: while this finding affects the interpretation of our results and somewhat complicates the concept of multiple-stressor impacts, it is also one of the most important outcomes of this thesis, with great ecological relevance. Acknowledging the fact that taxonomic groups differ in their sensitivity to environmental factors (e.g. acidity or substratum size), background geology may eventually determine how communities respond to land use. This means that ecosystem management cannot be based on a straightforward extrapolation from experiences about land use impacts in a different environmental context. In particular, this should be taken into account in the Reference Condition Approach where communities of human-impacted freshwaters are compared to corresponding reference sites. If reference sites are poorly chosen (i.e. from a region with a strongly differing geology), the interpretation of the biotic effect of land use will likely be biased. Our results also argue for the primacy of a multi-taxon approach to environmental impact assessment, particularly if the actual impact of a stressor on the stream environment is unknown. It is also important not to rely too heavily on species richness as the sole indicator of ecosystem health but to use a wider array of ecological responses, particularly β diversity, abundance-based composition and ecosystem processes, which may be able to detect responses that go unnoticed by conventional richness-based measures.

The fact that we found additive and also some synergistic responses to multiple stressors indicates that environmentally distinct regions, such as regions with extensive occurrences of black shale, are extremely vulnerable to human impact. The exploitation of these areas for human purposes will have great ecological costs. Moreover, the synergistic responses by fungi may suggest that other microbes, and ecosystem processes controlled by them, might show similar responses.

We found naturally acid stream communities to be consistently more species poor than those in circumneutral streams. Low species richness may result in limited buffer against natural variation and anthropogenic disturbances, potentially impairing ecosystem functioning and services (Schwartz *et al.* 2000). However, this is not always the case (e.g. Dang *et al.* 2005), and studies assessing the biodiversity-ecosystem functioning relationships are inconsistent on how changes in alpha

diversity affect ecosystem functions (Covich *et al.* 2004). We also found human disturbance to change community composition and reduce beta diversity in naturally acidic streams. Sustaining natural heterogeneity of communities would protect against local species extinctions (due to source-sink dynamics; Bond and Chase 2002) and maintain ecosystem functions through functional compensation between species (Loreau *et al.* 2003), that is, species not considered functionally important in one stream may become important in others as environmental conditions and/or community compositions change.

Extreme habitats often support unique organisms and genes that are not only of high value to biodiversity, but also may serve humankind in multiple ways in the future (Colwell 1997). Changes in ecosystem processes and impoverished gene banks will eventually lead to impaired ecosystem services (Isbell *et al.* 2015), emphasizing the need to conserve biodiversity and ecosystem functionality in such special habitat types as naturally acidic streams.

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