ORIGINAL ARTICLE

Complex effects of acidification, habitat properties and fish stock on littoral macroinvertebrate assemblages in montane standing waters

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ABSTRACT

Littoral macroinvertebrates in acidified waterbodies are affected by the interaction of acidification and local environmental conditions. Understanding the interplay of these factors in the structuring of communities is essential for interpreting responses to and/or recovery from acidification. Here, we analyse the species composition and richness of littoral macroinvertebrates in a range of acidified montane standing waters in relation to water chemistry, littoral characteristics and fish stock. The main species composition gradients were related to pH and conductivity; however, considerable variation along these gradients was associated with local habitat characteristics (changing water levels and littoral structure) and concentration of ionic aluminium and dissolved organic carbon. Although fish stock effects were confounded by correlated acidity, we observed a significant decline in abundance of macroinvertebrates vulnerable to fish predation at sites with fish stock. Overall, littoral macroinvertebrates of acidic waterbodies were diverse due to the heterogeneity of local habitat properties, despite they were dominated by acid-tolerant species. Acidic humic sites with dense, heterogeneous littoral vegetation were species-rich, hosting numerous habitat specialists and rare species, while chronically acidified lakes with high aluminium concentrations and sparse littoral vegetation had species-poor assemblages, characteristic of strong acid-stress. Water level manipulation resulted in serious assemblage impoverishment, overriding the effects of more favourable water chemistry. This study shows that the littoral fauna of acidic waterbodies is structured by complex effects induced by local factors in addition to acidity, resulting in acid-stressed assemblages with relatively high variability, emphasising a need to analyse local habitat factors when evaluating the impact of acidification on macroinvertebrates.

INTRODUCTION

Acidification is an important environmental stressor affecting littoral communities in standing waters (Vrba *et al*., 2003; Lento *et al*., 2008; Johnson *et al*., 2018). Acidification arising from atmospheric deposition of strong inorganic acids can cause significant reductions in diversity and changes in the composition of communities and food webs that persist for decades after acid deposition has declined (Hildrew, 2018). On the other hand, naturally acidic waters, where the source of acidity is mostly organic, can host diverse, acid-tolerant communities, particularly when widespread and persistent in the landscape (Dangles *et al*., 2004). In many cases, natural acidity is exacerbated by anthropogenic acidification, making the effects difficult to disentangle (Schartau *et al*., 2008; McFarland *et al*., 2010; Johnson *et al*., 2018).

Numerous studies have explored the effects of low pH and associated increases in toxic aluminium on the structuring of littoral invertebrate communities (Johnson *et al*., 2007; Lento *et al*., 2008; Schartau *et al*., 2008). However, the role of other key community drivers, which can also be modified by acidification and act as indirect effects of acidification, are often not considered (Appelberg *et al*., 1993; Hildrew, 2018). Littoral habitat composition is a prime factor shaping benthic communities in shallow lakes and littoral areas (Brown *et al*., 1988; Tolonen *et al*., 2003; Rennie and Jackson, 2005); however, acidification can alter littoral vegetation and primary production by altering nutrient availability and water transparency, favouring the development of littoral zones dominated by *Sphagnum* and emergent vegetation preferring nutrient-poor conditions (Farmer, 1990; Appelberg *et al*., 1993; Vrba *et al*., 2006). Slow decomposition in acidic waters often results in an accumulation of coarse detritus (Grahn *et al*., 1974) which, together with changes to primary pro-

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duction, alters the food supply for consumers in the littoral zone (Appelberg *et al*., 1993; Layer *et al*., 2010; Hildrew, 2018). As a consequence, littoral macroinvertebrate communities are influenced by availability of their preferred substrate and modified food resources (Wesolek *et al*., 2010).

Littoral macroinvertebrates can also be influenced by the elimination of susceptible fish through acid toxicity, leading to the release of acid-tolerant, large-bodied invertebrates from predation and cascading effects on the food web (Appelberg *et al*., 1993; Knapp *et al*., 2001; Hildrew, 2018). Fishless and fish-containing lakes differ in macroinvertebrate community composition, abundance and species richness; hence, fish presence or absence can be a stronger determinant of community structure than other predictors (Schilling *et al*., 2009). Consequently, the re-establishment of fish populations in recovering lakes is often regarded as crucial for the structuring of macroinvertebrate communities (Wesolek *et al*., 2010; Vrba *et al*., 2016). On the other hand, fish predation pressure can be mitigated by high littoral habitat complexity providing refuges (Tolonen *et al*., 2003; Rennie and Jackson, 2005). This suggests that macroinvertebrates in acidic standing waters are influenced by highly interactive effects that can be locally specific, depending on bank and littoral vegetation and concentration of aluminium and humic substances, the latter determine toxicity and nutrient conditions (cf. Wesolek *et al*., 2010; Vrba *et al*., 2016).

Here, we investigate the interplay of acidification, habitat structure and fish presence on littoral macroinvertebrates in lakes, ponds and reservoirs (some of them naturally acidic) affected by anthropogenic acidification over the second half of the 20th century (Stuchlík *et al*., 1997; Křeček and Hořická, 2001; Vrba *et al*., 2003, 2016). Thanks to recent chemical recovery, the waterbodies range in pH from extremely acidic (pH <5, negative alkalinity, high concentrations of ionic aluminium, Al_i >80 $\mu g\ L^{-1}$) to weakly acid (pH 6.2-6.8) and near neutral (pH 6.9-7), and differ in dissolved organic matter (DOM), fish stock and littoral properties. In exploring this environmental variability, we aim to assess the relative roles of water chemistry, habitat properties and fish stock on the structuring of macroinvertebrate assemblages. We expect that direct effects of acidity will be modified by local habitat properties, such as littoral substrate and vegetation, DOM and fish stock. We hypothesize that littoral properties will have stronger effects on species composition and richness than acidity itself. Acidification will have adverse effects on macroinvertebrates only in waterbodies with high aluminium toxicity. We further expect that fish stock will influence species composition of macroinvertebrates in less acidified waterbodies by elimination of species vulnerable to fish predation.

METHODS

Study area and waterbodies

We investigated 23 mountain waterbodies (natural lakes, artificial ponds and reservoirs) located in the Krušné hory Mts., the Jizerské hory Mts. and the Bohemian Forest in the Czech Republic and Germany (Tab. 1, Fig. 1). These areas were affected by heavy atmospheric pollution in the second half of the 20th century, resulting in acidification of both terrestrial and aquatic ecosystems. Acidifying compounds (SO_2-S, NO_x-N) in the atmosphere increased sharply from the 1950s, peaking in the first half of the 1980s (Kopáček and Veselý, 2005). The effects of deposition were enhanced by high geological sensitivity (granite, mica-schist and gneiss bedrocks low in base cations), shallow soils with low buffering capacity, predominantly coniferous forests and relatively high precipitation (Kopáček *et al*., 1995; Stuchlík *et al*., 1997).

The Krušné hory and Jizerské hory Mts, situated in the northern part of the study area, form part of the so-called "Black triangle" comprising the Czech-German-Polish borderlands (Fig. 1), the epicentre of acidification in Europe in the second half of the 20th century (Grübler, 2002). This area had the highest emission loads in Europe due to numerous local power plants burning brown coal and local topography that resulted in extended inversion events (Eliassen *et al*., 1988; Bridges *et al*., 2002; Kopáček and Veselý, 2005). The resultant acidic deposition of SO_2 caused catastrophic dieback of spruce forests above 750 m asl (Ardö *et al*., 1997; Kolář *et al*., 2015) and acidification of montane surface waters and soils (Oulehle and Hruška, 2009; Vašát *et al*., 2015). The Bohemian Forest, along the Czech-German-Austrian border in the southern part of the study area (Fig. 1), became acidified later than the northern region owing to its distance from the emission sources (Cerny, 1995; Stuchlík *et al*., 1997).

A dramatic decrease in sulphur and nitrogen deposition (by more than 80 % and 50 %, respectively) from the late 1980s was caused by a decrease in energy consumption, sulphur emission controls and regional socioeconomic changes (Kopáček and Veselý, 2005; Kopáček *et al*., 2012). Chemical recovery of surface waters commenced in the 1990s and was later followed by biotic recovery, documented through recolonisation by some species of zooplankton, aquatic insect and fish (Křeček and Hořická, 2001; Vrba *et al*., 2003, 2016). Nevertheless, numerous montane standing waterbodies in both areas still suffer from acid stress (Vrba *et al*., 2016; Křeček *et al*., 2017).

The study sites are all located at altitudes of 730-1100 m asl and include 15 artificial waterbodies (six reservoirs and nine ponds) and eight natural lakes of glacial origin (Fig. 1, Tab. 1). The littoral zones of lakes, reservoirs and ponds all had similar depth profiles and temperatures and had natural substrates and vegetation, providing comparable mesohabitats for macroinvertebrates (Fig. S1). As such, abiotic characteristics were comparable in both natural and artificial waterbodies and in southern and northern study areas (Fig. S1, Tab. 1).

All sites were located in acid-sensitive, nutrient-poor catchments with a prevalence of coniferous forest, and all are recovering from acidification or remain naturally acidic. The sites range from dystrophic to mesotrophic, with differing acidity (average pH 4.7-7), acid neutralising capacity (−15-174 µmol L^{-1}) and aluminium toxicity (Al_i 0-231 µg L^{-1}) (Tab. 1). Littoral zone characteristics cover a wide gradient from stony-gravel substrate and limited vegetation to organic substrate and dense, diverse vegetation (Tab. 1).

Field sampling and processing of samples

Macroinvertebrate sampling was performed in 2014 and 2015, with each site sampled in spring (late May-early June) and autumn (early September). Two samples were taken by one person at each site in each period: i) a semi-quantitative sample taken by 3-minutes sampling by a hand net (mesh size 0.5 mm) proportionally distributed in various mesohabitats (e.g., patches with stony substrate, moss vegetation, coarse organic sediment, *etc.*) in lake littoral, and ii) a qualitative sample obtained by individual collecting of macroinvertebrates using a metal strainer in lake littoral zone for 60 minutes. Macroinvertebrates were determined to the lowest possible taxonomic level, with most identified to species (234 species, 80 % of all taxa). Higher levels included species groups (22), genera (34), subfamilies (2) and families (2), especially in Diptera. Macroinvertebrate larvae, pupae and aquatic adults (Heteroptera, Coleoptera) were all identified; however, Oligochaeta (not abundant in littoral zones) were not included in the dataset.

Water samples, obtained at the same time as macroinvertebrate sampling, were filtered through 0.4-µm pore size glass-fibre filters (MN-5, Macherey Nagel) and analysed for dissolved compounds. Concentrations of major ions (Ca^{2+}, Mg^{2+}, Na^+, K^+, Cl^-, SO_4^{2-}, F^-) were assessed using ion chromatography and dissolved organic

Tab. 1. List of waterbodies studied, with selected characteristics shown. Water chemistry variables are displayed as mean values.

Site	Code	Water-body type	Wat. level manip.	Area (ha)	pH	ANC (µmol L^{-1})	Al_i (µg L^{-1})	Conductivity (µS cm^{-1})	SO_4^{2-} (mg L^{-1})	P_t (µg L^{-1})	DOC (mg L^{-1})	Vegetation belt (m)	Sedges (%)	Mosses (%)	Organic substrate (%)	Fish stock
Černé jezero	CernJ	Lake	No	18.8	4.9	-2	168.9	20.5	2.6	3.8	1.9	0	2	0	60	1
Čertovo jezero	CertJ	Lake	No	10.5	4.7	-15	232.4	20.5	2.5	6.1	3.8	0.3	5	0	30	1
Grosser Arbersee	GrosA	Lake	No	7.7	5.6	24	41.2	14.5	2.2	6.6	4.8	2	65	10	90	2
Kleiner Arbersee	KleiA	Lake	No	6.4	6.2	41	30.4	14.3	2.0	6.3	4.1	3	20	40	90	2
Laka	Laka	Lake	No	2.4	6	30	24.7	19.4	1.1	9.7	4.9	7	95	5	90	1
Plešné jezero	PlesJ	Lake	No	7.4	5.5	16	81.6	16.8	2.3	18.5	6.5	8	60	20	75	1
Prášilské jezero	PrasJ	Lake	No	4.1	5.1	4	90.5	16.3	1.5	5.7	6.2	2	50	10	45	1
Rachelsee	Rachel	Lake	No	5.7	5.4	6	43.5	11.2	2.2	7.0	5.1	6	60	20	80	1
Jelení jezírko	JeleJ	Pond	No	0.5	4.9	-5	68	35	7.7	34.2	15.9	3	80	60	95	1
Polecká nádrž	PoleN	Pond	No	1.2	6.5	59	16	28	5.2	34.8	11.9	8	90	75	100	3
Tokaniště	Tokan	Pond	No	0.2	6.8	79	30	23	4.7	14.1	12.9	10	95	5	97	3
Ždárské jezírko	ZdarJ	Pond	No	1.6	6.3	53	38	26	5.8	33.6	7	5	85	50	70	3
Černý rybník	CernR	Pond	No	0.6	7	174	0	81.7	10.6	30.7	5.6	5	100	50	100	3
Lieche	Liech	Pond	No	1.3	4.7	11	37.9	34.1	15.6	22.2	7.7	6	95	80	75	1
Mrtvý rybník	MrtvR	Pond	No	1.6	4.7	21	10.9	13.1	18.0	160.0	15.1	4	100	90	100	1
Starý rybník	StarR	Pond	No	4.6	6.1	87	7.4	53.5	9.8	31.7	13.8	4	95	5	100	3
Volárenský rybník	VolarR	Pond	No	2.2	4.8	-4	43.4	42	20.6	70.3	11	6	80	40	99	1
Blatný rybník	BlatR	Pond	Yes	1.3	6.6	173	17.9	62.9	6.7	19.1	2.8	0	0	1	40	1
Bedřichov	Bedr	Reserv.	Yes	29.0	5.8	32	9.9	38.7	21.3	14.6	5.5	0	5	10	80	2
Fláje	Flaje	Reserv.	Yes	139.9	6.7	129	0.4	71.4	8.8	12.2	4.5	2	90	0	60	3
Josefův Důl	JoseD	Reserv.	Yes	131.6	6.3	33	0.6	36.3	0.7	6.1	3.5	0	5	0	60	2
Přísečnice	Prise	Reserv.	Yes	314.4	6.9	313	0	96.8	12.8	28.3	5.5	0	20	0	30	3
Souš	Sous	Reserv.	Yes	65.1	7	150	8.4	37.9	11.3	8.4	5.1	3	95	20	95	2

Wat. level manip., water level manipulation, Reserv., reservoir; Fish stock - categories: 1, fishless; 2, low fish abundance, only or predominantly salmonids; 3, high fish abundance, complex fish community, cyprinids prevailing.

carbon (DOC) was analysed with a LiquiTOC analyser (Foss/Heraeus). Total phosphorus (P_t) was determined by the molybdate method after perchloric acid digestion. Fractionation of Al was analysed in non-filtered samples (total reactive Al; Al_t), filtered samples (dissolved Al; Al_d) and cation-exchange treated samples (organically bound Al; Al_o) after filtration. Values for ionic Al (Al_i) were obtained as the difference between Al_d and Al_o. Acid neutralising capacity (ANC; Gran titration), pH and total phosphorus (P_t) were all analysed within 24 h of sampling.

The littoral zone of each waterbody was described on site using a range of vegetation descriptors (percentage cover of sedges and rushes, mosses, mostly brown mosses and *Sphagnum*, and other macrophytes, mostly *Nuphar* and *Potamogeton*), the average width of the vegetated littoral belt, average littoral water depth and a series of bed substrate descriptors (percentage cover of stones, gravel and sand, and organic substrate). The variable ‘water level manipulation’ was treated as two-level factor: ‘manipulated’ included waterbodies affected by water storage activities, where a decline in water level exposed 2–5 metres of previously flooded littoral zone, while the remaining sites were categorized as ‘stable’. Data on fish stocks were obtained from local authorities (Šumava National Park, Bayerische Landesanstalt für Landwirtschaft), published reports (Peterka *et al*. 2009; Šanda *et al*., 2015) and our own investigations in the field. Owing to the differing quality of data and descriptors measured, the fish stock data were not quantifiable; hence, fish stocks were described using an ordinal variable that took into consideration fish abundance, species richness and dominant species, i.e. 1 = fishless; 2 = low abundance, only or predominantly salmonids (maintained by fish stocking in some waterbodies); 3 = high abundance, complex fish community, cyprinids prevalent (waterbodies used for recreational fishing or aquaculture).

Data analysis

The species data from spring and autumn sampling were merged before analysis. Species composition analysis was based on species abundance, obtained from the semi-quantitative samples, and species richness (presence-absence data), which was obtained by combining the semi-quantitative and quantitative samples. Species abundance was log-transformed, and some explanatory vari-

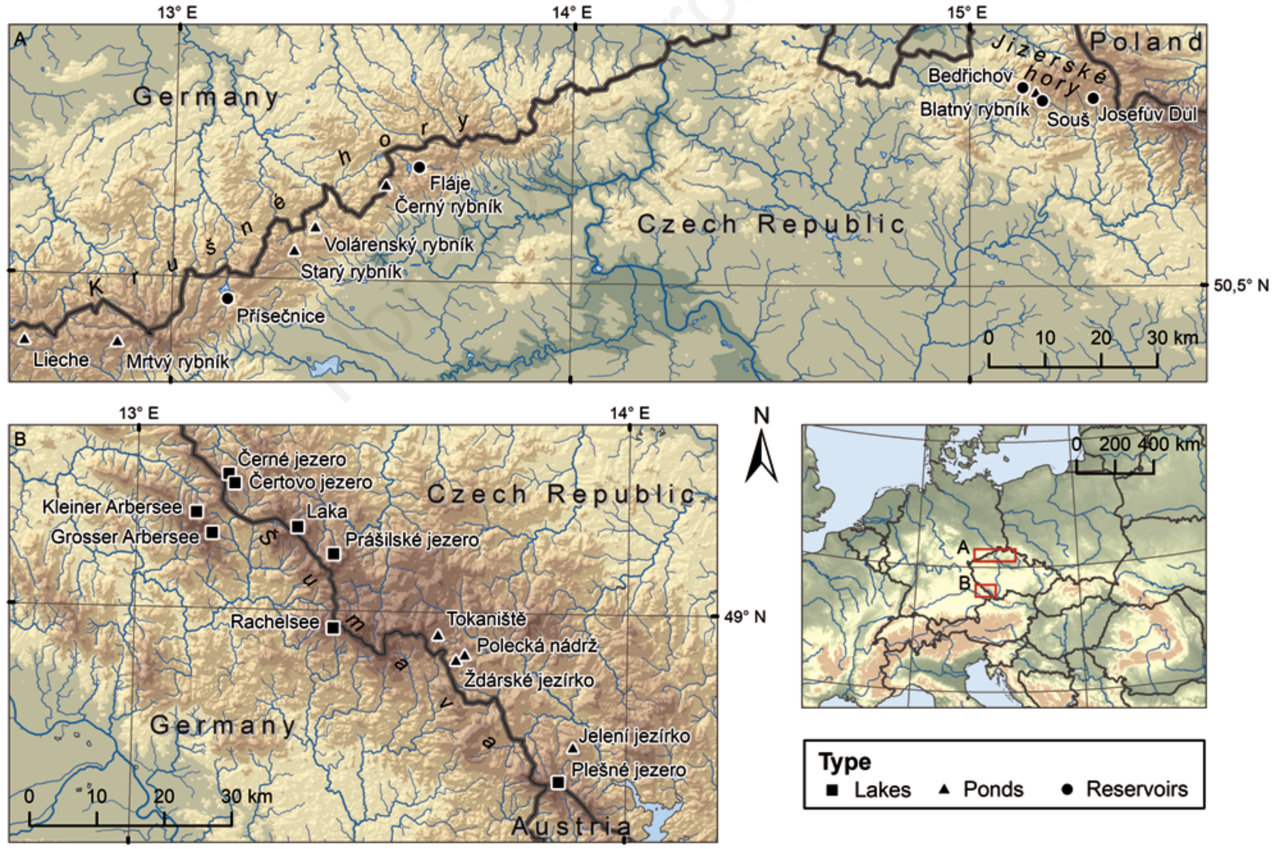

Fig. 1. Location of the study area, with the positions of individual waterbodies indicated. Symbols represent different types of waterbodies.

ables were transformed (square-root, logarithmic, arcsin or logit transformation) to achieve their normal distribution. From total 30 variables measured, 15 were selected for subsequent analyses. First, variables constant among lakes were excluded. Conductivity was used as a proxy of strongly correlated concentrations of ions (R_S >0.90). Acidity and Al toxicity were described by pH and Al_i, and nutrients and humic substances by P_t, NO_3-N, and DOC. From numerous littoral descriptors, cover of organic substrate differentiating littorals with organic and inorganic substrate, and three specific descriptors of vegetation (cover of sedges, mosses, and total width of vegetation belt) were included. Fish stock and water level manipulation were included as well. Relationships between selected variables were explored by Spearman's correlation matrix (using the "rcorr" function in the R "Hmisc" package) (Tab. S1).

Non-metric Multidimensional Scaling (NMDS) on Bray-Curtis distances was used ("metaMDS" function, "vegan" package) to visualise the main patterns in species composition, using explanatory variables with a significant fit in the ordination. The explanatory variables were selected using the "envfit" function ("vegan" package), which fits variables on ordination scores using multiple linear regression and tests the significance of each variable using a permutation test (9999 permutations). Forty percent of non-rare species (those with total abundance higher than 100 and frequency higher than three sites) with best fit in the ordination were selected by the "ordiselect" function ("goeveg" package) and also displayed in the NMDS diagram. Differences in abiotic variables, species richness and abundance between sites with manipulated water levels and those with stable water levels were tested using the Mann-Whitney U test. Indicator analysis with 1,000 randomisations was applied to species with a total abundance > 40 in order to identify indicator species for sites with manipulated and stable water levels ("indval" function, "labdsv" package).

The effects of three types of variable accounting for species data variation (chemical, littoral and fish stock) were tested by db-RDA (Legendre and Anderson, 1999). Significant variables were selected by forward selection (Blanchet *et al*., 2008) for chemical and littoral variables separately, with fish stock entered the analysis as a single variable. Significant variables from each group were then included into the final model. The adjusted explained variation for each explanatory variable was calculated i) without the effect of other variables (gross effect), ii) after excluding the effect shared with all explanatory variables within the partial model (chemical or littoral variables), and iii) within the final model (pure effect). The significance of gross and pure variable effects, as well as significance of the effects of explanatory variable groups, was tested using a 9999-permutation procedure. The adjusted R^2, corrected for the number of explanatory variables, was used as an unbiased estimate of explained variation (Peres-Neto *et al*., 2006). Functions "capscale", "ordistep", "RsquareAdj" ("vegan" package) were used in these analyses. A Venn diagram was constructed to display the final model.

We performed simple linear regression analysis testing the relationships of species richness and total abundance at sites with each environmental variable. Based on the NMDS results showing significant effect of water level manipulation on species composition, we performed tests separately the whole dataset (23 sites) and dataset including only sites with stable water levels (17 sites) to explore its effect on these relationships. Selected significant relationships between the variables and species richness were displayed as scatter plots.

The anticipated effect of fish stock was explored by comparing abundance and species richness of macroinvertebrate groups known to be vulnerable to fish predation, i.e., Aeshnidae, Libellulidae, Notonectidae, Corixidae, Gyrinidae, Dytiscidae and Chaoboridae (Schilling *et al*., 2009), in waterbodies with (Starý rybník, Černý rybník, Polecká nádrž, Žďárské jezírko and Tokaniště ponds) and without (Lake Laka and the Lieche, Jelení jezírko, Volárenský rybník and Mrtvý rybník ponds) fish stocks. These macroinvertebrate groups included solely acid-tolerant species. Sites with fish stock included both the categories 1 and 2 to acquire two groups with same number of sites for the analysis. Additionally, only waterbodies with stable water levels and with dense littoral (i.e., cover of sedges and rushes 80-100 %) were compared in order to avoid possible influence of lacking vegetation on macroinvertebrates vulnerable to predation (as many of them are phytophilous). Differences in species richness and abundance between waterbodies with and without fish were tested using the unpaired *t*-test.

Relationships between the matrices of environmental variable and species composition, and the matrix of spatial distances were tested by Mantel test ("mantel.test" function, "ape" package) to explore the spatial structuring of environmental and species composition data.

All statistical analyses were carried out in R (R Core Team 2019) using the following packages: "vegan" (Oksanen *et al*., 2019), "Hmisc" (Harrell, 2019), "labdsv" (Roberts, 2019), "goeveg" (Goral and Schellenberg, 2018) and "ape" (Paradis and Schliep, 2019). All plots were created using the R packages "ggplot2" (Wickham, 2016) and "eulerr" (Larsson, 2019).

RESULTS

Species richness and abundance of macroinvertebrate groups

We recorded a total of 35,739 individuals from 294 macroinvertebrate taxa in the 23 montane standing waterbodies examined (Tab. S2). The most species-rich groups were Coleoptera (86 taxa) and Chironomidae (69 taxa). While both had a similar median number of taxa per site (19 and 18, respectively), Coleoptera exhibited high variance between sites (4–36 taxa per site; Fig. 2). Two other species-rich groups, Trichoptera and Heteroptera (42 and 37 taxa, respectively), had a lower number of species per site (Fig. 2). Chironomidae were the most abundant group at nearly all sites (med. 768 ind. per site), comprising more than a half of all individuals recorded. The remaining groups were markedly less abundant (Fig. 2), with non-insect fauna represented by just six species with low total abundance (Tab. S2, Fig. 2).

Explanatory variables

While pH and metal toxicity were significantly correlated ($R_S = -0.71$), Al_i showed considerable variability at acidic sites (11–234 µg L^{-1} at $pH \leq 5.1$), which could potentially influence their biota. Conductivity was used as a proxy for ionic concentration as all measured ions (Ca^{2+}, Mg^{2+}, Na^+, SO_4^{2-}, and Cl^-) were highly intercorrelated. The concentration of divalent cations (Ca^{2+}, Mg^{2+}) was positively correlated with pH. In contrast, concentration of SO_4^{2-} was not negatively correlated with pH, indicating that it was independent of acidic conditions present at the sites. Sulphates were generally higher in northern waterbodies (Tab. 1) as they were closer to emission sources; however, they showed a wide distribution, ranging from 0.7 to 21.3 mg L^{-1}, reflecting local conditions such as altitude and waterbody retention time. The organic substrate chosen for analysis from the group of correlated substrate descriptors was partly correlated with the vegetation descriptors, and especially with sedges (Tab. S1), presumably as vegetation directly influences the amount of organic matter in the littoral substrate. Despite a strong correlation with pH, fish stock was partly independent, as some waterbodies with suitable pH were not colonised by fish yet, hence fish stock may have independent effects on macroinvertebrates. No correlation between matrix of explanatory variables and matrix of spatial distances was found (Mantel test, p=0.415). Ranges of variable values were always similar or highly overlapping when comparing sites according to location or waterbody type (examples in Tab. 1), except for water level manipulation, where manipulated sites were located only in the northern part of the study area.

Main compositional gradients in species data

NMDS analysis of macroinvertebrate assemblages resulted in two significantly distinct groups along the first axis (db-RDA: $p<0.001$, adjusted $R^2 = 13.9\%$), with manipulated water level sites on the left (all reservoirs and one pond) and stable water level sites on the right (nine ponds and all lakes) (Fig. 3). Manipulated water level sites were characterised by significantly higher pH, conductivity (positively correlated with other ions) and oxygen saturation, and lower Al_i, than stable sites (Tab. S3). In both cases, nutrients (P_t, NO_3-N) and DOC were comparable. Despite considerably improved water chemistry (in terms of acidity), macroinvertebrate species richness and abundance were significantly lower at manipulated water level sites (median 50 taxa compared to 73 taxa at stable water level sites), presumably as macroinvertebrates were af-

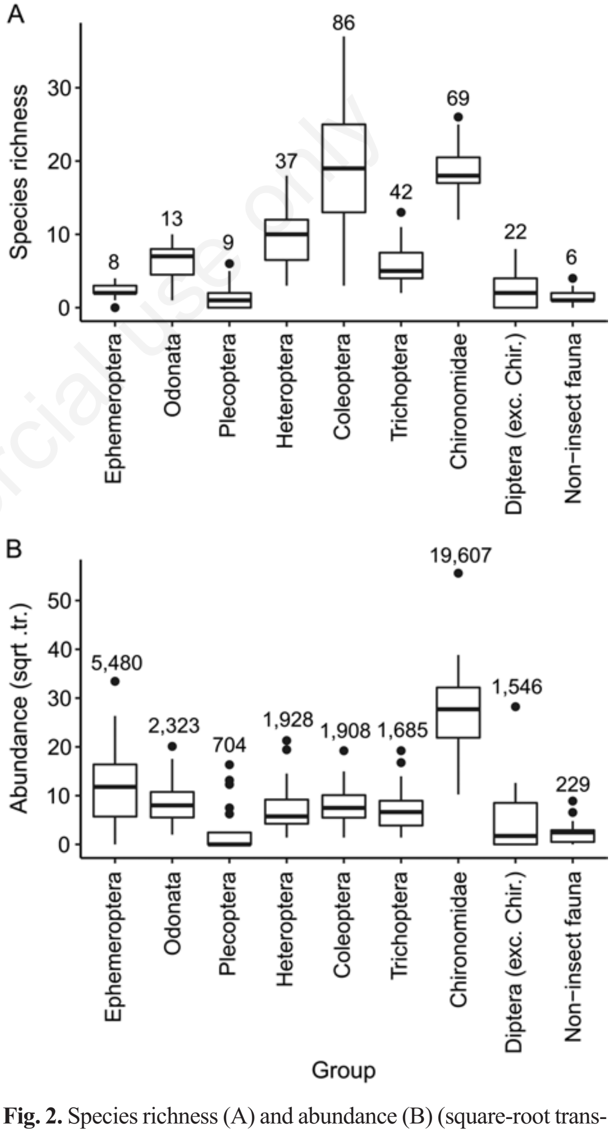

Fig. 2. Species richness (A) and abundance (B) (square-root transformed) of macroinvertebrate groups found at the study sites. Total number of species and total abundance of each group are given above the boxplots. Chironomidae are shown separately from other Diptera; non-insect fauna includes Crustacea, Mollusca, Hirudinea and Tricladida (Platyhelminthes). Boxes represent the median (horizontal line) and inter-quartile range (25th –75th percentiles), whiskers represent the range of values under 1.5 box-lengths and outliers are shown as dots.

fected by significantly narrower belts of vegetation and lower moss cover in littoral zones affected by water management (sedges and organic substrate did not differ significantly) (Fig. 3A, Tab. S3). Lowered species richness was particularly evident as regards Odonata, Heteroptera, Coleoptera and Diptera, i.e. those groups containing numerous phytophilous species, while Heteroptera, Trichoptera and Diptera all showed lowered abundance (Tab. S3). Non-insect fauna (mainly molluscs) was the only group more abundant at manipulated water level sites. Four fine-sediment dwelling taxa, *Pisidium casertanum*, *Caenis horaria*, *Dicrotendipes* sp. and *Ablabesmyia longistyla*, were evaluated as significant indicators for waterbodies with manipulated water levels (Tab. 2). Thirty Trichoptera, Coleoptera and Chironomidae taxa found exclusively at manipulated sites (Tab. S2) usually occurred at very low abundance (med. 2 inds. per site; e.g. *Molanna angustata*, *Limnephilus decipiens*, *Nebrioporus elegans*, *Helophorus nanus*, *Synorthocladius semivirens*, and *Larsia* sp.). In contrast, 12 Odonata, Coleoptera, Trichoptera and Diptera indicator species were found at sites with non-manipulated water levels (Tab. 2).

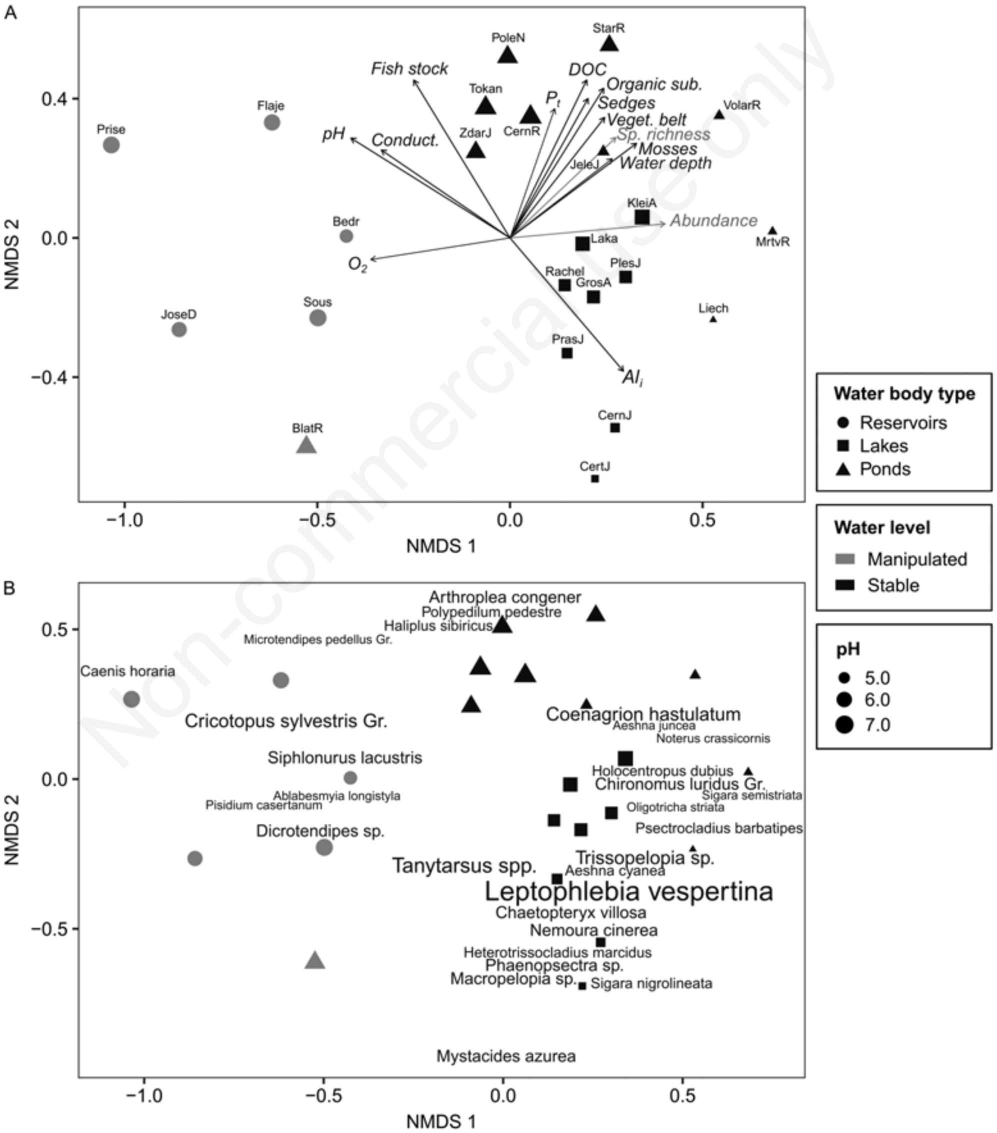

Fig. 3. NMDS ordination diagrams (stress value = 0.16) showing the main gradients in assemblage species composition. A) Assemblages in waterbodies in relation to variables significantly fitting ($p<0.05$) into the ordination (shape, colour and size of symbols represent waterbody type, water level manipulation and pH, respectively); total species richness and abundance are displayed by grey arrows. B) The positions of selected taxa with highest fit in the ordination. The size of taxa names represents their total abundance (ranging from 107, *Noterus crassicornis*, to 3,326, *Leptophlebia vespertina*).

The second compositional gradient in species data, displayed diagonally in the NMDS diagram, was associated with acidity (pH, Al_i), conductivity and fish stock (Fig. 3A). Macroinvertebrate assemblages were arranged as fishless acidic lakes and ponds in the lower right part of the diagram to circum-neutral ponds and reservoirs with diverse and stable fish stocks in the upper left part (Fig. 3A). Strongly acidic waterbodies (pH $\leq$5) with high toxic Al_i concentrations (>90 µg L^{-1}) and limited vegetation (CertJ, CernJ, PrasJ) were dissimilar to those with lower Al_i concentrations and well-developed littoral vegetation (JeleJ, Liech, MrtvR, VolarR) (Fig. 3A), thereby indicating the interplay of water quality and littoral zone properties. Variation in assemblage composition associated with littoral vegetation, organic substrate, DOC and P_t (second NMDS axis) was considerable in all habitat types (i.e., lakes, reservoirs and ponds). Macroinvertebrate assemblages in acidic waterbodies with limited vegetation were dominated by eurytopic acid-tolerant species (*Aeshna cyanea*, *Nemoura cinerea*, *Sigara nigrolineata*, *Chaetopteryx villosa* and *Mystacides azurea*) and species preferring cold oligotrophic or humic conditions (*Leptophlebia vespertina*, *Heterotrissocladius marcidus*, *Phaenopsectra* sp.) (Fig. 3B). In contrast, the vegetated littoral zones of acidic and near-neutral waterbodies were characterised by numerous phytophilous species (e.g. *Coenagrion hastulatum*, *Aeshna juncea*) and species preferring organic substrates (*Noterus crassicornis*) and peaty pools (*Holocentropus dubius*, *Oligotricha striata* and *Psectrocladius barbatipes*) (Fig. 3B).

Tab. 2. Indicator species/genera for sites with manipulated water levels (Manip.) and sites with stable water levels (Stable). Only taxa with a probability value less than 0.05 are shown.

Taxon	Cluster	Indicator value	Probability
Pisidium casertanum	Manip.	0.8054	0.001
Dicrotendipes sp.	Manip.	0.7563	0.010
Caenis horaria	Manip.	0.4587	0.037
Ablabesmyia longistyla	Manip.	0.3996	0.030
Pyrrhosoma nymphula	Stable	0.8871	0.001
Endochironomus sp.	Stable	0.7640	0.002
Anacaena lutescens	Stable	0.7217	0.002
Aeshna juncea	Stable	0.7073	0.014
Coenagrion hastulatum	Stable	0.7059	0.018
Enochrus ochropterus	Stable	0.7059	0.014
Holocentropus dubius	Stable	0.6471	0.020
Oligotricha striata	Stable	0.6471	0.017
Aeshna cyanea	Stable	0.6072	0.037
Leptophlebia vespertina	Stable	0.5882	0.041
Noterus crassicornis	Stable	0.5882	0.037

No significant relationship between species composition and spatial distances between sites was found (Mantel test, p=0.335).

Determinants of species composition variation

Most variables had a significant ($p<0.01$) influence on macroinvertebrate species composition when tested separately in db-RDA (i.e., gross effects). The highest gross effects were found in Al_i, conductivity, pH, water level manipulation and fish stock (Tab. 3). The four variables

Table 3. Results of db-RDA analysis performed on the whole dataset, showing percentage values of adjusted explained variation, variance explained by each variable separately (gross effect), variance explained by each variable using all other variables from the final model, and partial models (chemical or littoral variables) as conditions (pure effect). The order of selection for individual variables (using the forward selection method) is in brackets.

	Gross effect	Pure effect in the final model (order of selection)	Pure effect in partial model
Chemical variables			
Al_i	10.6^{***}	4.4^{**} (1)	4.4^{***}
Conductivity	10.3^{***}	2.6^{*} (4)	4.0^{**}
pH	9.5^{***}	3.7^{**} (3)	4.3^{**}
DOC	6.2^{**}	1.7^{ns} (2)	7.5^{***}
P_t	5.7^{**}	-	-
O_2 saturation	4.6^{**}	-	-
Water temperature	2.7	-	-
NO_3-N	1.4	-	-
Littoral variables			
Water level manipulation	13.9^{***}	4.2^{**} (1)	12.7^{***}
Organic substrate	6.9^{***}	2.1^{*} (2)	5.7^{***}
Mosses	6.9^{***}	-	-
Vegetation belt	6.4^{**}	-	-
Sedges	5.1^{**}	-	-
Water depth	2.5	-	-
Fish stock	8.2^{***}	1.9^{ns}	-

*Significance based on a 9999-permutation test: ***$p<0.001$; **$p<0.01$; *$p<0.05$; $^{ns}p>0.05$.*

chosen by forward selection for the water chemistry model (Al_i, DOC, pH, conductivity) explained 26.1% of total variance. Pure effects of Al_i, pH and conductivity were all significant ($p<0.05$) in the final model (Tab. 3). The model including littoral variables explained 19.6% of total variance, with significant ($p<0.05$) pure effects of water level manipulation and organic substrate included in the model. Fish stock explained 8.2% of total variance and its pure effect was marginally non-significant (1.9%, $p=0.06$). The three partial explanatory models together explained 34.7% of total variance, with a relatively large proportion of the explained variation shared among the models. The pure effect of water chemistry (9.8%) was higher than that of the littoral variables (7.3%) (Fig. 4), though considerable variation was shared by the two groups (12.9%), probably associated with the shared effect of DOC (not significant in the final model) with organic substrate and the higher pH and conductivity of waterbodies with manipulated water levels (Tab. S3). The effect of fish stock was almost completely shared with that of water chemistry (6.9%) owing to the absence of fish in strongly acidic waterbodies.

Relationships between species richness and abundance with environmental variables

When using the whole dataset, macroinvertebrate species richness increased significantly ($p<0.01$) with width of the vegetated littoral belt, sedge vegetation, organic substrate, moss cover, water depth and DOC (Fig. 5, Tab. S4). Relationships of species richness to pH and Al_i concentration were non-significant. When excluding waterbodies influenced by water level manipulation, species richness increased significantly ($p<0.05$) with increasing pH and decreasing Al_i concentration (Fig. 5 E, F), while relationships with some littoral characteristics (sedges, vegetation belt and organic substrate) and DOC were almost the same as for the whole dataset (Fig. 5 A-D). Total macroinvertebrate abundance was negatively related to conductivity, pH and fish stock and positively to Al_i and vegetation belt in the whole dataset, with no significant relationships found when excluding manipulated waterbodies (Tab. S4), due to a significantly lower abundance observed in manipulated waterbodies with higher conductivity and pH (Tab. S3).

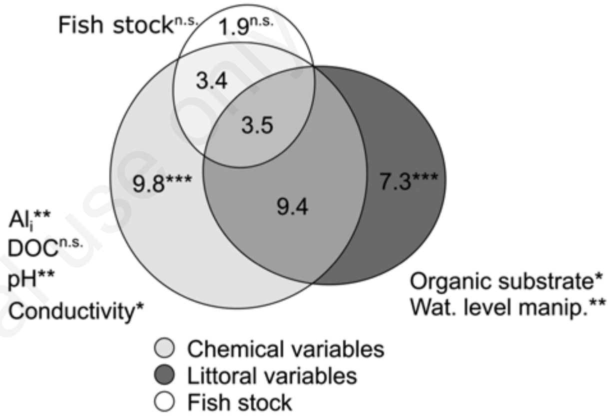

Fig. 4. Partitioning of explained adjusted variation (adj. R^2) in 23 macroinvertebrate assemblages between water chemistry, littoral, and fish stock variables. Chemical and littoral variables in this model were selected by forward selection (Tab. 3). The model explained 34.7 % of total variance. The significance of explained variation for fractions or individual variables is marked with asterisks: $^{***}p<0.001$; $^{**}p<0.01$; $^{*}p<0.05$; $^{n.s.}p>0.05$.

Tab. 4. Mean (+SD) abundance and richness of taxa vulnerable to fish predation in fishless (Laka, JeleJ, Liech MrtvR, VolarR) and fish-containing (PoleN, Tokan, ZdarJ, CernR, StarR) waterbodies. Differences between waterbodies tested using the unparied t-test. Significant differences are in bold.

	Abundance			Species richness		
	Fishless	Fish-containing		Fishless	Fish-containing	
	Mean (SD)	Mean (SD)	p (*t*-test)	Mean (SD)	Mean (SD)	p (*t*-test)
Odonata	51.4 (43)	9.6 (6.2)	0.093	3.4 (0.8)	2.6 (0.5)	0.134
Aeschnidae	19.4 (17.7)	6.4 (3.4)	0.173	1.4 (0.5)	1.8 (0.4)	0.243
Libellulidae	32.0 (44.2)	3.2 (5.9)	0.164	2 (1.1)	0.8 (0.4)	0.094
Nepomorpha	**229.4 (158.1)**	**14.6 (13.3)**	**0.008****	**8.2 (2.6)**	**4.2 (1.5)**	**0.033***
Notonectidae	**31.6 (18.2)**	**4.0 (2.1)**	**0.005****	1.8 (0.4)	1.2 (1)	0.306
Corixidae	**197.8 (158)**	**10.6 (12.5)**	**0.032***	**6.4 (2.2)**	**3.0 (1.4)**	**0.039***
Coleoptera	76 (56.4)	31.6 (13)	0.199	14 (3.6)	13.8 (2.6)	0.931
Gyrinidae	0.6 (1.2)	1.4 (2.8)	0.790	–	–	–
Dytiscidae	75.4 (56.6)	30.2 (15.5)	0.179	13.4 (3.2)	13.4 (2.9)	1.000
Chaoboridae	161 (281.9)	0 (0)	0.188	–	–	–
All groups	**517.8 (242)**	**55.8 (9.6)**	**<0.001*****	**26.2 (3.7)**	**20.6 (2.1)**	**0.037***

$^{***}p<0.001$; $^{**}p<0.01$; $^{*}p<0.05$.

Effect of fish stock on macroinvertebrates

We observed a distinctly higher abundance of macroinvertebrate groups vulnerable to fish predation in fishless waterbodies, with a significant ($p<0.05$) difference in Notonectidae, Corixidae, and all Nepomorpha (Tab. 4). Species richness was slightly higher in Corixidae and Nepomorpha, which contributed to a marginally significant ($p=0.04$) difference when comparing all groups (Tab. 4).

DISCUSSION

Species richness and its environmental correlates

Littoral properties and DOC had a decidedly stronger influence on macroinvertebrate species richness in the littoral zone than acidity (Tab. S4, Fig. 5), with richness being positively related to vegetation belt width and sedge cover, even when including sites with manipulated water levels, where littoral vegetation could temporarily occur above the water. These factors both refer to habitat heterogeneity and size, which are known to be important correlates of diversity in littoral macroinvertebrates (Brown *et al*., 1988; Heino, 2000). Extensive macrophyte stands provide environmental complexity, resources and refuges, allowing them to support highly diverse macroinvertebrate assemblages (Newman, 1991; Tolonen *et al*., 2003; Rennie and Jackson, 2005; Sychra *et al*., 2010). The positive relationship between species richness and DOC is most likely attributable to the complexing of toxic metals by DOM and other positive effects on invertebrate metabolisms in acidic conditions (Hargeby and Petersen, 1988; Gensemer and Playle, 1999), conditions that make humic lakes more favourable for acid-sensitive taxa than clear-water lakes at

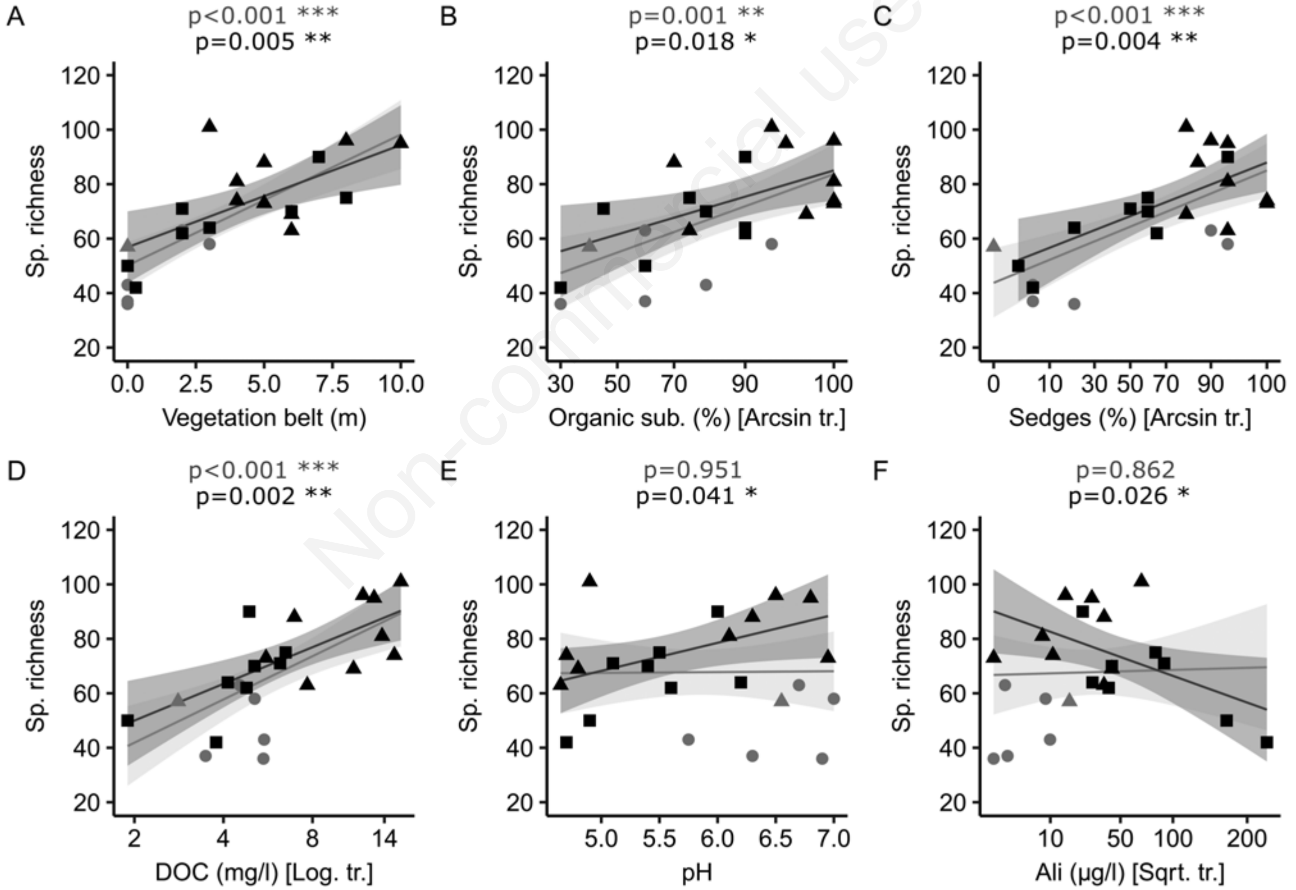

Fig. 5. Relationships between assemblage species richness and selected variables. Each line is the result of simple linear regression. The light line shows the relationship based on the whole dataset, while the dark line shows the relationship based on sites with stable water level only (shaded areas represent 95% confidence intervals). Axes transformations used are in square brackets. Symbols represent the different types of sites: black = sites with stable water levels, grey = sites with manipulated water levels; squares = lakes, triangles = ponds, circles = reservoirs. P-values from testing the significance of simple linear regression models are given for the whole dataset (in grey) and for sites with stable water levels (in black). All relationships between species richness and abundance with the different variables are given in Tab. S4. ***p<0.001; **p<0.01; *p<0.05.

a given pH (Schartau *et al*., 2008). The positive effects of humic conditions and vegetation on species richness were collinear to some extent as vegetation belt width and sedge cover were positively correlated ($R_s = 0.6$) with DOC concentration. Consequently, influences of these variables on macroinvertebrates were joined.

Owing to its interplay with other factors, the relationship between pH and species richness was weak (Fig. 5E). High variability in species richness at pH >6 was related to the adverse effect of water level manipulation at some sites. Species richness in lakes with lower pH was influenced by aluminium toxicity (Fig. 5F), which constrains biotic recovery (Vrba *et al*., 2006, 2016), and littoral structure, which limited habitat availability for some invertebrates.

Overall, strongly acidic and circum-neutral humic ponds supported the most species-rich macroinvertebrate assemblages, including several rare and threatened species, e.g. the mayfly *Arthroplea congener*; the water bugs *Notonecta obliqua*, *Cymatia bonsdorffii* and *Sigara scotti* and the aquatic beetles *Hydrovatus cuspidatus*, *Ilybius crassus*, *Laccobius obscuratus* and *L. ytenensis*. These waterbodies are likely to contribute significantly to regional biodiversity in low mountains in a similar manner as those in small high-mountain ponds above the timberline (Martínez-Sanz *et al*., 2012; Novikmec *et al*., 2015). Though they are artificial, their original use as water storage for timber floating and tin mines, and for fish farming ceased many decades ago and they have now turned into near-natural habitats. Further, owing to their low retention time, they recovered rapidly from acidification following the reduction in anthropogenic acid deposition (cf. Vrba *et al*., 2016). As such, these ponds could serve as refugia and/or as a source of colonisers for chronically acidified lakes or reservoirs in the region.

Effect of water level manipulation on littoral assemblages

Water level manipulation has the potential to alter sediment dynamics, habitat structure and littoral zone stability and, as a result, affects the diversity and composition of biotic communities (Wantzen *et al*., 2008). Macroinvertebrates dependent on organic substrates and vegetation attached to the substrate, and long-lived species are vulnerable to fluctuations in water level (Hynes, 1961; Furey *et al*., 2006; Aroviita and Hämäläinen, 2008), especially in shallow littoral zones where even small changes can affect large areas (Leira and Cantonati, 2008). In this study, water level fluctuations prevented stable conditions needed for the development of littoral vegetation stands. Moreover, suitable patches of littoral vegetation and coarse organic substrate were temporarily above the water level, hence, not available for aquatic invertebrates. This resulted in a lowered species richness and abundance (Tab. S3) and a dissimilar assemblage composition compared to stable waterbodies (Fig. 3), primarily due to a decline in phytophilous species (mostly from the orders Odonata, Trichoptera and Diptera) and swimming insects (Heteroptera, Coleoptera), which prefer the interior of macrophyte stands and use them as refuges from predation (Nilsson *et al*., 1994; Fairchild *et al*., 2000; Nosek *et al*., 2007). While manipulated waterbodies hosted 50 % of all taxa recorded, most occurred infrequently and at low abundance, resulting in increased dissimilarity between assemblages in unstable littoral zones. As such, it is difficult to identify common indicative taxa for water level manipulation as the assemblages are often defined by missing, rather than present, species (Aroviita and Hämäläinen, 2008). Typical taxa in unstable littorals were small gatherer/collectors and filter feeders able to use fine substrate as habitat and food (e.g., *P. casertanum*, *C. horaria*, and *Cricotopus sylvestris* Gr.), and Trichoptera that build cases from mineral particles (*M. angustata, Mystacides longicornis, Oecetis lacustris, O. ochracea*). Inorganic substrates were locally abundant at some sites and these supported some silicophilous or rhitral aquatic beetles, such as *N. elegans*, *Stictotarsus duodecimpustulatus* or *Oreodytes sanmarkii*.

Disentangling the effects structuring macroinvertebrate assemblages

Major compositional gradients in species data were associated with acidity, littoral characteristics (including water level fluctuation) and fish stock (Fig. 3). The effects were, to some extent, collinear; hence, variability among predictor groups was considerable. Nevertheless, we found a higher pure effect of water chemistry (pH, Al_i and conductivity significant) than littoral characteristics (Fig. 4). The effect of fish stock was confounded by its correlation with acidity, which resulted in high shared variability and a non-significant pure effect of fish stock on variation partitioning (Fig. 4, Tab. 3). Species composition showed predictable responses along the acidity gradient, however, though with considerable variability (Fig. 3) because of differing littoral structure (and correlated DOC) and aluminium toxicity.

Three chronically acidified lakes with high Al_i concentration (Černé, Čertovo and Prášilské) were dissimilar to the remaining acidic sites (Fig. 3) as they hosted species-poor assemblages, characteristic of strong acid-stress and reduced interspecific competition (Økland and Økland, 1986; Appelberg *et al*., 1993), and were dominated by a few acid-tolerant species (e.g., *Leptophlebia vespertina*, *N. cinerea*, *M. azurea*, *C. villosa*, *Macropelopia* sp., and *Phaenopsectra* sp.). However, their dissimilarity to other highly acidic sites was also associated with the absence of large sedge stands and mosses, resulting in a loss of phytophilous species and an increase in invertebrates preferring sandy substrates without vegetation, including the diving beetle *Nebrioporus assimilis* and the caddisfly *Molanna nigra*, both very rare species in Central Europe (Weinzierl, 1999; Soldán *et al*., 2012). As such, it was not possible to separate the effects of high Al_i and limited littoral vegetation at such sites.

At acidic sites with low Al_i concentrations, macroinvertebrate assemblages were structured by available littoral substrate, independent of pH (Fig. 3), and were arranged along a gradient comprising littorals with significant amounts of inorganic (fine sandy or coarse stony) substrate and partial sedge cover to humic sites with completely organic substrates and rich sedge and moss cover. This was reflected in the significant pure effect of organic substrate (positively correlated with vegetation) in variation partitioning (Fig. 4, Tab. 3). Such sites were typified by acid-tolerant species such as *C. hastulatum*, *A. juncea*, *Sigara semistriata*, *N. crassicornis* and *H. dubius*, with acid-sensitive species absent or very rare.

Assemblage variability in the circum-neutral section of the pH gradient was mostly associated with water level manipulation and its effect on littoral characteristics, which contributed to the high variability shared between water chemistry and littoral characteristics (Fig. 4). Several acid-sensitive or moderately acid-sensitive species (e.g., the mayflies *A. congener*, *C. horaria* and *Cloeon dipterum*, the caddisfly *Anabolia nervosa* and the water beetle *Hydraena britteni*) were recorded at such sites, though always at low abundance. As the db-RDA results showed, circum-neutral assemblages can also be affected by fish predation (Tab. 3), with stronger predation pressure expected in littoral zones with no or only sparse vegetation (Gilinsky, 1984; Diehl, 1992). On the other hand, such littorals were also affected by water level manipulation, which resulted in lowered presence of invertebrates that could serve as fish prey, i.e., large non-burrowing, free-swimming invertebrates (Knapp *et al*., 2001; Schilling *et al*., 2009; Tiberti *et al*., 2014). To clarify this, we assessed the effect of fish predation in waterbodies with stable water levels and rich littoral vegetation, focusing on the abundance and richness of macroinvertebrates vulnerable to predation (according to Schilling *et al*. 2009). Unlike Schilling *et al*. (2009), we only recorded a significantly lower abundance and richness in water bugs, presumably as they are more vulnerable to predation, and profit more from the absence of fish, than aquatic beetles and dragonflies, with show a stronger affinity to vegetation (Henrikson and Oscarson, 1985; Bendell and McNicol, 1995; Corbet, 1999). Nymphs and adults of the genus *Notonecta*, and several corixids such as *Glaenocorisa propinqua*, are predominantly pelagic and/or use pelagic habitats more often when fish are absent (Henrikson and Oscarson, 1985). Moreover, nymphs of the corixid genus *Sigara* can be heliophilous, preferring open water more than the adults (Teyrovský, 1956; Sychra *et al*., 2010). Other groups of predation-vulnerable invertebrates contributed to the higher total abundance of vulnerable invertebrates in fishless waterbodies, but not to the (slightly) higher species richness (Table 4), indicating a significant effect of fish predation independent of acidity, though with a minor influence on assemblage composition (Fig. 3, Tab. 3, Tab. S4). This suggest that the return of fish to recovering lakes may not lead to substantial changes in littoral macroinvertebrate species composition where the availability of dense and heterogeneous vegetation protects the invertebrates from predation (Tolonen *et al*., 2003).

CONCLUSIONS

Our study showed that littoral macroinvertebrate assemblages in acidified waterbodies are structured by complex effects of local factors interacting with acidification. Considerable assemblage variability along the acidity gradient was associated with heterogeneous local habitat properties and water chemistry (particularly Al_i and DOC concentration). The effects of acidification were primarily modified by water level manipulation, which resulted in seriously impoverished macroinvertebrate assemblages and significant dissimilarity to those of non-manipulated sites, overriding the effects of more favourable slightly acidic to near neutral conditions. Acid-stressed communities were diversified by heterogeneous littoral structure (substrate and vegetation), even though they were predominated by acid-tolerant species. Chronically acidified lakes with a high Al_i concentration and very sparse littoral vegetation were inhabited by species-poor assemblages, characteristic for strong acid-stress. In contrast, humic sites, usually with dense and heterogeneous littoral vegetation, were the most species-rich and diverse, hosting numerous habitat specialists and rare species. The interactive effects of Al toxicity and lack of littoral vegetation, as well as high DOC and a rich littoral organic substrate, could not be disentangled and resulted in high shared effects of water chemistry and littoral characteristics in the variation partitioning. Likewise, the effect of fish stock was collinear with pH. Nevertheless, we observed an apparent decline in the abundance of macroinvertebrates vulnerable to fish predation, especially water bugs using open-water habitats. Based on these results, we emphasise the need to analyse the influence of local habitat factors when evaluating the impact of acidification on macroinvertebrates.

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REFERENCES

Appelberg M, Henrikson BI, Henrikson L, Svedäng M, 1993. Biotic interactions within the littoral community of Swedish forest lakes during acidification. Ambio 22:290-297.

Ardö J, Lambert N, Henzlik V, Rock BN, 1997. Satellite-based estimations of coniferous forest cover changes: Krusne Hory, Czech Republic 1972-1989. Ambio 26:158-166.

Aroviita J, Hämäläinen H, 2008. The impact of water-level regulation on littoral macroinvertebrate assemblages in boreal lakes. Hydrobiologia 613:45-56.

Bendell BE, McNicol DK, 1995. Lake acidity, fish predation, and the distribution and abundance of some littoral insects. Hydrobiologia 302:133-145.

Blanchet FG, Legendre P, Borcard D, 2008. Forward selection of explanatory variables. Ecology 89:2623-2632.

Bridges KS, Jickells TD, Davies TD, Zeman Z, Hunova I, 2002. Aerosol, precipitation and cloud water chemistry observations on the Czech Krusne Hory plateau adjacent to a heavily industrialised valley. Atmos. Environ. 36:353-360.

Brown CL, Poe TP, French JR, Schloesser DW, 1988. Relationships of phytomacrofauna to surface area in naturally occurring macrophyte stands. J. N. Am. Benthol. Soc. 7:129-139.

Cerny J, 1995. Recovery of acidified catchments in the extremely polluted Krusne Hory Mountains, Czech Republic. Water, Air, and Soil Poll. 85:589-594.

Corbet PS, 1999. Dragonflies: Behavior and Ecology of Odonata. Harley, Colchester: 829 pp.

Dangles O, Malmqvist B, Laudon H, 2004. Naturally acid freshwater ecosystems are diverse and functional: evidence from boreal streams. Oikos 104:149-155.

Diehl S, 1992. Fish predation and benthic community structure: the role of omnivory and habitat complexity. Ecology 73:1646-1661.

Eliassen A, Hov O, Iversen T, Saltbones J, Simpson D, 1988. Estimates of airborne transboundary transport of sulphur and nitrogen over Europe, p. 79. In: EMEP/MSC-W Report 1/88. The Norwegian Meteorological Institute, Oslo.

Fairchild GW, Faulds AM, Matta JF, 2000. Beetle assemblages in ponds: effects of habitat and site age. Freshwater Biol. 44:523-534.

Farmer AM, 1990. The effects of lake acidification on aquatic macrophytes – a review. Environ. Pollut. 65:219-240.

Furey PC, Nordin RN, Mazumder A, 2006. Littoral benthic macroinvertebrates under contrasting drawdown in a reservoir and a natural lake. J. N. Am. Benthol. Soc. 25:19-31.

Gensemer RW, Playle RC, 1999. The bioavailability and toxicity of aluminum in aquatic environments. Crit. Rev. Environ. Sci. Technol. 29:315-450.

Gilinsky E, 1984. The role of fish predation and spatial heterogeneity in determining benthic community structure. Ecology 65:455-468.

Goral F, Schellenberg J, 2018. goeveg: Functions for Community Data and Ordinations. R package version 0.4.2. https://CRAN.R-project.org/package=goeveg.

Grahn O, Hultberg H, Landner L, 1974. Oligotrophication – a self-accelerating process in lakes subjected to excessive supply of acid substances. Ambio 3:93-94.

Grübler A, 2002. Trends in global emissions: carbon, sulphur, and nitrogen, p. 35-53. In: I. Douglas (ed.), Encyclopedia of Global Environmental Change. Volume 3. John Wiley & Sons, Chichester.

Hargeby A, Petersen Jr RC, 1988. Effects of low pH and humus on the survivorship, growth and feeding of *Gammarus pulex* (L.) (Amphipoda). Freshwat. Biol. 19:235-247.

Harrell Jr. FE with contributions from Charles Dupont and many others, 2019. Hmisc: Harrell Miscellaneous. R package version 4.2-0. https://CRAN.R-project.org/package=Hmisc.

Heino J, 2000. Lentic macroinvertebrate assemblage structure along gradients in spatial heterogeneity, habitat size and water chemistry. Hydrobiologia 418:229-242.

Henrikson L, Oscarson HG, 1985. Waterbugs (Corixidae, Hemiptera-Heteroptera) in acidified lakes: Habitat selection and adaptations. Ecol. Bull. 37:232-238.

Hildrew A, 2018. Freshwater acidification. Natural history, ecology and environmental policy. Excellence in ecology 27. International Ecology Institute, Oldendorf: 194 pp.

Hynes HBN, 1961. The effect of water-level fluctuations on littoral fauna. Verh. Internat. Verein. Limnol. 14:652-656.

Johnson RK, Goedkoop W, Fölster J, Wilander A, 2007. Relationships between macroinvertebrate assemblages of stony littoral habitats and water chemistry variables indicative of acid-stress. Water Air Soil Pollut.: Focus 7:323-330.

Johnson RK, Hallstan S, Zhao X, 2018. Disentangling the response of lake littoral invertebrate assemblages to multiple pressures. Ecol. Indic. 85:1149-1157.

Knapp RA, Matthews KR, Sarnelle O, 2001. Resistance and resilience of alpine lake fauna to fish introductions. Ecol. Monogr. 71:401-421.

Kolář T, Čermák P, Oulehle F, Trnka M, Štěpánek P, Cudlín P, Hruška J, Büntgen U, Rybníček M, 2015. Pollution control enhanced spruce growth in the “Black Triangle” near the Czech–Polish border. Sci. Total Environ. 538:703-711.

Kopáček J, Posch M, Hejzlar J, Oulehle F, Volková A, 2012. An elevation-based regional model for interpolating sulphur and nitrogen deposition. Atmos. Environ. 50:287-296.

Kopáček J, Procházková L, Stuchlík E, Blažka P, 1995. The nitrogen phosphorus relationship in mountain lakes: influence of atmospheric input, watershed, and pH. Limnol. Oceanogr. 40:930-937.

Kopáček J, Veselý J, 2005. Sulfur and nitrogen emissions in the Czech Republic and Slovakia from 1850 till 2000. Atmos. Environ. 39:2179-2188.

Křeček J, Hořická Z, 2001. Degradation and recovery of mountain watersheds: the Jizera Mountains, Czech Republic. Unasylva 207:43-49.

Křeček J, Palán L, Stuchlík E, 2017. Acid atmospheric deposition in a forested mountain catchment. iForest 10:680–686.

Larsson J, 2019. eulerr: Area-Proportional Euler and Venn Diagrams with Ellipses. R package version 5.1.0. https://cran.r-project.org/package=eulerr.

Layer K, Hildrew A, Monteith D, Woodward G, 2010. Long-term variation in the littoral food web of an acidified mountain lake. Glob. Chang. Biol. 16:3133-3143.

Legendre P, Anderson MJ, 1999. Distance-based redundancy analysis: testing multispecies responses in multifactorial ecological experiments. Ecol. Monogr. 69:1-24.

Leira M, Cantonati M, 2008. Effects of water-level fluctuations on lakes: an annotated bibliography. Hydrobiologia 613:171-184.

Lento J, Dillon PJ, Somers KM, Reid RA, 2008. Changes in littoral benthic macroinvertebrate communities in relation to water chemistry in 17 Precambrian Shield lakes. Can. J. Fish. Aquat. Sci. 65:906-918.

Martínez-Sanz C, Cenzano CSS, Fernández-Aláez M, García-Criado F, 2012. Relative contribution of small mountain ponds to regional richness of littoral macroinvertebrates and the implications for conservation. Aquatic Conserv: Mar. Freshw. Ecosyst. 22:155-164.

McFarland B, Carse F, Sandin L, 2010. Littoral macroinvertebrates as indicators of lake acidification within the UK. Aquatic Conserv: Mar. Freshw. Ecosyst. 22(S1):105-116.

Newman RM, 1991. Herbivory and detritivory on freshwater macrophytes by macroinvertebrates: a review. J. N. Am. Benthol. Soc. 10:89-114.

Nilsson AN, Elmberg J, Sjöberg K, 1994. Abundance and species richness patterns of diving beetles (Coleoptera, Dytiscidae) in Swedish lakes. J. Biogeogr. 21:197-206.

Nosek JN, Vásárhelyi T, Bakonyi G, Oertel N, 2007. Spatial pattern of water bugs (Nepomorpha, Gerromorpha) at different scales in the Szigetköz (Hungary). Biologia 62:345-350.

Novikmec M, Veselská M, Bitušík P, Hamerlík L, Matúšová Z, Reduciendo Klementová B, Svitok M, 2015. Checklist of benthic macroinvertebrates of high altitude ponds of the Tatra Mountains (Central Europe) with new records of two species for Slovakia. Check List 11:1522.

Økland J, Økland KA, 1986. The effects of acid deposition on benthic animals in lakes and streams. Experientia 42:423-432.

Oksanen J, Blanchet FG, Friendly M, Kindt R, Legendre P, McGlinn D, Minchin PR, O'Hara RB, Simpson GL, Solymos P, Stevens MHH, Szoecs E, Wagner H, 2019. vegan: Community Ecology Package. R package version 2.5-5. https://CRAN.R-project.org/package=vegan.

Oulehle F, Hruška J, 2009. Rising trends of dissolved organic matter in drinking-water reservoirs as a result of recovery from acidification in the Ore Mts., Czech Republic. Environ. Pollut. 157:3433-3439.

Paradis E, Schliep K, 2019. ape 5.0: an environment for modern phylogenetics and evolutionary analyses in R. Bioinformatics 35:526-528.

Peres-Neto PR, Legendre P, Dray S, Borcard D, 2006. Variance partitioning of species data matrices: estimation and comparison of fractions. Ecology 87:2614-2625.

Peterka J, Čech M, Draštík V, Frouzová J, Jankovský M, Muška M, Prchalová M, 2009. Průzkum rybí obsádky nádrže Fláje v roce 2008. Biologické centrum AV ČR, v.v.i., Hydrobiologický ústav, České Budějovice: 14 pp.

R Core Team, 2019. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. https://www.R-project.org/

Rennie MD, Jackson LJ, 2005. The influence of habitat complexity on littoral invertebrate distributions: patterns differ in shallow prairie lakes with and without fish. Can. J. Fish. Aquat. Sci. 62:2088-2099.

Roberts DW, 2019. labdsv: Ordination and Multivariate Analysis for Ecology. R package version 2.0-1. https://CRAN.R-project.org/package=labdsv.

Šanda R, Švátora M, Vukič J, Hořická Z, Sychrová O, Marič S, 2015. Evaluation of the temporal development of three introduced populations of brook trout, *Salvelinus fontinalis*, in Jizerské mountains, Czech Republic. Arch. Biol. Sci. 67:775-784.

Schartau AK, Moe SJ, Sandin L, McFarland B, Raddum GG, 2008. Macroinvertebrate indicators of lake acidification: analysis of monitoring data from UK, Norway and Sweden. Aquatic Ecol. 42:293-305.

Schilling EG, Loftin CS, Huryn AD, 2009. Macroinvertebrates as indicators of fish absence in naturally fishless lakes. Freshwat. Biol. 54:181-202.

Soldán T, Bojková J, Vrba J, Bitušík P, Chvojka P, Papáček M, Peltanová J, Sychra J, Tátosová J, 2012. Aquatic insects of the Bohemian Forest glacial lakes: Diversity, long-term changes, and influence of acidification. Silva Gabreta 18:123-283.

Stuchlík E, Hořická Z, Prchalová M, Křeček J, Barica J, 1997. Hydrobiological investigation of three acidified reservoirs in the Jizera Mountains, the Czech Republic, during the summer stratification. Can. Tech. Rep. Fish. Aquat. Sci. 2155:56-64.

Sychra J, Adámek Z, Petřivalská K, 2010. Distribution and diversity of littoral macroinvertebrates within extensive reed beds of a lowland pond. Ann. Limnol. 46:281-289.

Teyrovský V, 1956. [Fotopathie larev klešťanek (Corixinae)].[Article in Czech]. Sborník Vysoké školy pedagogické v Olomouci, Přírodní vědy 2:147-177.

Tiberti R, von Hardenberg A, Bogliani B, 2014. Ecological impact of introduced fish in high altitude lakes: a case of study from the European Alps. Hydrobiologia 724:1-19.

Tolonen KT, Hämäläinen H, Holopainen IJ, Mikkonen K, Karjalainen J, 2003. Body size and substrate association of littoral insects in relation to vegetation structure. Hydrobiologia 499:179-190.

Vašát R, Pavlů L, Borůvka L, Tejnecký V, Nikodem A, 2015. Modelling the impact of acid deposition on forest soils in North Bohemian Mountains with two dynamic models: the Very Simple Dynamic Model (VSD) and the Model of Acidification of Groundwater in Catchments (MAGIC). Soil & Water Res. 10:10-18.

Vrba J, Bojková J, Chvojka P, Fott J, Kopáček J, Macek M, Nedbalová L, Papáček M, Rádková V, Sacherová V, Soldán T, Šorf M, 2016. Constraints on the biological recovery of the Bohemian Forest lakes from acid stress. Freshwat. Biol. 61:376-395.

Vrba J, Kopáček J, Bittl T, Nedoma J, Štrojsová A, Nedbalová L, Kohout L, Fott J, 2006. A key role of aluminium in phosphorus availability, food web structure, and plankton dynamics in strongly acidified lakes. Biologia 61:S441-S451.

Vrba J, Kopáček J, Fott J, Kohout L, Nedbalová L, Pražáková

M, Soldán T, Schaumburg J, 2003. Long-term studies (1871–2000) on acidification and recovery of lakes in the Bohemian Forest (central Europe). Sci. Total Environ. 310:73-85.

Wantzen KM, Rothhaupt KO, Mörtl M, Cantonati M, Tóth LG, Fischer P, 2008. Ecological effects of water-level fluctuations in lakes. Springer, Dordrecht: 184 pp.

Weinzierl A, 1999. [Neues über *Molanna nigra* und einige seltenere Leptoceridae aus Bayern (Insecta: Trichoptera)].[Article in German]. Lauterbornia 36:9-12.

Wesolek BE, Genrich EK, Gunn JM, Somers KM, 2010. Use of littoral benthic invertebrates to assess factors affecting biological recovery of acid- and metal-damaged lakes. J. N. Am. Benthol. Soc. 29:572-585.

Wickham H, 2016. ggplot2: Elegant Graphics for Data Analysis (2nd Edition). Springer-Verlag, New York: 260 pp.