Hydrobiologia (2017) 793:161–173
DOI 10.1007/s10750-016-2760-4

SMALL WATER BODIES

Does habitat restoration enhance spring biodiversity and ecosystem functions?

Kaisa Lehosmaa · Jussi Jyväsjärvi · Risto Virtanen · Pekka M. Rossi · Dimitrios Rados · Tatiana Chuzhekova · Annamari Markkola · Jari Ilmonen · Timo Muotka

Received: 31 October 2015 / Revised: 1 April 2016 / Accepted: 2 April 2016 / Published online: 17 May 2016

Abstract Degradation of groundwater-dependent ecosystems has raised a need for their restoration, but ecological responses to restoration are largely unknown. We evaluated the effectiveness of spring restoration using data from near-natural, restored, and human-impacted springs, the major impact being degradation of spring hydrology by forest drainage. We used both taxonomic (bryophytes, macroinvertebrates, and leaf-decomposing fungi) and functional (leaf breakdown) measures of restoration success. We expected that by reducing surface water input, restoration will improve spring hydrology and place spring ecosystems in a trajectory towards more natural conditions. Restored springs were thermally more stable than impacted springs and the contribution of surface water was greatly reduced. Bryophytes were more abundant in restored than in impacted springs but did not differ among restored and natural springs. Similarly, macroinvertebrate communities differed between restored and impacted springs whereas no difference was detected between restored and natural sites. Species diversity and functional attributes showed weaker responses to restoration. Our results suggest that restoration enhances spring habitat quality, and the first signs of biodiversity enhancement were also detectable only a few years post-restoration. Restoration clearly bears great promise as a conservation tool for the protection of this valuable component of regional freshwater biodiversity.

Keywords Boreal springs · Macroinvertebrates · Bryophytes · Fungi · Leaf litter breakdown · Crenophiles

Electronic supplementary material The online version of this article (doi:10.1007/s10750-016-2760-4) contains supplementary material, which is available to authorized users.

Guest editors: Mary Kelly-Quinn, Jeremy Biggs & Stefanie von Fumetti / The Importance of Small Water Bodies: Insights from Research

K. Lehosmaa (✉) · J. Jyväsjärvi · R. Virtanen · D. Rados · A. Markkola · T. Muotka
Department of Ecology, University of Oulu, P.O. Box 3000, 90014 Oulu, Finland
e-mail: kaisa.lehosmaa@oulu.fi

P. M. Rossi
Water Resources and Environmental Engineering Research Group, University of Oulu, P.O. Box 3000, 90014 Oulu, Finland

T. Chuzhekova
St. Petersburg State University, Vasil'evsky Island 16 line 29, St. Petersburg, Russia 199178

J. Ilmonen
Metsähallitus, P.O. Box 94, 01301 Vantaa, Finland

T. Muotka
Natural Environment Centre, Finnish Environment Institute, 90014 Oulu, Finland

Introduction

The alarming rate of degradation and loss of freshwater habitats (Dudgeon et al., 2006) has increased the importance of ecosystem restoration (Palmer et al., 2014). Most freshwater restoration projects, and monitoring of their impacts, have focused on rivers and lakes, while little is known about the ecological consequences of restoration of groundwater-dependent ecosystems such as cold-water springs. During recent years, there has been a considerable increase in scientific attention towards small water bodies, including springs (Céréghino et al., 2008; Cantonati et al., 2012; Kristensen & Globevnik, 2014), but monitoring of the structural and functional responses of biota to restoration in these environments is at its infancy.

Springs are important ecotones between surface water and groundwater, and aquatic and terrestrial habitats (Ward & Tockner, 2001; Cantonati et al., 2012). The combination of diverse habitat structure and continuous influx of cold and thermally and chemically stable groundwater provides a unique environmental setting that supports diverse plant and invertebrate communities and hosts several rare and threatened endemic (i.e., crenobiontic and crenophilous) species (Cantonati et al., 2012; Ilmonen et al., 2012). These fragile ecosystems are of great importance to regional biodiversity, yet are currently threatened by groundwater abstraction and pollution, habitat degradation, and land use and global warming (von Fumetti et al., 2006; Juutinen, 2011; Cantonati et al., 2012; Ilmonen et al., 2012; Jyväsjärvi et al., 2015). In Finland, springs are included among the 13 habitats of special importance in the National Forest Act (Pykälä, 2007). However, the act does not specify any guidelines to improve the sustainable management of springs. Similarly, while the Water Framework Directive (European Commission, 2000) has developed bioassessment protocols for surface waters, management, and conservation guidelines for springs and other small water bodies (catchments <10 km^2) are largely non-existent (Barquín & Scarsbrook, 2008; Ilmonen et al., 2012).

The poor current status of springs in Finland has led to an increasing number of restoration projects, with more than 1,000 springs having been restored by 2015. The most typical restoration practices include (i) removal of water abstraction structures such as pipes and dams, (ii) filling and damming of inflow ditches to reduce surface water influence, and (iii) heightening the point of discharge to increase water level in the spring pool. These actions aim to provide natural, groundwater-dominated hydrological conditions and to increase spring habitat area, particularly the ecologically critical land–water interface (Barquín & Scarsbrook, 2008).

We assessed the hydrological and ecological consequences of spring habitat restoration based on a space-for-time substitution design with nine near-natural, seven restored, and seven human-degraded springs. Structural responses to restoration were evaluated using the taxonomic composition of bryophytes, benthic invertebrates, and aquatic fungi while leaf litter breakdown assays were used to measure functional responses. We hypothesized that (i) habitat restoration reduces surface water inflows and elevates spring pool water levels, resulting in thermally and chemically stable conditions and increased spring habitat size. We further hypothesized that, as a result of (i), (ii) biodiversity and spring ecosystem functions have improved (restored vs. disturbed springs), and (iii) are approaching those in near-pristine springs (restored vs. near-natural springs).

Materials and methods

Selection of study sites

We conducted the study in eastern Finland (64°–65°N, 27°–29°E, Fig. 1). The area is characterized by mixed forests and peatlands. The main land use is silviculture, particularly drainage of peatlands. Drainage ditching in Finland began in the nineteenth century, with a peak during the 1970s and 1980s. Drainage was practiced to channel surplus water to streams to enhance forest growth but, as a side effect, it increased sediment load and nutrient concentrations and altered catchment-scale hydrology (Vuori et al., 1998; Holden et al., 2004; Jyväsjärvi et al., 2014). Drainage also impaired, and in some cases completely destroyed, spring habitats (Heino et al., 2005).

From a spring database (690 springs) maintained by the Finnish Forest Centre, we selected more than 100 potentially suitable springs for site visits; 23 of these springs were eventually selected for the study (Fig. 1). The springs were classified in three categories: restored ($n = 7$), disturbed (by forest ditching)

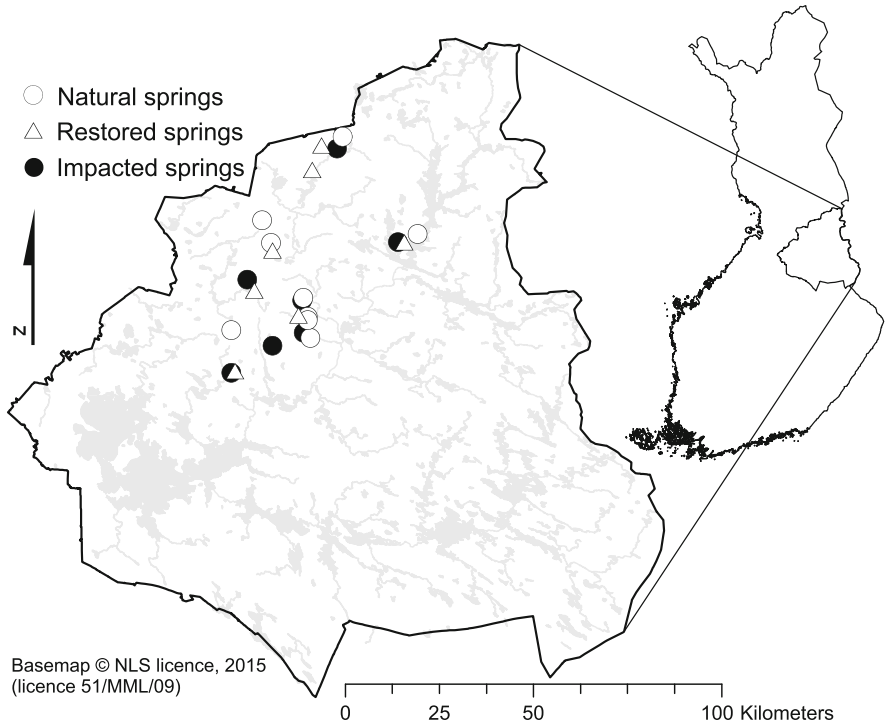

Fig. 1 Location of the study springs in eastern Finland

($n = 7$), and minimally disturbed (hereafter 'natural') springs ($n = 9$). Site selection was based, in addition to accessibility, on the following a priori criteria: (i) sites in each status class had to be spatially interspersed, (ii) all springs were of comparable size and structure and contained a distinct spring pool and an outflowing stream (i.e., limno-rheocrenes) (see Online Resource 1). While a before–after control-impact design, with multiple restored and control springs being monitored for several years pre- and post-restoration (see Underwood, 1994), would have been preferable, no before-restoration data were available for any of our study sites and we therefore had to resort to a space-for-time substitution design. This design may suffer from spatial variability between sites (Kappes et al., 2010) but, provided that the signal of human intervention (in our case, restoration) is strong enough, it often performs well in environmental impact detection (e.g., Lepori et al., 2005; Turunen et al., 2016).

The restored and impacted springs were in most cases directly altered by forest ditching, i.e., a forest ditch drained directly into the spring pool and/or spring outflow. In addition, some of the impacted springs had man-made structures for water abstraction. Natural springs were located in areas protected by national forest legislation (Pykälä, 2007) and thus lacked any visible human impact within at least 30 m distance from the spring. Restoration activities were supervised by the Finnish Forest Centre and mainly executed by local landowners. Consequently, restoration measures were closely similar at all sites: inflowing ditches were filled and the spring outflow was dammed with wooden constructions, stones, and mosses to facilitate raising of the water table in the spring pool to the original level. In many cases, spring pools were also dug deeper. Restorations were carried out between 2009 and 2011, and all sampling was conducted during May–October 2014; thus, the recovery period prior to our sampling was 3–5 years. Systematic spring restoration in the study area started in 2009 and any older restoration attempts comparable to our restored springs were thus unavailable.

Environmental data

For each study spring, we estimated the integrity of the spring habitat using the classification procedure by

Table 1 Averages and ranges of the measured environmental variables of the spring groups

	Natural		Restored		Impacted	
	Mean	Range	Mean	Range	Mean	Range
Spring habitat area[a]	2	1–2	1	1–2	1	1–2
Habitat integrity[b]	3	2–3	2	1–2	1	0–1
Spring pool depth (cm)	20	5–28	52	29–80	25	11–41
Forest shading (%)	55	36–76	52	6–80	44	10–70
Mean temperature	3.5	2.9–4	3.8	3.2–4.6	4.1	2.9–6.9
Temperature range	1.3	0.1–3.4	1.3	0.9–2.4	4.5	1.6–12.6
pH	6.1	5.5–6.8	5.7	4.9–6.4	5.9	5.1–7.1
Electrical conductivity (mS m^{-1})	5.7	2–16.1	4.5	3.4–6.1	5.2	1.4–13.5
Alkalinity (mmol l^{-1})	0.4	0.1–1	0.3	0.1–0.4	0.5	0.1–1.4
DOC (mg org C l^{-1})	1.9	1–2.8	2.3	1.2–5.8	3.2	1.8–7.8
TP (μg l^{-1})	11	1–39	6	1–15	42	1–240
TN (μg l^{-1})	617	90–1,840	729	140–1,790	294	62–730
Discharge (m^3 day^{-1})	22	0–78	57	3.5–181	38	0–78
Isotope vector lengths	0.5	0.1–1.1	0.5	0.3–0.8	0.8	0.5–1.1

[a] Spring habitat area in a logarithmic scale, see "Materials and methods" section

[b] Habitat integrity (0–3) based on visual assessment, see "Materials and methods" section

Heino et al. (2005). Sites that were completely destroyed by, for example, having their groundwater outflow disrupted and lacking distinct spring habitat were assigned into class 0. Severely (e.g., drainage ditches in the immediate neighborhood of a spring) or moderately (e.g., minor structures for water extraction, or logging, or ditching more than 50 m from the spring) altered sites belonged to classes 1 and 2, respectively. Pristine (or nearly so) springs with no visible human impact were assigned into class 3 (Table 1). Spring area was classified on a logarithmic scale ($1 = <10$ m^2, $2 = 10$–99 m^2, $3 = 100$–999 m^2, $4 = 1{,}000$–$9{,}999$ m^2, $5 = \geq 10{,}000$ m^2) (Ilmonen et al., 2012). Areal coverage (m^2) of different habitat types (i.e., spring pools, helocrenes, and spring brooks with either minerogenic or organogenic substrate) were classified using the logarithmic scale described above. Percentage cover of forest shading was visually estimated using a gridded microscope ocular. Depth of the spring pool and depth and width of the outflowing stream were measured at five locations and mean values across these measurements were used in data analyses. Spring discharge (m^3 day^{-1}) was measured at one location. Water samples were taken from the spring pool in August and were analyzed for alkalinity, dissolved organic carbon (DOC), total phosphorus (TP), and total nitrogen (TN) using Finnish national standards (National Board of Waters, 1981) (Table 1). In addition, electrical conductivity, pH, and water temperature were measured with a field meter (WTW Multi 350i meter) at each visit to a spring (Table 1) and averages of these measurements were used in the analyses. Water temperature variation was measured in May–July 2014 with temperature loggers (iButton, Thermochron; Maxim Integrated, San Jose, CA, USA) set to measure water temperature at 30 min intervals.

Stable isotopes of spring water

Stable isotopes (SIs) of water (^{18}O, ^{2}H) are commonly used as tracers in hydrology (Clark & Fritz, 1997). Isotopic fractionation by, for example, evaporation and precipitation induces changes in isotopic abundances, thus enabling their use in hydrological studies (Gat, 2010). We determined the $^{2}H/^{1}H$ and $^{18}O/^{16}O$ isotope ratios of the spring water using cavity ring-down spectroscopy with a Picarro L2120-i analyzer (Picarro, Santa Clara, CA, USA). The measured ratios are given as δ (‰) notations relative to Vienna Standard Mean Ocean Water. The measurement precision of the $\delta^{18}O$ values was 0.1 ‰ while that of the $\delta^{2}H$ values was 1.0 ‰.

Temporal variation of the isotopic composition of spring water was studied using a vector analysis. The $\delta^{18}O$ and $\delta^{2}H$ isotopes were sampled four times in 2014 (May, June, August, and October). Variation of the δ-values between two consecutive samples (e.g., between May and June samples) was calculated as the length of a vector along the $\delta^{18}O$ and $\delta^{2}H$ dimensions, and all vectors for each study site were then summed

(Fig. 2). As $\delta^{18}O$ and $\delta^{2}H$ have a different variance ratio, samples were standardized to vary between 0 and 1. The standardized vectors were then summed to define the overall variation of SIs of the spring water for each study site across all sampling surveys (see Fig. 2). Finally, the total length of a vector was used to assess the relative contributions of surface water and groundwater. Short SI vectors indicate stable groundwater influx driven by a deep regional groundwater source with negligible contribution of precipitation or snow melt, whereas long vectors suggest a more direct connection to surface waters or a short, local flow route of groundwater to a spring.

Biological sampling

We sampled spring bryophytes in August 2014 at 1 m intervals from the point of discharge along the main course of the flow in seven 0.5 × 0.5 m quadrats. In the smallest springs (<10 m^2), only five quadrats were sampled. For each plot, we identified all bryophyte species, including semiaquatic taxa, and estimated their percentage cover (%) visually.

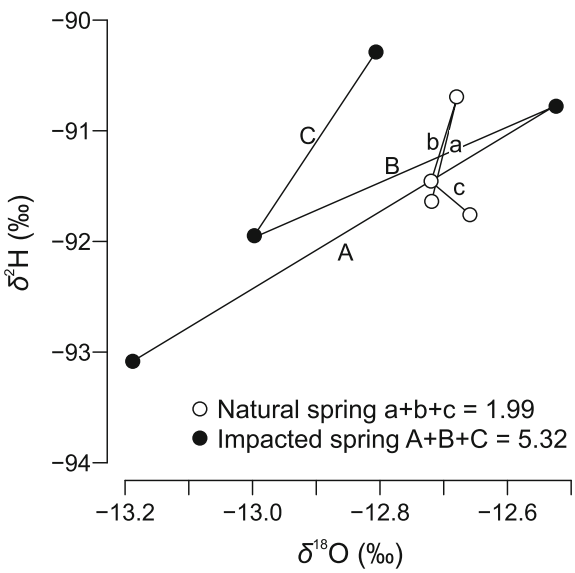

Fig. 2 An illustrative graph showing the variation of $\delta^{18}O$ and $\delta^{2}H$ isotopes across the four sampling visits (May, June, August, and October 2014) for one natural and one impacted spring. Stable isotope vector lengths for each spring were calculated by summing the lengths of the three consecutive subvectors. Note that the initial vector lengths were standardized prior to statistical analyses (see "Materials and methods" section)

Benthic invertebrates were sampled in early June 2014 using a 20-cm wide D-frame hand net (mesh size 500 µm). Five 20 s subsamples representing all available habitat types (spring pools, helocrenes, spring-fed streams with minerogenic or organogenic substrate) were taken in 2 m intervals within the first 10 m from the spring source by sweeping submerged substrates or by pressing mossy or muddy substrates and collecting loose material into the net (see Ilmonen & Paasivirta, 2005). All macroinvertebrate samples were collected by the same person. Animals were preserved in 70% ethanol in the field and later identified (including chironomid midges) to the lowest feasible taxonomic level (usually species). Due to their excessive numbers, only 300 chironomids were sorted and identified in each sample; thus chironomids were excluded from all abundance analyses.

Leaf decomposition

Leaf decomposition was measured with leaf breakdown assays using 15 × 15 cm mesh bags. Alder (*Alnus incana*) leaves were collected in August 2014 prior to abscission and were air-dried for 1 week. Four grams of leaves were then weighed into each mesh bag. Two different mesh sizes were used; fine bags (0.2 mm) were used to measure microbial decomposition rates, while coarse bags (8 mm) also allowed leaf-shredding invertebrates to enter the bags. Previous studies have not detected any signs of hypoxia in similar fine-mesh bags in streams (Tolkkinen et al., 2013). At each site, house bricks were used to anchor three bags of each type into the outflow channel and two bags into the spring pool. The bags were placed in each spring in late August and they were removed after 62 days of incubation. In the laboratory, the remaining leaf material was carefully cleaned of invertebrates and other material. From each fine-mesh bag a subsample of 1 g of frozen leaf litter was taken for DNA extraction and measurement of the fungal biomass (see below). The remaining material was dried at 60°C for 48 h, then ashed for 4 h at 550°C to determine ash-free dry mass. Leaf breakdown rate (k) was calculated using the negative exponential decay model (Benfield, 1996). There were no differences in initial or final water temperature among the spring types and thus the decomposition rates were not adjusted for water temperature. Decomposition rate in coarse bags was calculated as coarse minus fine-mesh

bags, representing decomposition caused by macroinvertebrate feeding and/or physical abrasion.

Fungal DNA isolation, sequencing, and library construction

Fungal assemblages were determined using DNA sequencing techniques. Subsamples of frozen leaf material were freeze-dried and pulverized for the extraction of fungal DNA. DNA was extracted from 0.07 g of leaf material using PowerSoil DNA isolation kit (Mo Bio Laboratories, Carlsbad, CA, USA). In each sample, DNA was diluted to 5 ng μl^{-1}. The rDNA coding was amplified using fungal ITS primers ITS1F 5′-CTTGGTCATTTAGAGGAAGTAA-3′ and 58A2R-P1 5′-CCTCTCTATGGGCAGTCGGTGAT CTGCGTTCTTCATCGAT-3′ (Gardes & Bruns, 1993). The amplicons were sequenced using the Ion Torrent next-generation sequencing in the BioSer Laboratory of University of Oulu. All sequences were analyzed using quantitative insights into microbial ecology (QIIME) pipeline (Caporaso et al., 2010). We used default settings for analyzing sequences in QIIME. In addition, the sequence library was split by samples and quality filtered based on quality scores for every sequence. Quality scores below 25 were removed, and minimum and maximum sequence lengths were 200 and 1000 bp, respectively. Sequences with ambiguity, more than two mismatches in the primer, or maximum homopolymer run exceeding eight were also removed. Sequences were clustered into operational taxonomic units (OTUs) using the *Uclust* algorithm, which clusters OTUs at 97% identity (Edgar, 2010). As sequence numbers varied among samples, OTU data were rarefied to the lowest shared sample size (2,118). OTU composition was determined using basic local alignment search tool (BLAST) findings of National Center for Biotechnology Information, USA GenBank's non-redundant nucleotide database. The naming of OTUs to genus or species level was based on the best and second-best BLAST hits.

Ergosterol content

Ergosterol content, a proxy of metabolically active fungal biomass (Gessner, 2005), was determined from 50 mg of freeze-dried and pulverized leaf litter using a modified ergosterol assays (Nylund & Wallander, 1992). Ergosterol extracts were quantified with high-pressure liquid chromatography (HPLC) using a reverse-phase C18 column (Agilent 1100 Series HPLC, Agilent Technologies, Waldbronn, Germany). The HPLC was equipped with a pre-cartridge and methanol (1.0 ml min^{-1}, column temperature 30°C). Ergosterol, 5,7,22-ergostatrien-3β-ol; Fluka AG, Sigma-Aldrich, St. Louis, MO, USA, was used as a standard. Ergosterol concentration is given as $\mu g\ g^{-1}$ litter dry weight.

Statistical analyses

Principal component analysis (PCA) was used to summarize the environmental data and to assess environmental differences among the spring groups. Prior to analysis, all variables were standardized to zero mean and unit variance. The final number of PCs retained was determined using the broken-stick model (Jackson, 1993) whereby eigenvalues from the PCA were compared to values given by broken-stick distribution. Since each eigenvalue represents a measure of the component's variance, a component is retained if its eigenvalue is larger than the value given by the broken-stick model.

Among-group differences in environmental variables and taxonomic richness, abundance and leaf breakdown rate were tested using generalized linear models with a priori contrasts (restored vs. impacted, restored vs. natural). As our study focused on restoration impacts, these two comparisons are of prime importance; however, as the difference between impacted and natural springs is also of interest, we included this additional contrast in our analysis. In cases of count data (taxa richness), Poisson error distributions were used, whereas in cases of overdispersed abundance data, we used quasi-Poisson models. Otherwise, Gaussian error distribution was used. Abundances and species diversities were examined separately for all taxa and for crenophilous taxa only, the latter being identified based on Eurola et al. (1984) and Ulvinen et al. (2002) for bryophytes and Ilmonen et al. (2009) for macroinvertebrates.

Statistical differences in bryophyte, macroinvertebrate, and fungal species composition among the spring groups were tested using one-way nonparametric permutational multivariate analysis of variance (PERMANOVA; Anderson, 2001) with the *adonis* function of the vegan package (Oksanen et al., 2015) in the R program (R Core Team, 2014). PERMANOVA

was run using the Bray–Curtis similarity coefficient for abundance data, and statistical significance was estimated based on 9,999 permutations. After global tests, pairwise differences between the spring groups were tested with separate PERMANOVAs. The among-group differences in environmental conditions were also tested with PERMANOVA using a Euclidean distance matrix. The bryophyte, macroinvertebrate, and fungal taxa primarily responsible for the among-group community-level differences were identified using similarity percentage (SIMPER; Clarke, 1993) analysis. SIMPER sorts the contribution of each species to sample similarity and allows the identification of the taxa contributing most to the observed pattern of among-site similarities. SIMPER was performed on Bray–Curtis dissimilarities using *simper* function in vegan.

Results

Environmental conditions of the study springs

The first three PCs were retained, explaining 52% of the total variance of environmental variables. The first PC correlated positively with spring habitat area and habitat integrity, and negatively with average water temperature, thermal variation (CV), and vector lengths of the SI measurements (Fig. 3a). The second PC correlated most with pH, alkalinity, DOC, and conductivity. This was due mainly to the atypically dolomite-rich bedrock of three of the study springs, each belonging to a different spring group. The three spring groups were positioned along the first PC axis, the impacted springs being located on the left-hand side of the ordination space and the restored and natural springs on the right-hand side, with considerable overlap among the last two (Fig. 3b). According to PERMANOVA, environmental conditions differed significantly among the spring groups ($F_{2,22} = 2.75$, $P = 0.001$). Both natural and restored springs differed from the impacted ones ($F_{1,15} = 2.64$, $P = 0.003$ and $F_{1,13} = 2.89$, $P = 0.001$, respectively), but there was also a significant difference between the natural and restored springs ($F_{1,15} = 2.00$, $P = 0.002$). The distinction between impacted springs and the other two spring groups was mainly due to considerable differences in their surface water/groundwater interactions, resulting in higher average water temperature, as well as higher temperature variation and isotopic composition variation in the impacted springs (Fig. 4a–c). The difference in environmental conditions between natural and restored springs was mainly due to differing depth of the spring pool (Fig. 4d).

Leaf decomposition

Leaf decomposition rate in fine-mesh bags ranged from 0.011 to 0.029 day^{-1}, and from 0.024 to 0.051 day^{-1} in coarse mesh bags, average values being 0.019 and 0.037 day^{-1}, respectively (Fig. 5). Decomposition in fine-mesh bags was faster in the impacted than in restored springs (a priori contrast, $P = 0.03$), while neither restored nor impacted springs differed from the natural springs (both $P > 0.20$; Fig. 5). Leaf decomposition rates in coarse leaf bags did not differ among the spring groups (all $P > 0.45$; Fig. 5).

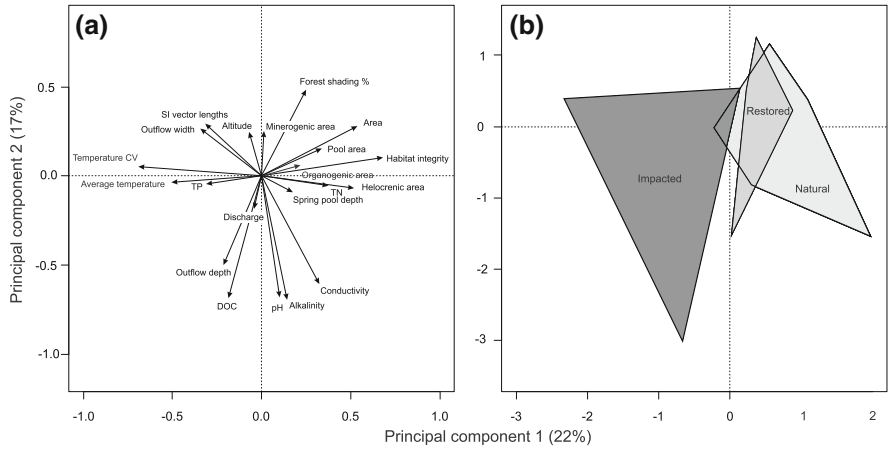

Fig. 3 PCA ordination of the environmental characteristics of the study springs, showing **a** correlations (i.e., *arrow* lengths) between the principal components and environmental variables and **b** separation of the three spring groups in the ordination space. Each *polygon* encloses all sites within a group

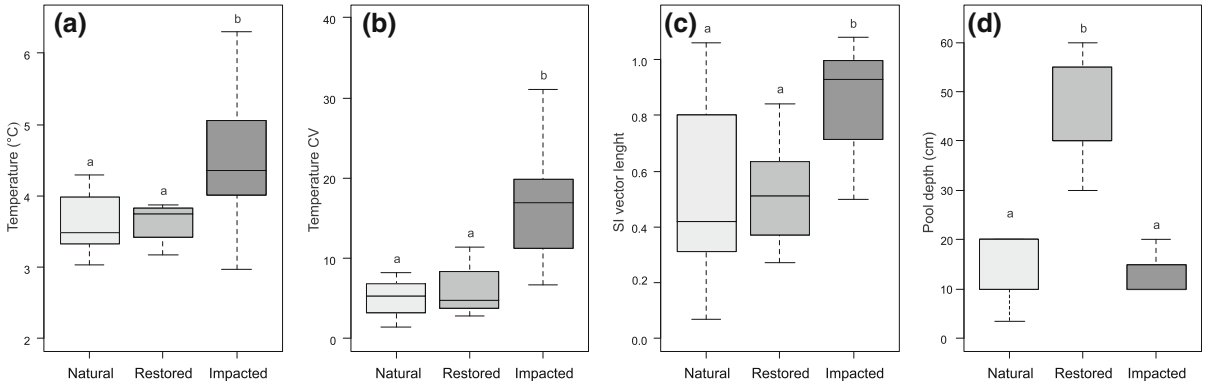

Fig. 4 Spring water temperature (**a**), coefficients of variation (CV) of spring water temperature (**b**), stable isotope (SI) vector lengths (**c**), and depth of the spring pool (**d**) in each spring group. SI vector lengths represent the relative contributions of surface water and groundwater in the study springs (see "Materials and methods" section). *Boxplots* show median values with interquartile range, *whiskers* indicate maximum and minimum values. Sites sharing a *letter* do not differ significantly according to a priori contrasts ($P > 0.05$)

Species abundance, richness, and community composition

Macroinvertebrate abundance was slightly (though non-significantly; $P = 0.08$) lower in the impacted than in restored springs (Fig. 6a) whereas bryophyte abundance (% cover) was significantly higher in natural ($P = 0.003$) and restored ($P = 0.02$) springs compared to impacted springs (Fig. 6b). Fungal biomass (ergosterol content) did not differ among the spring groups (Fig. 6c). For crenophilous invertebrates, the difference between restored and impacted springs bordered at significance ($P = 0.08$; Fig. 6d) whereas, similar to all bryophytes, crenophilous bryophyte abundance was much lower in impact than in either natural ($P = 0.005$) or restored ($P = 0.03$) springs (Fig. 6e).

Altogether 78 macroinvertebrate taxa were identified, the 5 most common taxa (and their percentage frequency of occurrence) being the plecoptera larva *Nemurella pictetii* (47%), and the chironomids *Chaetocladius* spp. (10%), *Rheocricotopus effusus* (7%), *Paratrichocladius skirwithensis* (4%), and *Micropsectra junci*-agg. (4%) (Online Resource 2). We detected 46 bryophyte taxa from the study springs, and the mosses *Brachythecium rivulare* (18%), *Warnstorfia exannulata* (16%), *Rhizomnium magnifolium* (14%), *Plagiomnium ellipticum* (5%), and the liverwort *Chiloscyphus polyanthos* (12%) were the five most common bryophyte taxa (Online Resource 2). Of the 233 fungal OTUs, 64% belonged to Ascomycota,

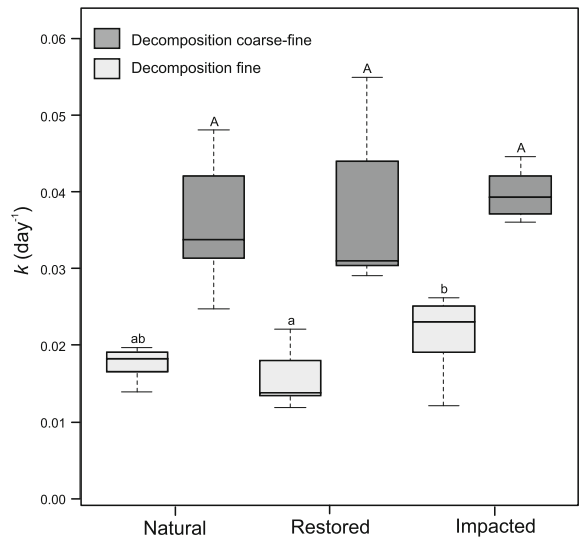

Fig. 5 Leaf litter decomposition rates in coarse and fine bags in each spring group. For other explanations, see Fig. 4

followed by Basidiomycota (27%), Chytridiomycota (5%), Glomeromycota (2.6%), and Zygomycota (1.4%). The three most common fungal taxa were *Varicosporium elodea* (44%), *Lemonniera centrosphaera* (28%), and *Helotiales* sp. (20%). Species richness of macroinvertebrates, bryophytes, and fungi did not differ among the site groups, although crenophilous bryophyte richness was somewhat ($P = 0.10$) higher in natural (7 ± 0.52; mean $\pm$ 1 SE) than in impacted (5 ± 0.85) springs.

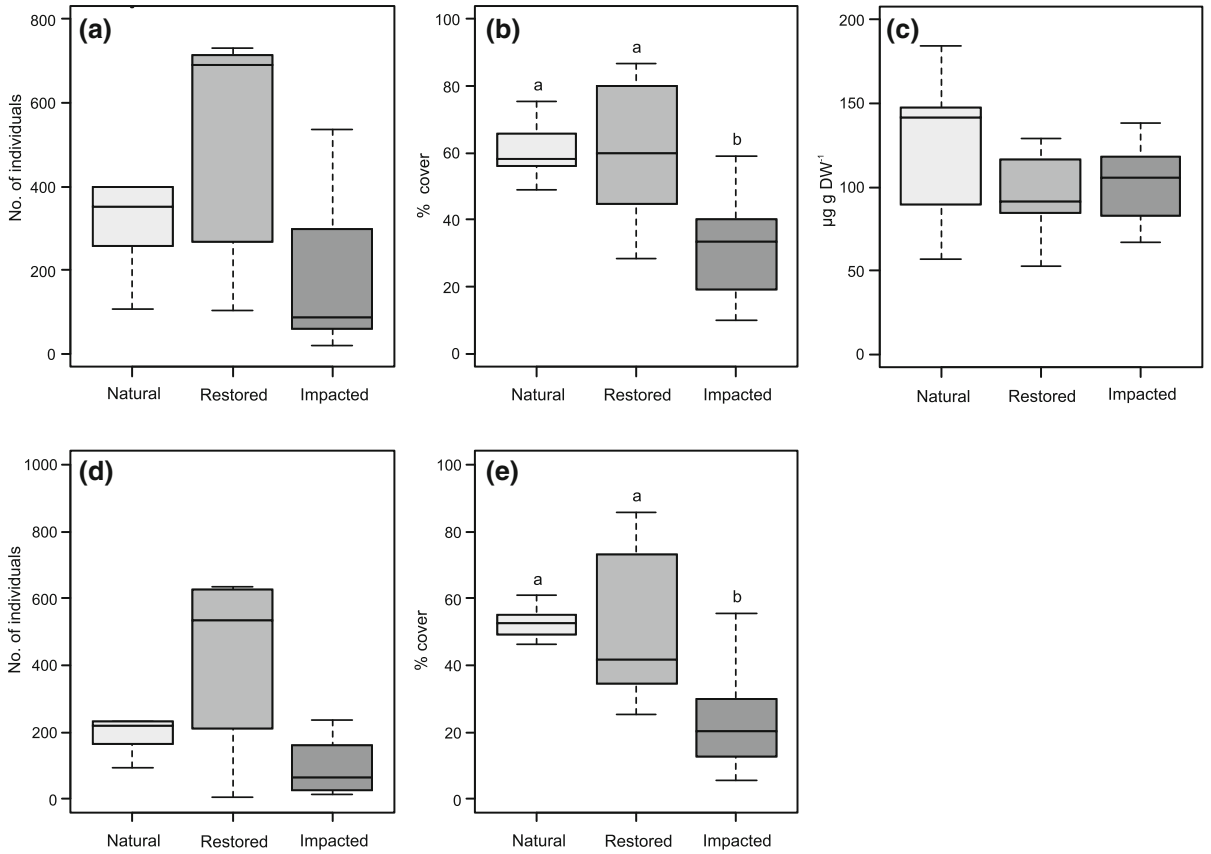

Fig. 6 Total macroinvertebrate abundance (excluding chironomids, **a**), total bryophyte cover (%, **b**), fungal biomass (**c**), and abundances of crenophilous invertebrates (**d**) and bryophytes (**e**) in each spring group. For other explanations, see Fig. 4

Macroinvertebrate assemblage composition differed significantly between natural and impacted, and between impacted and restored sites (PERMANOVA $F_{1,15} = 1.8$, $P = 0.03$ and $F_{1,13} = 2.02$, $P = 0.01$, respectively), but not between natural and restored sites (PERMANOVA $F_{1,15} = 0.93$, $P = 0.47$). The difference in bryophyte community composition between natural and impacted springs bordered at significance (PERMANOVA $F_{1,15} = 1.64$, $P = 0.09$) whereas restored and impacted springs did not differ. Fungal communities did not differ between natural and impacted springs (PERMANOVA $F_{1,12} = 0.72$, $P = 0.53$), but differed slightly among restored and impacted springs ($F_{1,13} = 3.2$, $P = 0.06$) and more so among restored and natural springs ($F_{1,12} = 9.24$, $P = 0.002$).

Similarity percent analysis (SIMPER) suggested that the crenophilous stonefly *N. pictetii* contributed most to differences between natural and impacted, and between restored and impacted sites (SIMPER contributions: 20 and 32%, respectively), indicating lower abundance of *N. pictetii* in impacted sites. The crenophilous chironomid larvae *Chaetocladius* spp. and *R. effusus* were also less abundant in impacted sites (SIMPER contributions: 11 and 7%, respectively). Of bryophytes, two crenophilous species, *B. rivulare* and *R. magnifolium*, contributed most to differences between natural and impacted sites (SIMPER contributions: 15 and 13%, respectively). The fungus *V. elodea* was found in higher abundances in natural (SIMPER contribution 17%) and impacted (49%) than in restored springs, while *Helotiales* sp. and *L. centrosphaera* were more abundant in restored sites (SIMPER contributions between restored and natural springs: 8% for both).

Discussion

The poor current status of spring ecosystems has sparked wide interest in their restoration, but ecological responses to restoration are not well understood. Our work aimed to fill this gap by being, to our knowledge, the first study to assess the effectiveness of spring restoration using both taxonomic and functional indicators. Our results suggest that the restoration actions, despite their recentness, have been largely successful in improving the hydrological conditions and habitat quality of the restored springs to a trajectory towards natural conditions. In particular, our results showed that restoration effectively prevented surface water inflow from nearby drainage ditches. This resulted in stabilized thermal and SI composition of the spring water, suggesting groundwater-dominated hydrological conditions, a prerequisite for the restoration of the biodiversity and ecosystem functions of groundwater-dependent ecosystems. Moreover, restoration increased spring habitat size, particularly of the moss-dominated helocrenic habitat. However, despite the markedly improved hydrology and habitat quality, we observed relatively slight and inconsistent effects on spring biota. Restoration did increase bryophyte abundance and a similar, although non-significant, trend was observed also for macroinvertebrates. More importantly, restoration had a positive effect on endemic species assemblages. Macroinvertebrate species composition differed between restored and impacted springs, suggesting that restoration facilitated the formation of native invertebrate assemblages. For example, the abundance of the crenophilous stonefly *N. pictetii* was lower in impacted than in near-natural and restored springs. Ilmonen et al. (2012) showed that *N. pictetii* is highly tolerant of habitat disturbances, provided that at least some groundwater discharge remains, yet our results showed that improved habitat conditions do have a positive effect on this species too.

The relatively weak and inconsistent biological responses to restoration are probably due partly to the fact that restoration activities at our study sites were relatively recent. Evidence from multiple ecosystem types suggests that dispersal limitation may be a key factor limiting the recovery of communities at restored sites. For example, Hasselquist et al. (2015) suggested recently that the recovery of riparian vegetation to near-natural levels after stream restoration may take several decades, and a similar time lag was suggested for vegetation recovery in restored peatlands by Haapalehto et al. (2011). One can easily envisage that recolonization of such small and weakly connected habitats as springs can be a very slow (see Juutinen, 2011; Ilmonen et al., 2013; Chuzhekova, 2015) and largely stochastic process, particularly for organisms such as bryophytes and fungi that rely on spores in their long-distance dispersal (Astorga et al., 2012). This may be less so for aquatic insects which have a winged adult stage with less stochastic among-site dispersal (Bilton et al., 2001). Other aquatic invertebrates (e.g., water mites; Wieçek et al., 2013) are much weaker dispersers and their recovery in restored springs should therefore be a very slow process. Unfortunately, such a long-term impact assessment is not currently possible in Finland, due to the short history of spring restoration and lack of adequate biological data.

As regards the restoration targets, the most encouraging finding of our study was that the restored springs exhibited species diversity and community composition largely comparable to pristine springs, particularly for macroinvertebrates. Our study was conducted in a sparsely populated area where the quantitative and qualitative status of groundwater is excellent, thus resulting in negligible water chemistry differences among the spring groups. Thus, as restored springs clearly provide groundwater-dependent organisms with improved habitat conditions, the potential for the recovery of spring biodiversity should be high, assuming adequate dispersal from near-natural springs. Spring restoration thus bears great promise as a conservation tool for the protection of a valuable component of regional freshwater biodiversity, particularly at a time when cold-water springs are facing novel threats caused by changing climate (Jyväsjärvi et al., 2015).

Restoration did not affect the biomass or species diversity of leaf-decomposing fungi, whereas their species composition was noticeably altered. Similarly, microbial decomposition rate was reduced after restoration. Fungal communities in restored springs differed from both near-natural and impacted springs, indicating that changes in fungal community may have resulted in changes in ecosystem functioning. Three fungal taxa (*Helotiales* sp., *V. elodea,* and *L. centrosphaera*) explained 92% of community dissimilarity among the spring groups. *V. elodea* was more

abundant in near-natural and impacted sites, while *L. centrosphaera* and an unidentified taxa from the same polyphyletic genera, *Helotiales* sp., dominated in the restored sites. *V. elodea* and *L. centrosphaera* belong to the same group of hyphomycetes and they both occur frequently in freshwater habitats of the boreal region (Baschien et al., 2013; Jabiol et al., 2013). Spring pool depth was the only environmental variable that clearly differed between near-natural and restored springs, suggesting that deepening of the spring pool during the restoration work may have partly disrupted the terrestrial–aquatic linkage for microbial communities (see Chauvet et al., 2015; Ruiz-González et al., 2015). Overall, little is known about fungal communities in springs and other groundwater-dependent ecosystems but, based on our work, spring fungal communities seem to be dominated by hyphomycetes that typically occur in both terrestrial and aquatic environments. For example, the most common fungal species in our springs, *V. elodea*, occurs in streams but is also found in subarctic soils (Saravesi et al., unpublished), and has been recorded to be equally common in soils and streams (Mäkelä, 1972). The relatively high proportional occurrence of Chytridiomycota also suggests a greater role for terrestrial fungi in springs compared to streams (see Tolkkinen et al., 2015). The presence of Glomeromycota in springs may also be of terrestrial origin, as they typically occur as arbuscular mycorrhizal (AM) symbionts of riparian herbs and grasses (Beauchamp et al., 2006). Glomeromycota have also been recorded as AM symbionts of epigeic bryophytes (Liepina, 2012), thus their presence in springs may refer to aquatic bryophyte–AM fungal associations. Clearly, the terrestrial–aquatic linkage of fungal taxa needs further research to better understand their role to leaf decomposition in springs and other freshwater habitats.

Although restoration measures were generally similar across sites, they were somewhat adjusted for site-specific conditions. For example, damming of the outflow channel with wooden constructions in some sites created more complex flow pathways and provided suitable habitat and moisture conditions for aquatic mosses. In some cases, spring pools were cleared of mosses and wood, which resulted in atypically deep spring pools compared to natural springs. This operation was likely used to improve the visual and aesthetical appearance of springs. However, our results suggest that such actions had altered biological (particularly fungal) communities and may thus be harmful to biota. Therefore, more coherent guidelines for ecologically sustainable spring restoration are clearly needed. Also, the overall need for restoration measures should be carefully considered before any actions are taken, as forestry activities often have relatively minor effects on spring ecosystems if groundwater quality and quantity remain at a sufficient level (Ilmonen et al., 2012). In our study area, land drainage was conducted mainly in the 1980s, and the relatively weak differences between the near-natural and impacted sites may have resulted from the fact that drainage ditches were partly revegetated and the effect of drainage on spring hydrology was therefore relatively minor.

Boreal spring ecosystems are currently threatened, and are likely to face novel threats in the future (Jyväsjärvi et al., 2015). Therefore, restoration efforts are needed to halt the continuing loss of spring biodiversity. Our work showed that spring restoration can indeed be beneficial, with immediate positive effects on some elements of spring biota, but more rigorous assessment of the ecological effects of spring restoration requires long-term post-restoration monitoring that includes, in addition to restored sites, also degraded and near-natural reference sites.

Acknowledgments We are grateful to Elina Isokangas for assistance in the field and with the SI data, and Leevi Heikura and Antti Leinonen for helping us select the study sites. We also thank Peggy Lommatzsch for her assistance in the field, Marko Suokas for his help in bioinformatics, and Lauri Paasivirta for checking our chironomid identifications. Finally, we acknowledge the two anonymous referees for their helpful and constructive comments on the previous draft of the manuscript. The study was supported by Maj and Tor Nessling Foundation (Project Number 201500223), University of Oulu (Thule Institute), and Academy of Finland (AKVA Program, Project Number 128377).

References

Anderson, M. J., 2001. A new method for non-parametric multivariate analysis of variance. Austral Ecology 26: 32–46.

Astorga, A., J. Oksanen, M. Luoto, J. Soininen, R. Virtanen & T. Muotka, 2012. Distance decay of similarity in freshwater communities: Do macro- and microorganisms follow the same rules? Global Ecology and Biogeography 21: 365–375.

Barquín, J. & M. Scarsbrook, 2008. Management and conservation strategies for coldwater springs. Aquatic Conservation: Marine and Freshwater Ecosystems 18: 580–591.

Baschien, C., C. K.-M. Tsui, V. Gulis, U. Szewzyk & L. Marvanova, 2013. The molecular phylogeny of aquatic hyphomycetes with affinity to the Leotiomycetes. Fungal Biology 117: 660–672.

Beauchamp, V. B., J. C. Stromberg & J. C. Stutz, 2006. Arbuscular mycorrhizal fungi associated with *Populus–Salix* stands in a semi-arid riparian ecosystem. New Phytologist 170: 369–380.

Benfield, E. F., 1996. Leaf breakdown in stream ecosystems. In Hauer, F. R. & G. A. Lamberti (eds), Methods in Stream Ecology. Academic, San Diego: 579–589.

Bilton, D. T., J. R. Freeland & B. Okamura, 2001. Dispersal in freshwater invertebrates. Annual Review of Ecology and Systematics 32: 159–181.

Cantonati, M., L. Füreder, R. Gerecke, I. Jüttner & E. J. Cox, 2012. Crenic habitats, hotpots for freshwater biodiversity conservation: toward an understanding of their ecology. Freshwater Science 31: 463–480.

Caporaso, J. G., J. Kuczynski, J. Stombaugh, K. Bittinger, F. D. Bushman, E. K. Costello, N. Fierer, A. Gonzalez Peña, J. K. Goodrich, J. I. Gordon, G. A. Huttley, S. T. Kelley, D. Knights, J. E. Koenig, R. E. Ley, C. A. Lozupone, D. McDonald, B. D. Muegge, M. Pirrung, J. Reeder, J. R. Sevinsky, P. J. Turnbaugh, W. A. Walters, J. Widmann, T. Yatsunenko, J. Zaneveld & R. Knight, 2010. QIIME allows analysis of high-throughput community sequencing data. Nature Methods 7: 335–336.

Céréghino, R., J. Biggs, B. Oertli & S. Declerck, 2008. The ecology of European ponds: defining the characteristics of a neglected freshwater habitat. Hydrobiologia 597: 1–6.

Chauvet, E., J. Cornut, K. R. Sridhar, M. A. Selosse & F. Bärlocher, 2015. Beyond the water column: aquatic hyphomycetes outside their preferred habitat. Fungal Ecology 19: 112–127.

Chuzhekova, T. A., 2015. Dynamics of macrozoobenthos structure in urban spring brooks of the Middle Volga Basin. Inland Water Biology 3: 259–268.

Clark, I. D. & P. Fritz, 1997. Environmental Isotopes in Hydrogeology. Lewis Publishers, New York.

Clarke, K. R., 1993. Non-parametric multivariate analysis of changes in community structure. Australian Journal of Ecology 18: 117–143.

Dudgeon, D., A. H. Arthington, M. O. Gessner, Z. I. Kawabata, D. J. Knowler, C. Lévêque & C. A. Sullivan, 2006. Freshwater biodiversity: importance, threats, status and conservation challenges. Biological Reviews 81: 163–182.

Edgar, R. C., 2010. Search and clustering orders of magnitude faster than BLAST. Bioinformatics 26: 2460–2461.

Eurola, S., S. Hicks & E. Kaakinen, 1984. Key to Finnish mire types. In Moore, P. D. (ed.), European Mires. Academic, London: 11–117.

European Commission, 2000. Directive 2000/60/EC of the European Parliament and of the Council of 23 October 2000 establishing a framework for Community action in the field of water policy. Official Journal of the European Communities L327: 1–72.

Gardes, M. & T. D. Bruns, 1993. ITS primers with enhanced specificity for basidiomycetes – application to the identification of mycorrhizae and rusts. Molecular Ecology 2: 113–118.

Gat, J. R., 2010. Isotope Hydrology: A Study of the Water Cycle. Imperial College Press, London.

Gessner, M. O., 2005. Ergosterol as a measure of fungal biomass. In Methods to Study Litter Decomposition. Springer, Dordrecht: 189–195.

Haapalehto, T. O., H. Vasander, S. Jauhiainen, T. Tahvainen & J. S. Kotiaho, 2011. The effects of peatland restoration on water-table depth, elemental concentrations and vegetation: 10 years of changes. Restoration Ecology 19: 587–598.

Hasselquist, E. M., C. Nilsson, J. Hjältén, D. Jørgensen, L. Lind & L. E. Polvi, 2015. Time for recovery of riparian plants in restored northern Swedish streams: a chronosequence study. Ecological Applications 25: 1373–1389.

Heino, J., R. Virtanen, K.-M. Vuori, J. Saastamoinen, A. Ohtonen & T. Muotka, 2005. Spring bryophytes in forested landscapes: land use effects on bryophyte species richness, community structure and persistence. Biological Conservation 124: 539–545.

Holden, J., P. J. Chapman & J. C. Labadz, 2004. Artificial drainage of peatlands: hydrological and hydrochemical process and wetland restoration. Progress in Physical Geography 28: 95–123.

Ilmonen, J. & L. Paasivirta, 2005. Benthic macrocrustacean and insect assemblages in relation to spring habitat characteristics: patterns in abundance and diversity. Hydrobiologia 533: 99–113.

Ilmonen, J., L. Paasivirta, R. Virtanen & T. Muotka, 2009. Regional and local drivers of macroinvertebrate assemblages in boreal springs. Journal of Biogeography 36: 822–834.

Ilmonen, J., H. Mykrä, R. Virtanen, L. Paasivirta & T. Muotka, 2012. Responses of spring macroinvertebrate and bryophyte communities to habitat modification: community composition, species richness and red-listed species. Freshwater Science 31: 657–667.

Ilmonen, J., R. Virtanen, L. Paasivirta & T. Muotka, 2013. Detecting restoration impacts in inter-connected habitats: spring invertebrate communities in a restored wetland. Ecological Indicators 30: 165–169.

Jabiol, J., A. Bruder, M. O. Gessner, M. Makkonen, B. G. McKie, E. T. H. M. Peeters, V. C. A. Vos & E. Chauvet, 2013. Diversity patterns of leaf-associated aquatic hyphomycetes along a broad latitudinal gradient. Fungal Ecology 6: 439–448.

Jackson, D. A., 1993. Stopping rules in Principal Components Analysis: a comparison of heuristical and statistical approaches. Ecology 74: 2204–2214.

Juutinen, R., 2011. The decrease of rich fen bryophytes in springs as a consequence of large-scale environmental loss. A 50-year re-sampling study. Lindbergia 34: 2–8.

Jyväsjärvi, J., H. Suurkuukka, R. Virtanen, J. Aroviita & T. Muotka, 2014. Does the taxonomic completeness of headwater stream assemblages reflect the conservation status of the riparian forest? Forest Ecology and Management 334: 293–300.

Jyväsjärvi, J., H. Marttila, P. M. Rossi, P. Ala-Aho, B. Olofsson, J. Nisel, B. Backman, J. Ilmonen, R. Virtanen, L. Paasivirta, R. Britschgi, B. Kløve & T. Muotka, 2015. Climate-induced warming imposes a threat to North European

spring ecosystems. Global Change Biology 21: 4561–4569.
Kappes, H., A. Sundermann & P. Haase, 2010. High spatial variability biases the space-for-time approach in environmental monitoring. Ecological Indicators 10: 1202–1205.
Kristensen, P. & L. Globevnik, 2014. European small water bodies. In Biology and Environment: Proceedings of the Royal Irish Academy 114. Royal Irish Academy: 281–287.
Lepori, F., D. Palm, E. Brännäs & B. Malmqvist, 2005. Does restoration of structural heterogeneity in streams enhance fish and macroinvertebrate diversity? Ecological Applications 15: 2060–2071.
Liepina, L., 2012. Occurrence of fungal structures in bryophytes of the boreo-nemoral zone. Environmental and Experimental Biology 10: 35–40.
Mäkelä, K., 1972. Some aquatic hyphomycetes on grasses in Finland. Karstenia 13: 16–22.
National Board of Waters, 1981. The Analytical Methods Used by National Board of Waters. Report 213. National Broad of Waters, Helsinki.
Nylund, J. E. & H. Wallander, 1992. Ergosterol analysis as a means of quantifying mycorrhizal biomass. Methods in Microbiology 24: 77–88.
Oksanen, J., F. G. Blanchet, R. Kindt, P. Legendre, P. R. Minchin, R. B. O'Hara, G. L. Simpson, P. Solymos, M. H. H. Stevens & H. Wagner, 2015. Vegan: Community Ecology Package Version 2.3-0 [available on internet at http://cran.r-project.org/web/packages/vegan/vegan.pdf].
Palmer, M. A., K. L. Hondula & B. J. Koch, 2014. Ecological restoration of streams and rivers: shifting strategies and shifting goals. Annual Review of Ecology, Evolution and Systematics 45: 247–269.
Pykälä, J., 2007. Implementation of Forest act habitats in Finland: Does it protect the right habitats for threatened species? Forest Ecology and Management 242: 281–287.
R Core Team, 2014. R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna.
Ruiz-González, C., J. P. Niño-García & P. A. Del Giorgio, 2015. Terrestrial origin of bacterial communities in complex networks. Ecology Letters 18: 1198–1206.
Tolkkinen, M., H. Mykrä, A.-M. Markkola, H. Aisala, K.-M. Vuori, J. Lumme, A.-M. Pirttilä & T. Muotka, 2013. Decomposer communities in human-impacted streams: species dominance rather than richness affects leaf decomposition. Journal of Applied Ecology 50: 1142–1151.
Tolkkinen, M., H. Mykrä, M. Annala, A. M. Markkola, K.-M. Vuori & T. Muotka, 2015. Multi-stressor impacts on fungal diversity and ecosystem functions in streams: natural vs. anthropogenic stress. Ecology 96: 672–683.
Turunen, J., T. Muotka, K.-M. Vuori, S. M. Karjalainen, J. Rääpysjärvi, T. Sutela & J. Aroviita, 2016. Disentangling the responses of boreal stream assemblages to low stressor levels of diffuse pollution and altered channel morphology. Science of the Total Environment 544: 954–962.
Ulvinen, T., K. Syrjänen & S. Anttila, 2002. Bryophytes in Finland: distribution, ecology and red list status. Finnish Environment Institute, Helsinki.
Underwood, A. J., 1994. On beyond BACI: sampling designs that might reliably detect environmental disturbances. Ecological Applications 4: 3–15.
von Fumetti, S., P. Nagel, N. Scheifhacken & B. Baltes, 2006. Factors governing macrozoobenthic assemblages in perennial springs in north-eastern Switzerland. Hydrobiologia 568: 467–475.
Vuori, K.-M., I. Joensuu, J. Latvala, E. Jutila & A. Ahvonen, 1998. Forest drainage: a threat to benthic biodiversity of boreal headwater streams? Aquatic Conservation: Marine and Freshwater Ecosystems 8: 745–759.
Ward, J. V. & K. Tockner, 2001. Biodiversity: towards a unifying theme for river ecology. Freshwater Biology 46: 807–819.
Wieçek, M., P. Martin & A. Lipinski, 2013. Water mites as potential long-term bioindicators in formerly drained and rewetted raised bogs. Ecological Indicators 34: 332–335.