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How to Assess the Ecological Status of Highly Humic Lakes? Development of a New Method Based on Benthic Invertebrates

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Abstract: Highly humic lakes are typical for the boreal zone. These unique ecosystems are characterised as relatively undisturbed habitats with brown water, high acidity, low nutrient content and lack of macrophytes. Current lake assessment methods are not appropriate for ecological assessment of highly humic lakes because of their unique properties and differing human pressures acting on these ecosystems. This study proposes a new approach suitable for the ecological status assessment of highly humic lakes impacted by hydrological modifications. Altogether, 52 macroinvertebrate samples from 15 raised bog lakes were used to develop the method. The studied lakes are located in the raised bogs at the central and eastern parts of Latvia. Altered water level was found as the main threat to the humic lake habitats since no other pressures were established. A multimetric index based on macroinvertebrate abundance, littoral and profundal preferences, Coleoptera taxa richness and the Biological Monitoring Working Party (BMWP) Score is suggested as the most suitable tool to assess the ecological quality of the highly humic lakes.

Keywords: highly humic lakes; macroinvertebrates; ecological status assessment

Citation: Ozoliņš, D.; Skuja, A.; Jēkabsone, J.; Kokorite, I.; Avotins, A.; Poikane, S. How to Assess the Ecological Status of Highly Humic Lakes? Development of a New Method Based on Benthic Invertebrates. *Water* **2021**, *13*, 223. https://doi.org/10.3390/w13020223

Received: 7 December 2020
Accepted: 14 January 2021
Published: 18 January 2021

1. Introduction

Humic lakes, also known as brown-water lakes, are typical for the boreal zone, located between 50° to 70° N latitude. These ecosystems are characterized by dark water colour, low water transparency, and low pH caused by the high concentration of dissolved organic matter (DOM), mostly originating from the catchment area and consisting of refractory humic substances [1,2]. Over the last decades, an impressive body of evidence has been accumulated which suggests that DOM is a major modulator of the structure and function of lake ecosystems, affecting numerous features such as light regime, thermal stratification, nutrient availability, primary production, and microbial metabolism [3–6]. Numerous studies have shown that the biological communities differ considerably from those of clear-water lakes (phytoplankton: [7]; macrophytes: [8]; periphyton: [9]; zooplankton: [10]; fish fauna: [11]). Moreover, their response to human stressors might differ too [12–14], asking for monitoring and assessment approaches different to those used for clear-water lakes [15,16].

Multiple human pressures, such as nutrient enrichment, hydrological and morphological alterations, invasion of non-native species and climate change, affect humic lakes [17,18]. Some of these pressures are similar to those impacting clear water lakes, but some are different, e.g., bog lakes are affected by artificial peatland drainage and peat extraction associated with habitat degradation, erosion, increased leaching of nutrients and dissolved organic carbon [19,20]. Similarly, peatland lakes are impacted by forestry practices (afforestation, fertilization, and clear-cutting) which have been shown to increase catchment loadings of nutrients, sediments and dissolved organic carbon (DOC) to these ecosystems [21–23]. These impacts can have a profound effect on the water quality, lake habitats, associated biological assemblages and conservation value of these lakes [22,24,25].

In Europe, the Water Framework Directive (WFD) [26] establishes a framework for the protection of inland and coastal waters. According to the WFD, lakes have to be classified into five status classes (high, good, moderate, poor and bad) based on four biological quality elements (BQEs)—phytoplankton, benthic flora, benthic invertebrates, and fish fauna. In addition, physico-chemical elements (e.g., nutrient conditions) and hydromorphological elements (e.g., flow conditions) are used to support ecological classification. The European Union (EU) member states have to identify degraded water bodies (i.e., less than good status) and to establish programmes of measures for each river basin district to reduce significant anthropogenic pressures and achieve good water status.

A large number of lake assessment methods have been intercalibrated and included in the European countries' monitoring toolkits [27]. In the recent years, all of these methods have been intercalibrated (i.e., compared and harmonized) among the EU member states [28,29]. Lake assessment methods include both primary producers, e.g., phytoplankton (e.g., [30,31]), macrophytes (e.g., [32,33]), phytobenthos (e.g., [34]), and heterotrophs such as benthic invertebrates and fish fauna (e.g., [33,35,36]).

However, two problems are still overlooked. At first, the majority of assessment systems target nutrient enrichment, while other key pressures are largely neglected. This is especially true regarding hydromorphological pressures, which affect a considerable number of lakes across Europe [37]. Only few assessment systems tackle the ecological effects of these pressures (shore degradation: [38]; water level fluctuations: [39]) and only two of these systems have been intercalibrated among member states [36].

Second, despite the well-known differences among clear and brown-water lakes [1], the current lake assessment systems are adopted mostly for clear-water lakes. Recently, several studies have raised the issue that assessment systems might not be appropriate for humic lake assessment [13,17,40]. Hence, there is an imperative need for the development of appropriate assessment methods targeting humic lakes.

Studies on macroinvertebrates in highly humic lakes are mainly focused either on biodiversity [41,42] or specific taxonomic groups, e.g., chironomids [43] or Coleoptera [44]. Raised bog water bodies are also known as habitats for rare and protected macroinvertebrate species [41].

Mires and bogs cover 4.9% of the territory of Latvia [45] ranking Latvia number 9 by the total area of peatlands among all the European countries [46]. Bog lakes are listed as protected habitats within the EU Habitats Directive emphasizing their high conservation value [47]. However, these lakes have been impacted by a range of anthropogenic activities, most importantly anthropogenic drainage and peat harvesting which can lead to water level fluctuations, loss of biodiversity and degradation of the lake ecological status [48,49]. Nevertheless, the effects of these impacts are poorly understood and there are no assessment tools in place to assess the ecological condition of humic lakes. According to the River Basin Management Plans (RBMPs) of Latvia, current methods reflect bog lakes at poor or bad status, though the anthropogenic pressures are irrelevant [50]. As a result, there is a need to develop new methods for the ecological assessment of the highly humic lakes.

The objectives of this study are (i) to explore littoral benthic invertebrate community response to hydrological alterations in highly humic lakes; (ii) to develop a biotic index for assessing hydrological alterations in these aquatic ecosystems.

2. Materials and Methods

2.1. Study Sites

Altogether 15 highly humic lakes were studied at seven raised bogs comprising the national monitoring data from the Latvian Environmental, Geology and Meteorology Centre and studies from the Institute of Biology, University of Latvia. The lakes were divided into two groups: lakes with altered water level due to drainage and lakes with natural or restored hydrological regime (see Figure 1).

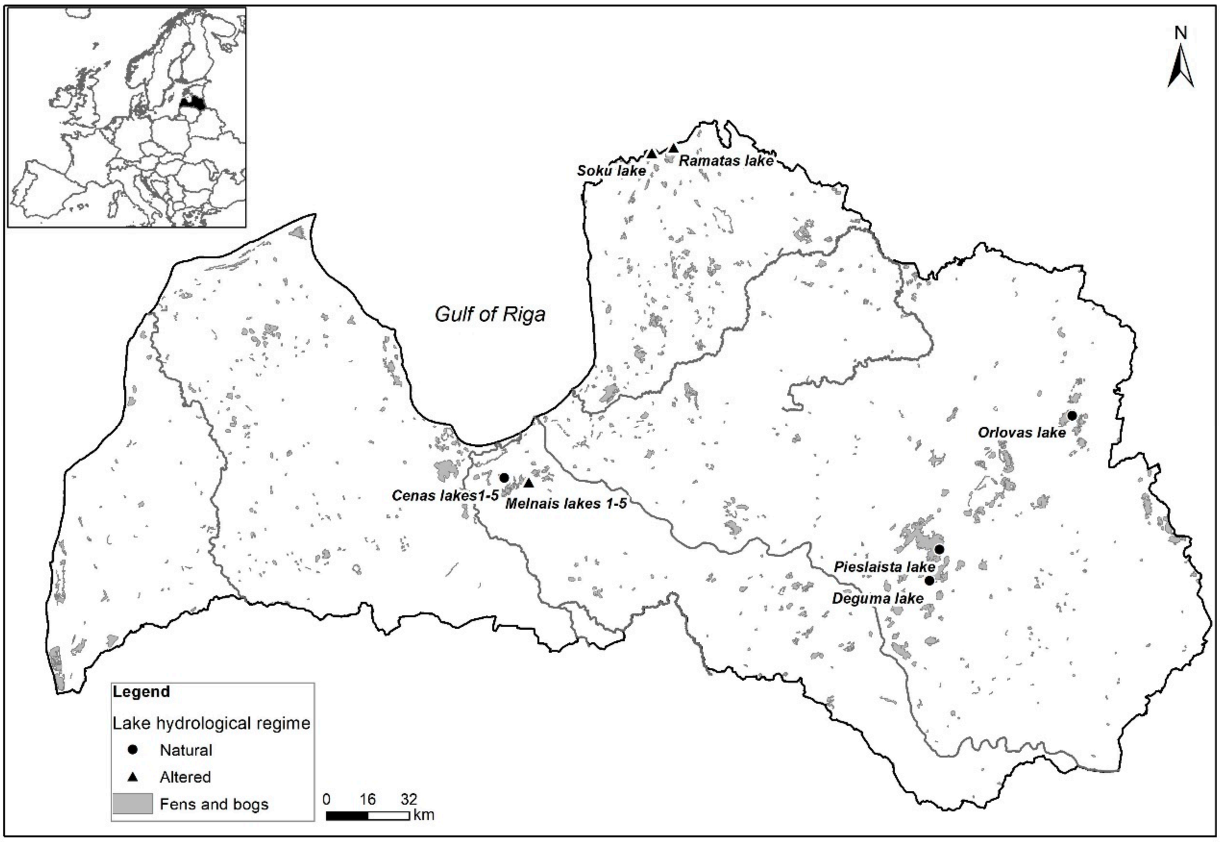


Figure 1. The macroinvertebrate sampling sites and distribution of fens in Latvia. Black triangles: highly humic lakes with altered water level; black dots: highly humic lakes with natural or restored water level.

All of the studied lakes are located in the territories included in the Natura 2000 network. In the central part of Latvia, Cena Mire and Melnais Lake Mire are represented by the samples from five small bog lakes each. In the Cena Mire, lakes are located in pristine areas, while in the Melnais Lake Mire—close to the peat excavation fields, thus representing a hydrologically disturbed state. In the northern part of Latvia, sampling was conducted in Lake Ramatas Lielezers and Lake Soku, both being with altered water level due to outflowing drainage ditches. The water level was receded at these lakes with visible open peat outcrops at the shoreline (see Figure 2).

Eastern part of Latvia is represented by three lakes (Pieslaista, Deguma and Orlovas) that have an unaltered or restored hydrological regime. The water level at these lakes was natural and not affected due to the drainage ditches or peat excavation (see Figure 3).

Figure 2. Lake Soku with receded water level and visible open peat outcrops at the shoreline in May 2019.

Figure 3. Lake Deguma with natural water level and mire vegetation at the shoreline in May 2019.

2.2. Physical and Chemical Parameters

Waterbodies of Melnais Lake Mire and Cena Mire were sampled in May 2015 and analyses were conducted at the Laboratory of Soils of the University of Latvia. Soku, Ramatas Lielezers, Deguma, Pieslaista and Orlovas lakes were sampled four times in different seasons in 2017 and analysis was conducted at the Laboratory of Latvian Environment, Geology and Meteorology Centre. In 2015, pH and electric conductivity (EC) were measured in-situ by using a portable pH tester (HI 98127, HANNA instruments, Sarmeola di Rubano, Italy) and conductivity tester (The Original Dist HI 98300, HANNA instruments, Sarmeola di Rubano, Italy). In 2017, these parameters were measured in-situ by using a portable probe (HQ40d, Hach Companies, Loveland, CO, USA). Total phosphorus (TP) was detected by ascorbic acid method after the digestion using potassium persulfate. Total nitrogen (TN) samples were digested by potassium persulfate, then nitrates were reduced to nitrites in a Cd column and analysed spectrophotometrically. Water colour was analysed spectrophotometrically using the Pt/Co scale [51].

2.3. Macroinvertebrate Sampling and Sample Processing

In larger lakes, sampling was conducted in a 100 m long, representative shoreline section, while in smaller lakes sampling was conducted around all of the shoreline. Benthic macroinvertebrate samples were taken by hand net (frame size 0.25 × 0.25 m, mesh of 0.5 mm) using the sweeping technique. A hand net was placed on the bottom parallel to the shore, if the depth was less than 0.8 m or under the vegetation overhang at the same depth and moved upwards over the vegetation stands to the surface. Five replicates of 0.5 m sweeps were chosen in proportion to habitat types, e.g., of stands of *Menyanthes trifoliata*, *Sphagnum cuspidatum*, *Batrachospermum turfosum*, *Carex* spp., bare littoral, etc. At the small lakes of Melnais Lake Mire and Cena Mire, samples were taken in May 2015. Sampling at Soku, Ramatas Lielezers, Deguma, Pieslaista and Orlovas lakes was conducted in May and October 2017. Additional samples were taken at lakes Soku, Ramatas Lielezers and Deguma in May 2019.

Sampled material was washed through a sieve with a mesh size of 0.5 mm at field. All replicates were placed in the polyethylene containers, labelled and preserved in 70% ethyl alcohol (final concentration).

Preserved samples were washed at the laboratory; all specimens were picked out from the vegetation, detritus and peat particles. Macroinvertebrates were identified to the smallest achievable taxonomical (species, genera) level, excluding Oligochaeta and juvenile Hydrachnidia. Specimens of Diptera were identified to the family level.

2.4. Data Analysis

2.4.1. Selection of Metrics

According to the WFD, the ecological quality assessment indices are required multimetric consisting of composition, abundance, sensitive/tolerant taxa and diversity metrics [26]. Macroinvertebrate metrics were calculated using ASTERICS 4.0.4. software (Wageningen Software Labs, Wageningen, The Netherlands) [52]. Numerically unsuitable metrics and majority of the metrics specific for the lotic habitats were excluded from further analysis. In total, 139 indices describing 52 samples from water bodies in open raised bogs were tested for a selection of multimetric index according to requirements of the EU WFD [26]. We generally followed the procedure described by Hering et al. [53], beginning with the reduction of dimensionality by the evaluation of each metric value distribution between the altered and the natural water bodies.

2.4.2. Sensitivity to Stressor

Only descriptors correlating to the stressor gradient can be used in the development of a multimetric index. We used Mann–Whitney–Wilcoxon U tests (each metric as dependent in groups of stressor, thereinafter U test) together with boxplots and simple binary logistic regression (groups of stressor as dependant: altered = 0, natural = 1, thereinafter binary logistic regression (BLR)) to select only statistically significant metrics for further processing. Metric values prior to BLR were centred by mean and scaled by standard deviation to ease the convergence, while unscaled values were used in the boxplots and U test.

With type I statistical error rate 0.05, the U test returned 66 statistically significant indices and BLR-44 (Table S1). All the indices found significant by BLR were significant also by U test. As a reason for this step is the reduction of dimensionality with selection of potentially most important indices, we did not account for possible false discoveries due to the multiple testing. We included metrics (except life index due to specific relation to lotic environment) found significant by both methods in further investigation of their suitability.

2.4.3. Numerical Suitability

The multimetric index must consist of metrics that tend to describe large gradients with possibly low skew and preferably without outliers [53]. These numeric properties can be the best evaluated graphically if values are from the same or a similar scale. We used the violin (density) plots with a point overlay (with stressor group indicated by a shape) to select one to six metrics per a metric type. Before plotting, each metric was scaled by its observed maximum value. Graphs used in evaluation are provided in Figure 4 with 14 metrics included in the further investigation marked with the dark background.

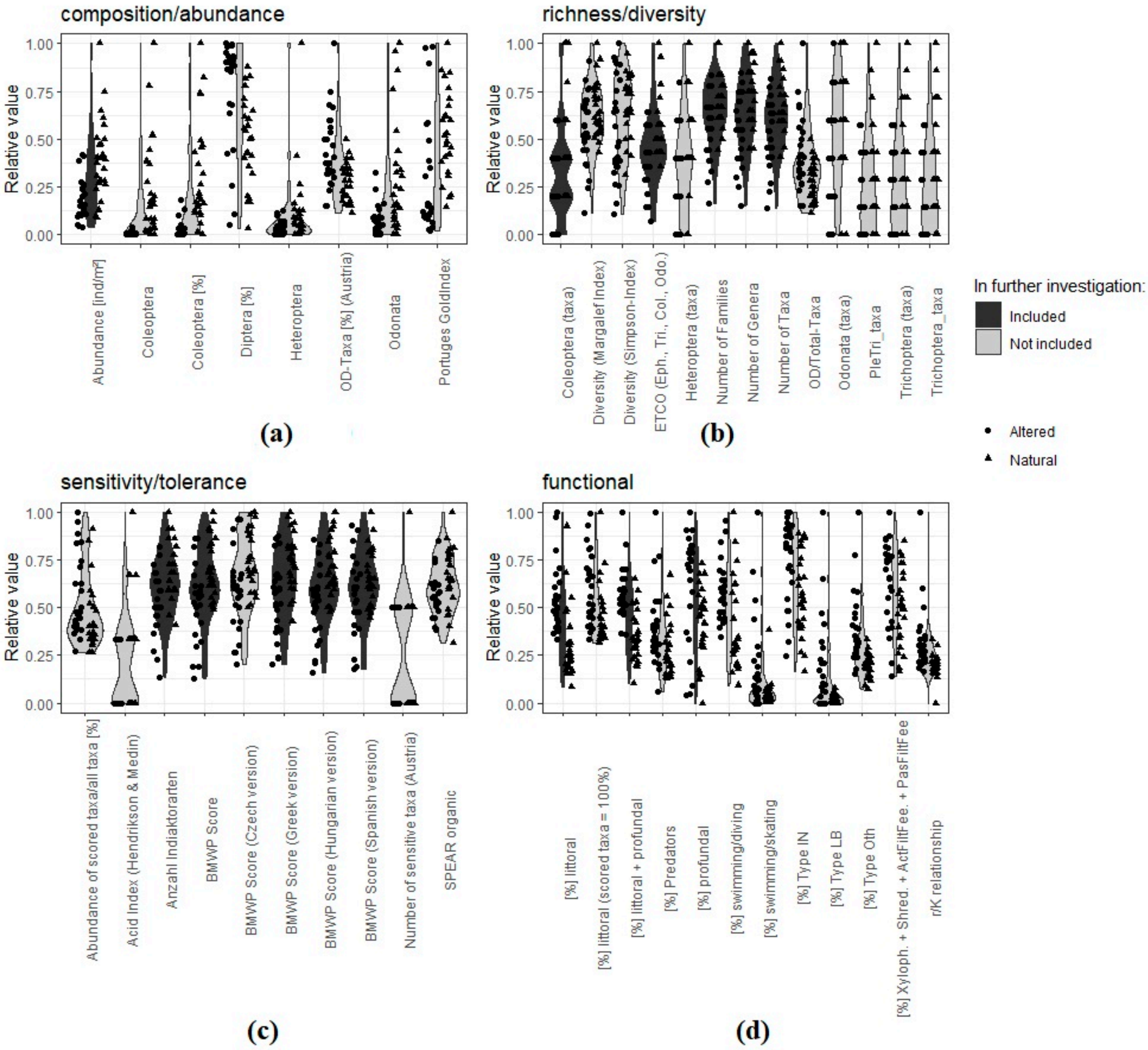


Figure 4. The evaluation graphs of (**a**) composition/abundance; (**b**) richness/diversity; (**c**) sensitive/tolerance and (**d**) functional metric groups of highly humic lakes.

2.4.4. Ecological Relevance

The group of experts authoring this paper evaluated each previously selected metric to exclude possibly non-explanatory correlations and those of ecologically low meaning. Additionally, metrics created for ecoregions other than boreal were excluded resulting in ten metrics for further use.

2.4.5. Correlations

Metrics with Spearman's correlation coefficient $|\geq 0.8|$ are considered redundant and only one of them must be used [53]. Additionally, we tried to avoid possible multicollinearity by selecting metrics with even lower ($|\leq 0.6|$) values [54] to obtain at least one metric per one WFD criteria. Correlation coefficients of the selected metrics are available in Table S2.

At this point, we decided to keep following metrics:

- For composition/abundance: abundance [ind/m^2];
- For richness/diversity: Coleoptera (taxa);
- For sensitivity/tolerance: BMWP (Biological Monitoring Working Party) Score;
- For functional metrics: (%) littoral, (%) littoral + profundal, (%) profundal.

As the selected functional metrics are highly correlated both statistically and ecologically, we decided to compare three models with inclusion of every previously mentioned metric from above and functional metrics as follows:

- (%) littoral;
- (%) littoral + profundal;
- (%) profundal.

As in every principal model where metric types have the same weights, we used the mean value as the result for the multimetric index.

2.4.6. Scaling

To ensure that the multimetric index is limited between 0 and 1, and to avoid potential influence of some extremely high- or low-quality sites anchoring is suggested [53]. We used the same approach for metrics decreasing with increasing impairment, but corrected the approach for the metrics increasing with increasing impairment to:

Value = 1—(Metric result—Lower Anchor)/(Upper Anchor—Lower Anchor). We corrected values >1 to 1 and negative values to 0.

To ensure good spread of sites within multimetric index, we compared several anchor values (Table S3):

- 5th percentile and 95th percentile;
- 10th percentile and 90th percentile;
- 10th percentile and 80th percentile;
- Each of the previous with prespecified values for "Coleoptera (taxa)" as 0 for lower and 4 for upper.

2.4.7. Quality Classification

We used the quality classes in accordance with WFD demands, following suggestion of Hering et al. [53]:

- Reference ≥ 0.8;
- Good $\geq 0.6 < 0.8$;
- Moderate $\geq 0.4 < 0.6$;
- Poor $\geq 0.2 < 0.4$;
- Bad <0.2.

We consider an index to be the best, if natural lakes are concentrated at the reference and good quality classes, while altered lakes are at bad- and poor-quality classes with some mixture present in a class of moderate quality.

We used the software R 4.0.3 (The R Foundation for Statistical Computing, Vienna, Austria) for data analysis [55]. Data processing and visualisations were performed within the tidyverse ecosystem [56].

3. Results

3.1. Characterisation of Chemical and Environmental Variables

The studied lakes are poly-humic lakes as indicated by high water colour values (114–666 mg Pt/L) and low pH values (3.35–6.09). Electric Conductivity (EC) is in the range of 21–65 µS/cm, concentrations of TN are 0.43–1.68 mg/L and TP are 0.017–0.061 mg/L. The highest conductivity, water color and total nitrogen values were observed in waterbodies of the Melnais Lake Mire (see Table 1).

Table 1. Mean annual chemical and environmental parameters at the studied highly humic lakes (Cond—electric conductivity, TN—total nitrogen, TP—total phosphorus).

Lakes	Altered Water Level (+)	Year	pH	Cond µS/cm	Colour mg Pt/L	TN mg/L	TP mg/L
Cenas Mire Lake 1		2015	3.93	28	124	0.95	0.017
Cenas Mire Lake 2		2015	4.44	29	144	0.99	0.020
Cenas Mire Lake 3		2015	4.49	26	114	0.90	0.019
Cenas Mire Lake 4		2015	3.75	32	189	0.83	0.021
Cenas Mire Lake 5		2015	3.46	43	304	0.92	0.020
Melnais Lake Mire Lake 1	+	2015	3.68	65	393	1.31	0.019
Melnais Lake Mire Lake 2	+	2015	3.53	49	402	1.25	0.022
Melnais Lake Mire Lake 3	+	2015	3.58	44	365	1.17	0.022
Melnais Lake Mire Lake 4	+	2015	3.43	45	505	1.36	0.017
Melnais Lake Mire Lake 5	+	2015	3.35	48	666	1.68	0.028
Deguma Lake		2017	5.09	31	222	0.95	0.032
Orlovas Lake		2017	5.42	21	205	0.87	0.037
Pieslaista Lake		2017	5.02	36	238	0.65	0.061
Ramatas Lielezers Lake	+	2017	6.09	23	134	0.65	0.032
Soku Lake	+	2017	5.88	25	130	0.43	0.035

3.2. Benthic Invertebrate Taxa

A list of benthic invertebrate taxa found in highly humic lakes is presented in the Supplemental Material (Table S4). Altogether, 18,808 individuals from 106 macroinvertebrate taxa are recorded at the studied bog lakes, of which the orders Coleoptera, Odonata and Trichoptera have the highest species richness. Mayflies (Ephemeroptera) are represented by four species, of which *Leptobphlebia vespertina* is the most widespread, missing only in the water bodies of the Cena Mire. In the humic lakes of Melnais Lake Mire and Cena Mire, larvae of dragonflies *Leucorrhinia albifrons* and *L. pectoralis* are recorded. These two species are included in the Bern Convention [57] and in the Habitats Directive [47]. The highest abundance of macroinvertebrates is recorded from waterbodies of Melnais Lake Mire and Cena Mire, ranging from 813 to 2014 individuals per sample, while the benthic invertebrate abundance at larger lakes ranges from 51 to 1202 individuals.

The macroinvertebrate orders Diptera and Coleoptera are the most abundant taxa at the lakes of the Cena Mire, while chironomids dominate in lakes of the Melnais Lake Mire. Taxonomic structure at lakes Orlovas, Deguma, Pieslaista, Ramatas Lielezers and Soku vary due to repeated sampling in different years and seasons, generally with Diptera, Coleoptera and Ephemeroptera as the dominant taxa. Molluscs (Gastropoda and Bivalvia) are completely absent at the studied lakes (see Figure 5).

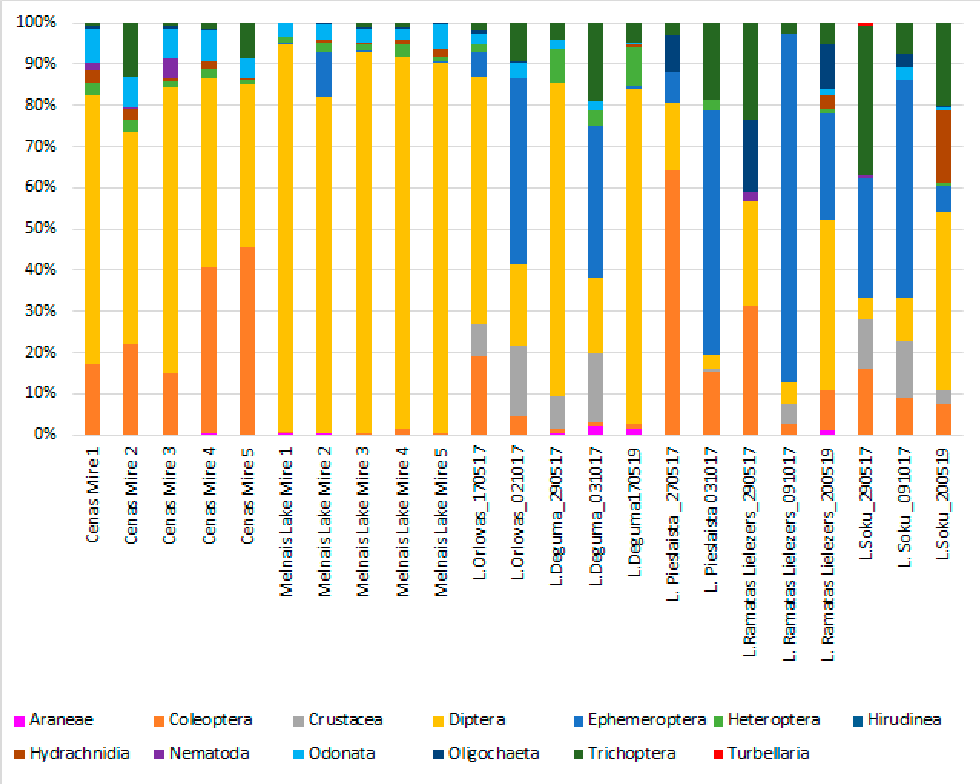
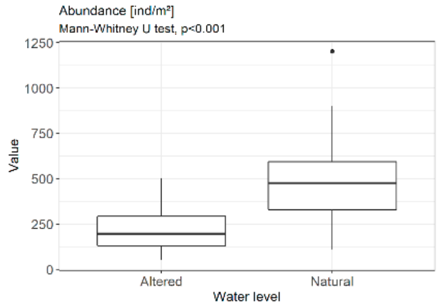


Figure 5. The dominance structure of macroinvertebrate assemblages in highly humic lakes of Latvia.

3.3. Metrics

The comparison of macroinvertebrate abundance (ind/m^2) between samples of bog lakes with altered water level and those with natural water level show significant differences ($p < 0.001$) (Figure 6). The macroinvertebrate abundance in lakes with natural level varies from 109 to 1202 individuals, while in lakes with altered level from 51 to 502. Similar significant differences are found between the number of taxa varying from 9 to 22 taxa in natural lakes and 3 to 18 taxa in altered lakes ($p < 0.001$). Additionally, our results show higher values of BMWP Score at natural lakes rather than the altered ones ($p = 0.002$). The BMWP values in natural lakes vary from 33 to 74, whereas from 10 to 67 in bog lakes with altered water level. Taxa richness of Coleoptera and ETCO (Ephemeroptera, Trichoptera, Coleoptera, Odonata) show the same significant differences between the studied lakes ($p < 0.001$). Natural bog lakes are represented by 1 to 5 Coleoptera species, while in the altered lakes 0 to 3 species are found. The number of ETCO varies from 5 to 14 species in natural lakes while 1 to 9 species in bog lakes with altered water level, respectively. Nevertheless, altered lakes are represented by taxa preferring littoral and profundal habitats ($p < 0.001$) and higher percentage of Diptera ($p < 0.001$). Number of taxa of Trichoptera ($p = 0.023$), Odonata ($p = 0.009$) and Heteroptera ($p = 0.007$) is higher at humic lakes with natural water level.



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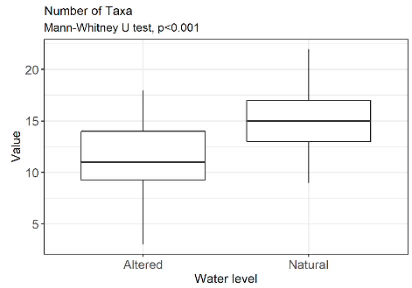

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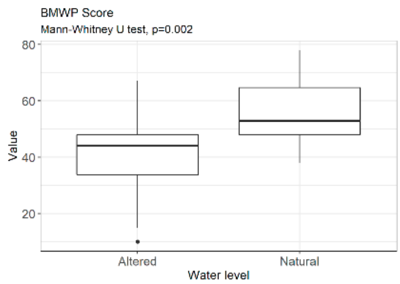

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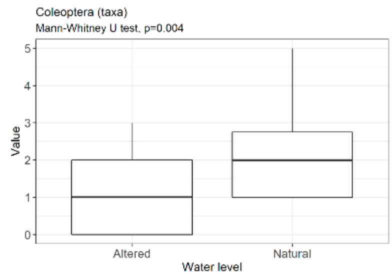

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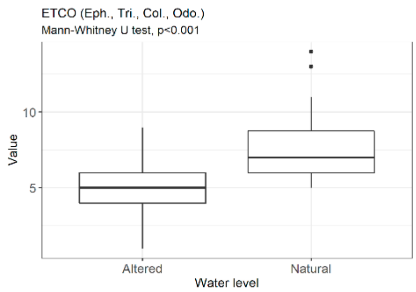

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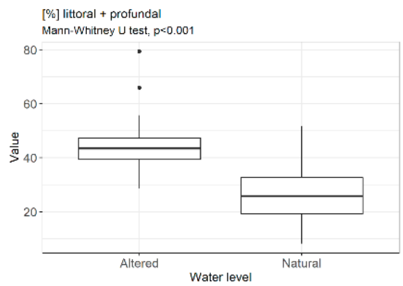

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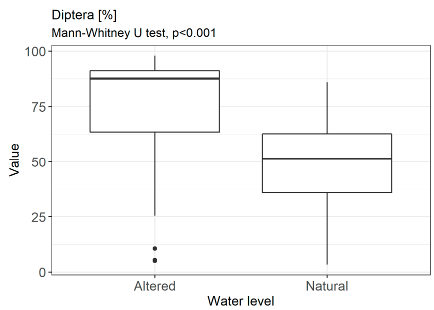

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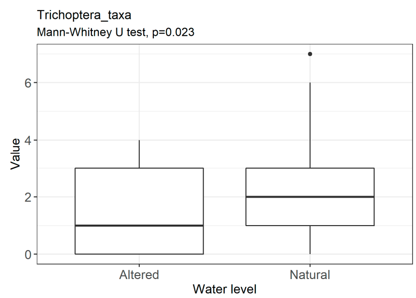
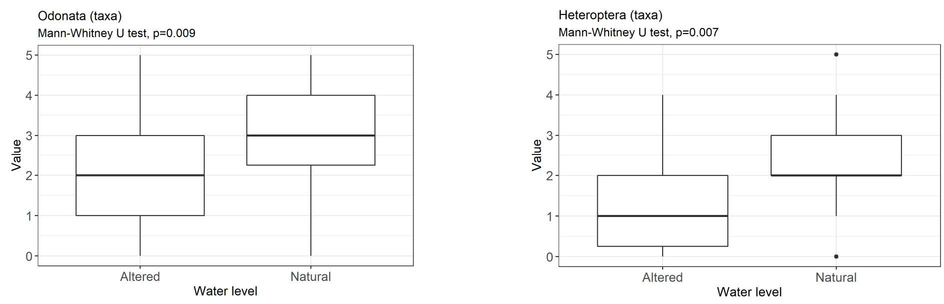


(**h**)

Figure 6. *Cont.*



(**i**) (**j**)

Figure 6. Differences of macroinvertebrate (**a**) abundance, (**b**) taxa richness, (**c**) BMWP (Biological Monitoring Working Party) Score, (**d**) Coleoptera taxa, (**e**) ETCO (Ephemeroptera, Trichoptera, Coleoptera, Odonata), (**f**) littoral + profundal preference, (**g**) % Diptera, (**h**) Trichoptera taxa, (**i**) Odonata taxa and (**j**) Heteroptera taxa between bog lakes with altered and natural level.

3.4. Multimetric Index

We found the model 2 (Figure 7) ((%) littoral + profundal and abundance (ind/m^2) and Coleoptera (taxa) and BMWP Score) with P10 and P80 anchoring and predefined Coleoptera (taxa) anchoring to be the best classifier for the highly humic lakes.

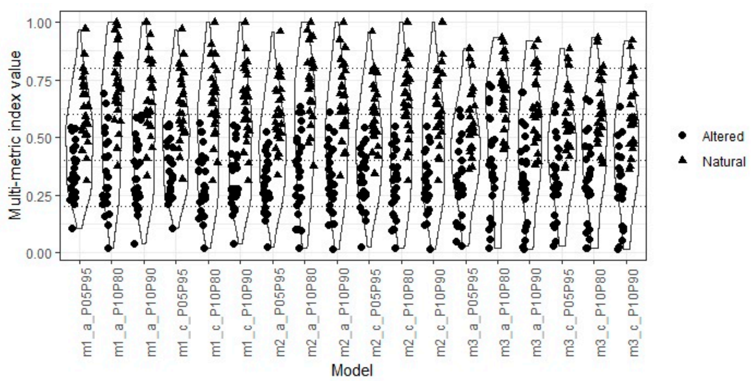


Figure 7. The model comparison of the humic lake multimetric indices at the lakes with natural and altered water level. The model caption consists of model_Coleoptera (c = yes; a = no) percentile anchors.

The distribution of values of the highly humic lake multimetric index are shown in Figure 8.

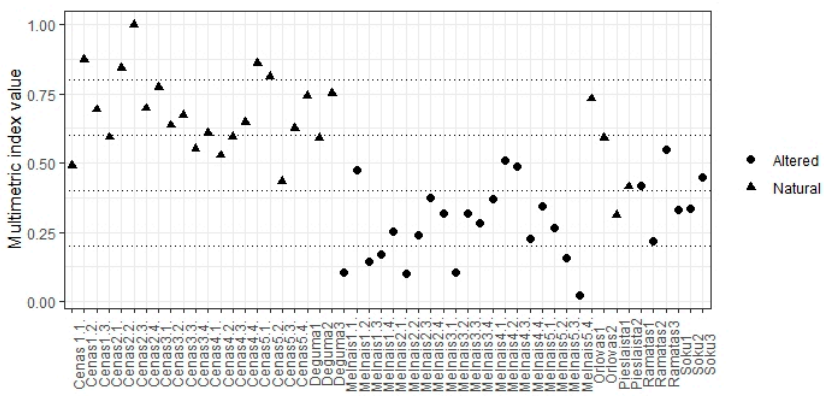


Figure 8. The distribution of values of the newly developed highly humic lake multimetric index at the lakes with natural and altered water level.

4. Discussion

The total number of taxa found in the studied highly humic lakes is relatively low and similar to those of other studies [40,41]. Desrochers and van Duinen [58] noted that the chemical constraints of humic lakes almost entirely exclude several macroinvertebrate taxa, e.g., lumbricid worms, isopods, and snails and the low nutrient availability may limit the presence of species with high nutrient preferences. In addition, acidity is known as a limiting factor for many macroinvertebrate groups, e.g., freshwater snails [59], mussels [60] and mayflies [61]. In general, macroinvertebrate species specialized on different mire habitats are obviously able to realize their life cycle at water pH of 4.0–5.0 [62]. The macroinvertebarte taxonomical composition and lack of molluscs indicate the peculiarities of the studied highly humic lakes.

The invertebrate assemblage in altered lakes showed a reduced taxonomic richness, especially in Trichoptera, Coleoptera, Heteroptera and Odonata, an increased proportion of Diptera and an overall lower numerical abundance.

The best-performing metrics were total abundance (for composition/abundance category), number of Coleoptera taxa (for richness/diversity category), BMWP score (for sensitivity/tolerance category), and % littoral and profundal (for functional metric category).

4.1. Use of Benthic Invertebrates in Lake Ecological Assessment

In lakes, phytoplankton and macrophytes are the most widely used communities for ecological assessment. However, in the last decades numerous systems using benthic invertebrates have been developed, following the requirements of the WFD [36]. These systems differ by habitat sampled (mostly littoral, but some sampled the profundal), putative pressure assessed and metrics used.

According to the concept of multimetric approach [53,63] different types of metrics should be included into the assessment system (composition/abundance metrics, richness/diversity metrics; sensitivity/tolerance metrics; functional metrics).

4.1.1. Sensitivity/Tolerance Metrics

Almost all lake benthic invertebrate assessment systems include some kind of sensitivity index, most common are Average Score per Taxon (ASPT) index [64], Acid Water Indicator Community (AWIC) index [65], Benthic Quality Index [66] and Fauna index [67].

Originally, ASPT and BMWP indices have been developed for river water quality in Britain [68], however they have proved suitable for lake assessment. For instance, Šidagytė et al. [69] have demonstrated relationships between ASPT and total phosphorus, biochemical oxygen demand (BOD) and tropho-morphoindex in lakes of Lithuania, while Mavromati et al. [70]—relationships between ASPT and the total phosphorus and shoreline modification in lakes for Greece. Similarly, relationships between ASPT and eutrophication and other pressures have been demonstrated in lakes of Denmark [71]. Currently, ASPT index is used in the lake assessment in Denmark, Greece, Estonia, Latvia, Lithuania, Poland, and Sweden [36] while BMWP in Hungary and Bulgaria [72]. Additionally, in our study, we found that ASPT and BMWP differentiate between natural and altered humic lakes, though we chose to include BMWP (p = 0.002) rather than ASPT (p = 0.45).

4.1.2. Richness/Diversity Metrics

Richness diversity metrics are the widely represented metric category in the lake assessment: almost all the countries use some of these metrics: total taxa richness, Shannon diversity, Margalef diversity or other. Many studies have revealed relationships between total taxa richness/diversity metrics and different human pressures: morphological degradation [73], water-level fluctuation [74], total phosphorus [70] and integrated pressure index [71]. However, in many cases, a pooled taxa number of stressor-sensitive macroinvertebrate orders can be more informative: for instance, CEP taxa richness (Coleoptera, Ephemeroptera, Plecoptera) has shown relatively strong relationships with a range of eutrophication indicators and hydromorphological index in Lithuania, so it was included in the Lithuanian assessment system [69]. Similarly, number of EPTCBO (Ephemeroptera, Plecoptera, Trichoptera, Coleoptera, Bivalvia, Odonata) taxa is used in Denmark, based on demonstrated relationships with eutrophication pressure [71]. In contrast, we found a significant difference in the total taxa richness, number of genera, number of Odonata taxa and the number of Coleoptera taxa ($p < 0.001$) between humic lakes with natural and altered water level. We selected Coleoptera taxa richness to include into the multimetric index. Additionally, Šidagytė et al. [69] demonstrated strong relationship between Coleoptera taxa richness and different pressure descriptors, both eutrophication and hydromorphological alterations.

4.1.3. Composition/Abundance Metrics

Composition/abundance metrics are widely used in lake assessment systems, mostly expressed as proportion (relative abundance) of specific taxa. Thus, Lithuanian and Danish assessment systems include % COP (Coleoptera, Odonata, Plecoptera) but Greek littoral assessment system—% of Diptera, and German assessment system—% Odonata. Several studies demonstrated between proportion of different taxa % Gastropoda, % Odonata and anthropogenic pressures [69,73]. We have found that % Coleoptera, % Odonata and % Diptera differ strikingly between natural and altered lakes; however, abundance of total community (expressed as ind/m^2) was selected as a core metric due to the strong inter-correlations between the proportion and the diversity metrics [53].

4.1.4. Functional Metrics

An alternative to species identity-based methods is the use of functional metrics based on species traits. This approach is recommended by many studies [75–77]. However, only few lake assessment systems include functional metrics, e.g., % abundance of feeding type collectors, is included in Austrian and German lake assessment systems, and % abundance of habitat type lithal in the German alpine lake assessment system. Percentage of feeding type predators is used in Sweden to assess acidification [78]. In our study, several functional metrics showed the difference between natural and altered lakes: % littoral, % profundal and % littoral + profundal ($p < 0.001$). Percentage of organisms with littoral and profundal preference was selected to be included in the core metrics. We assume that this group consists of generalist organisms that might indicate the altered water level.

4.2. Assessment of Hydrological Modifications

Traditionally, lake assessment has focused on eutrophication using primary producers—phytoplankton, macrophytes and, recently phytobenthos communities [79]. However, hydrological alterations, e.g., regulation of lake water level for power production and flood control is among the major anthropogenic disturbances in boreal aquatic ecosystems [80]. Several studies have demonstrated strong effects of water level fluctuations on lake biota, mainly benthic invertebrates, fish fauna and macrophytes [39,80,81]. For instance, Aroviita and Hämäläinen [74] showed a marked decrease in species richness of benthic macroinvertebrate with increasing intensity of water-level regulation, especially for Ephemeroptera, Trichoptera, Coleoptera or Megaloptera. Similarly, changes in species composition were reported in regulated lakes of Ireland, i.e., decrease of Crustaceans, increase of Chironomids, Oligochaetes, and invasive amphipods [82] and in regulated lakes in Italy—increase in mobile and/or feeding opportunistic taxa and decrease in sessile and/or herbivorous taxa [83]. Additionally, Brauns et al. [84] described the decrease in Coleoptera, Odonata, Trichoptera and functional groups of piercers, predators, shredders and xylophagous as the potential effects of lake water level fluctuation in lakes of Germany.

Furthermore, the decrease in benthic invertebrate biomass and/or abundance in the littoral area of regulated lakes has been reported by several studies [85–87]. However, other studies did not find any significant effect on numerical abundance of invertebrates [82], probably because water level regulation exerts stronger effect on the biomass of invertebrates than on the numerical abundance affecting larger taxa more [74].

So far, only two lake ecological assessment systems addressed hydromorphological alterations: Slovenian lake assessment system [38] and German assessment system for alpine lakes [36]. However, several lake assessments include multiple pressures including hydromorphological alterations (e.g., [69]). Similar to other studies, we found a marked change in composition and abundance of benthic invertebrates, which further can be used in the development of assessment systems, specifically addressing effect of hydrological alterations in highly humic lakes.

4.3. Assessment of Highly Humic Lakes

Humic lakes constitute a considerable portion of lakes in the boreal zone [1]. However, most assessment systems are developed for clear-water lakes characterized by neutral to alkaline pH, low level of DOM, and water transparency depending on the number of phytoplankton. Humic lakes differ substantially from these clear-water systems and therefore might need different assessment methods [13,17,40]. For instance, Benthic Quality Index (BQI)—a widely used metric in the assessment of lake status in Sweden and Finland [87] was deemed inadequate of assessment of the humic lakes as oxygen depletion and dominance by the tolerant species *Chironomus anthracinus* and *C. plumosus* are natural phenomena and not an effect of human impacts [17]. Further, several phytoplankton, macrophyte and fish metrics:

(i) Classified reference lakes as impacted;
(ii) Did not differentiate between reference and impacted lakes [17].

This can be explained by the fact that humic lake communities are more tolerant to the environmental fluctuations [88] and are less taxa-rich and diverse comparing to clear-water communities [8]. Further, light limitation due to high level of humic substances plays an important role in these lakes, so several widely used lake assessment parameters as macrophyte colonization depth cannot be used in these lakes [14].

In Latvia, lake assessment system is based on number of taxa, number of EPTCBO taxa, Shannon-Wiener diversity index, ASPT index and acidity index [89]. However, this assessment system was not appropriate for assessment of humic lakes, as also near-natural lakes were classified as impacted according to this system [50]. This problem was encountered also in lakes of Finland [17]. Unsuitability of certain metrics for highly humic lakes can be solved by developing an assessment system targeted to specific human pressure and lake types, as shown by this study. Whether this multimetric index is applicable to humic lakes in other regions needs to be tested in future studies.

Supplementary Materials: The following are available online at https://www.mdpi.com/2073-4441/13/2/223/s1, Table S1: The results of the binary logistic regression (BLR). Z and *p* values of the chosen metrics between natural and altered highly humic lakes, Table S2: Correlation coefficients of the selected metrics for the highly humic lake multimetric index, Table S3: Anchor values of the macroinvertebrate metrics for a potential multimetric index for the highly humic lake quality assessment, Table S4: Macroinvertebrate taxa found in the studied highly humic lakes.

Author Contributions: Conceptualization, D.O., S.P., I.K.; funding acquisition, D.O.; field works, D.O., A.S., J.J.; sample analysis, D.O., A.S.; data analysis, A.A., D.O., A.S., I.K., J.J.; Writing of original draft, D.O., S.P.; Review and editing, I.K., J.J., A.A., A.S. All authors have read and agreed to the published version of the manuscript.

Funding: The data were collected in the frame of ESF supported project PuReST, No. 1DP/1.1.1.2.0/13/APIA/VIAA/044 and a project supported by The Administration of Latvian Environmental Protection Fund, No. 1-08/202/2018.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: The data presented in this study are available on request from the corresponding author. The data are not publicly available due to the data acquisition from two different projects and the National Surface Water Monitoring program, all requiring different data availability policy.

Acknowledgments: Authors want to express their gratitude to the Latvian Environment, Geology and Meteorology Centre.

Conflicts of Interest: The authors declare no conflict of interest.

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