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Littoral eutrophication indicators are more closely related to nearshore land use than to water nutrient concentrations: A critical evaluation of stressor-response relationships

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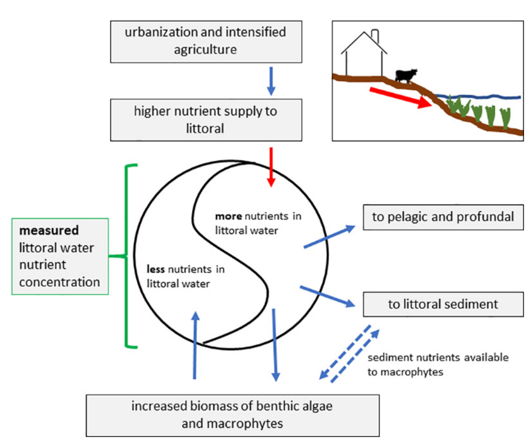
HIGHLIGHTS

- Stressor-response relationships for WFD lake assessment metrics are often poor.
- We estimated phosphorus runoff from the adjacent land from CORINE land use.
- Lakes with high P-runoff did not have higher water nutrient concentrations.
- Diatom indices were correlated with P-runoff but not with water chemistry.
- High primary producer biomass seemed to reduce dissolved nutrient concentrations.

GRAPHICAL ABSTRACT



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ABSTRACT

Biological assessment metrics and water chemistry measurements are used to quantify the link between stressors and their effects on lake ecosystems, for the Water Framework Directive. However, correlations between metrics and water chemistry are often poor. This is seen as major weaknesses of Water Framework Directive-related monitoring and assessment. We analyzed macrophytes, benthic algae, benthic macroinvertebrates, water chemistry and sediment total phosphorus content in the littoral of six lakes in the Western Balkans and used CORINE land use data to estimate nutrient enrichment via runoff from the adjacent land. Lakes with a higher estimated phosphorus runoff from the adjacent land did not have higher littoral water nutrient concentrations, but littoral diatom assemblages indicated more eutrophic conditions. These lakes also had higher abundances of littoral benthic primary producers, which in turn were associated with low concentrations of dissolved nutrients, but only in autumn, not in spring. This is consistent with primary producers taking up nutrients during the summer growth season. In lakes with high abundances of benthic primary producers, it is likely that the littoral vegetation plays a large role in the transfer of nutrients from the water to the benthos. This process impairs correlations between biological metrics and water nutrient concentrations. Our results suggest that CORINE land cover may be more useful to characterize littoral nutrient enrichment than lake water chemistry. Increased benthic primary producer biomasses and "eutrophic" diatom indices may indicate littoral nutrient enrichment even if water nutrient concentrations are low.

1. Introduction

The Water Framework Directive (WFD; European Commission, 2000) triggered the development of hundreds of biological metrics (Birk et al., 2012), which currently are used for an extensive and harmonized assessment of the ecological status of Europe's surface waters (Carvalho et al., 2019). Biological metrics used for status assessment according to the WFD are expected to be based on stressor - response relationships (Lyche-Solheim et al., 2013). This means that member states must show that a stressor (for example nutrient enrichment) leads to a quantifiable and consistent biological response (for example a change in the species composition of aquatic macrophytes). These stressor – response relationships were generally established by correlating indices (metrics) with water chemistry. Water nutrient concentrations – often total phosphorus – were generally used to approximate the stressor "nutrient enrichment" (e.g. Penning et al., 2008; Dudley et al., 2013; Phillips et al., 2013). In lakes, however, benthic primary producers are related to water nutrient concentrations in a fundamentally different way than are the planktonic primary producers (Eigemann et al., 2016). When phytoplankton take up phosphate from the water, total water phosphorus concentrations will remain unchanged, because the phosphorus is still present in the water, only incorporated in the phytoplankton biomass. In contrast, when macrophytes or benthic algae take up phosphate from water, it is removed from the water and transferred to benthic habitats. This process is called nutrient translocation (Vanni, 2002). Consequently, water nutrient concentrations are likely to decrease when nutrients are taken up by macrophytes or benthic algae. In principle, water nutrient concentrations may therefore be similarly low in lakes with low external nutrient loadings and little benthic primary production, as they are in lakes with higher loading and intense benthic primary production. This is relevant, because the poor linkage between stressors and effects on the ecosystem, assessed using water chemistry and biological WFD metrics, is seen as major weaknesses of WFD monitoring and assessment (Carvalho et al., 2019). Indeed, phytoplankton metrics developed for assessment according to the WFD were more closely correlated with water chemistry than were the benthic organism groups macroinvertebrates, macrophytes and phytobenthos (Birk et al., 2012).

We hypothesize that the reason for this perceived weakness is that benthic primary producers reduce water nutrient concentrations and thereby impair correlations between water chemistry and WFD metrics for benthic littoral organisms. We show here that a more direct descriptor of littoral nutrient enrichment, i.e. phosphorus runoff estimated from land use in the nearshore surroundings, is better linked to WFD assessment metrics than water chemistry. We analyzed macrophytes, benthic algae, benthic macroinvertebrates, water chemistry and sediment total phosphorus content in the littoral of six lakes in the Western Balkans and used CORINE land use data to estimate nutrient enrichment via runoff from the adjacent land. We explored how data of a type and quality as commonly recorded in WFD related monitoring may be used to address nutrient translocation in the littoral of lakes, and how this information may be used to improve stressor-response relationships. Specifically, we tested the following hypotheses:

1) Lakes with a higher nutrient runoff from the adjacent land will have a higher biomass of benthic primary producers (because nutrients will be used for increased growth of benthic primary producers)
2) Lakes with a high benthic primary producer biomass will have low dissolved nutrient concentrations in the littoral (because nutrients are taken up by benthic primary producers)
3) Indices used to assess nutrient enrichment will reflect nutrient runoff from the adjacent land more closely than water nutrient concentrations

2. Methods

2.1. Sampling sites

We sampled six lakes in the Western Balkans (Fig. 1, Table 1). These lakes were selected because we expected them to represent a gradient from oligotrophic to eutrophic conditions. In accordance with Dudley et al. (2013), water, sediment, and biological samples were taken at six sites around the perimeter of each lake (Table S1 in the appendix). The six sites per lake were selected to together represent the typical shoreline conditions in each lake (with respect to shoreline vegetation, land use, etc.), while at the same time aiming at an approximately even distribution of sites around the perimeter of the lake. None of the sites was situated close to a river mouth, because inlet water chemistry may differ substantially from overall lake water chemistry (e.g. in lake Ohrid; Matzinger et al., 2007). Samples of diatoms and macroinvertebrates were taken, macrophytes were recorded, and CORINE land use categories at the adjacent shoreline were registered at the same sites. All macroinvertebrate, water and sediment samples were collected in spring and autumn seasons, and diatoms and macrophytes in summer. Samples were taken between July 2016 and April 2018. All samples within one lake were collected in consecutive spring, summer and autumn, or, alternatively, in consecutive summer, autumn and spring seasons (Table 1).

2.2. Water and sediment chemistry

At each site, water samples were taken twice, in consecutive spring and autumn seasons (Table 1). We measured water chemical

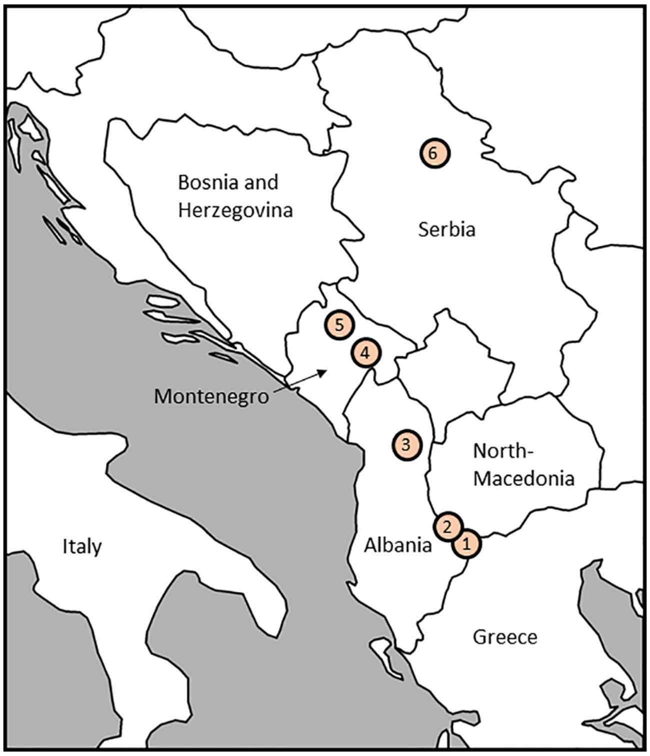

Fig. 1. Map showing the location of the sampled lakes; 1 = Prespa, 2 = Ohrid, 3 = Lura, 4 = Biogradsko, 5 = Crno, 6 = Sava.

parameters commonly related to eutrophication (phosphorus, nitrogen, biological oxygen demand). In addition, we measured sediment total phosphorus content, because sediment phosphorus may be a legacy indicator of past loading, and because aquatic macrophytes may take up phosphorus from the sediment (Barko and Smart, 1981).

At each site, a water sample was collected at a few meters distance from the shoreline at approximately 0.5 m depth. Chemical parameters were measured at laboratories accredited after ISO 17025 using intercalibrated standard procedures. Total phosphorus (TP) was measured spectrophotometrically after digestion of the water sample with peroxodisulfate (ISO 6878:2004 and APHA 4500-P,B,E) or using ICP-OES after digestion with nitric acid (ISO 11885:2007). Total nitrogen (TN) was measured chemically after oxidation with peroxodisulfate (ISO 11905:1997) or using a TOC/TN analyser after thermocatalytic oxidation (EN 12260:2008 and ISO 8245:1999). Water nitrate concentrations were measured spectrophotometrically (ISO 7890-3:1988 or APHA 4500-NO_3^- -E), or by liquid chromatography (ISO 10304-1:2007), and water ammonium concentrations were measured spectrophotometrically (ISO 7150-1:1984 or SRPS H.Z1.184:1974). Biochemical oxygen demand after 5 days (BOD) was measured according to EN 1899:2009.

In addition, a sediment sample was collected at a few meters from the shoreline from approximately 1 m depth using a grab, and sediment P content (in mg/kg dry weight) was determined after digestion with nitric acid (EN 16173:2012) by measuring TP in the extract using the same methods as described above for TP in water.

2.3. Littoral benthic organisms

2.3.1. Diatoms

At each site, epilithic diatoms were sampled from 5 cobbles with diameters of roughly 5–10 cm, collected from around 0.5 m water depth. The upper side of each cobble was brushed with a toothbrush, and the algae were transferred into a beaker. Samples were immediately fixed with formaldehyde to a final concentration of approximately 4%. In the laboratory, the organic content of the material was removed using hot HCl according to Taylor et al. (2007). Permanent slides were prepared from the cleaned suspensions using Naphrax ® (refractive index = 1.74, Brunel Microscopes Ltd). Microscopic examinations were done using a Zeiss AxioImagerM.1 light microscope (LM) with DIC optics and AxioVision 4.9 software. We used the following primarily identification guides and floras: Krammer, 2002; Krammer and Lange-Bertalot, 1988, 2004; Lange-Bertalot, 2001; Lange-Bertalot et al., 2017; Levkov et al., 2007, 2016. On each slide, 400 valves were counted using 1600 × magnification. Using the software OMNIDIA 6.05, we calculated the four most widely applied diatom indices for nutrient concentration/organic pollution/general pollution in Europe, i.e. the IPS (Coste in Cemagref, 1982), TDI (Kelly and Whitton, 1995; Kelly et al., 2001), SI (Rott et al., 1997) and TI (Rott et al., 1999), as well as the Trophic Diatom Index for Lakes TDIL (Stenger-Kovács et al., 2007) because the latter was specifically developed for lakes. To improve comparability, all indices were scaled from 1 to 20 in OMNIDIA, where low values indicate nutrient enriched conditions.

The methods we used for sampling and enumerating diatoms are in accordance with internationally accepted standards for ecological status assessment according to the WFD (EN 13946:2014, EN 14407:2014). However, this procedure evaluates relative abundance of diatom species only, and provides no information on diatom biomass. Diatoms were therefore not included in the estimation of macrophyte and benthic algal biomass. However, observed diatom abundances in the field were minor compared to other algae, particularly *Cladophora* sp., and macrophytes.

2.3.2. Macroinvertebrates

From each site, macroinvertebrate samples were taken from 0.5 and 2 m water depth in consecutive spring and autumn seasons, at the

Table 1
Lake morphometric data and sampling periods.

Lake	Location	Surface area (km^2)	Max depth (m)	Average depth (m)	References for morphometric information	Sampling of chemistry and macro-invertebrates	Sampling of macrophytes and diatoms
Prespa	Transboundary lake situated between Albania, North-Macedonia, and Greece	254	58	14	Matzinger et al. (2006)	October 2016, April 2017	July 2016
Ohrid	Transboundary lake situated between Albania and North-Macedonia	358	289	155	Matzinger et al. (2006)	October 2016, April 2017	July 2016
Lura	Albania	0.12	20	?	Unpublished information from a sign placed at the lake shore	June 2017, August 2017[a]	August 2017
Biogradsko	Montenegro	0.23	12	4.5	Stanković (1975)	May 2017, September 2017	July 2017
Crno	Montenegro	0.5	49	17	Stanković (1975)	May 2017, September 2017	July 2017
Sava	Serbia	0.9	12	4.5	Jovanović et al. (2017)	October 2017, April 2018	July 2017

[a] Due to logistical complications, sites LL4, LL5 and LL6 in Lake Lura were only sampled in August.

same time as the water samples were collected (Table 1). However, due to logistical complications, sites LL4, LL5 and LL6 in Lake Lura were not sampled in spring, and samples from 2 m water depth in the same lake were not collected in autumn. Samples from 0.5 m depth were collected using the kick-and-sweep method (D-shaped net with a metal frame holding a mesh bag of 500 μm size). A metal frame of one square meter was placed on top of the substratum, and the area within this frame was then sampled for a total of 1 min. At 2 m depth, a Van Veen Grab Sampler of 400 cm^2 was used. Two consecutive grab samples were collected and pooled. Grab samples were later sieved through a net of 500 μm mesh size. Samples were transported to the laboratory, rinsed and preserved in 70% ethanol. Specimens were identified to species or higher taxonomic level, where possible, using stereomicroscopes (10×–80× magnification), with the following primary identification guides: Snegarova, 1954; Hubendick, 1970; Brinkhurst and Jamieson, 1971; Radoman, 1983; Sket and Šapkarev, 1992; Nilsson, 1996; Bodon et al., 2001; Zwick, 2004; Lechthaler and Stockinger, 2005; Pillot, 2009; Waringer and Graf, 2011.

There was no difference in the abundance of macroinvertebrates at 0.5 and 2 m between spring and autumn (*t*-test for paired samples, all $p >> 0.05$, results not shown). Results from the same depth for spring and autumn were therefore averaged for some analyses, to reduce the number of tested variables. In all instances, individual spring and autumn results gave similar results as the averaged values.

The use of littoral benthic invertebrates for lake status assessment is relatively new, and no generally accepted single metric indices targeting nutrient enrichment/organic pollution exist (Poikane et al., 2016). We aimed at testing metrics within the four main types defined by Hering et al. (2006): composition/abundance, richness/diversity, sensitivity/tolerance and functional. However, we had to omit functional metrics because of a too high proportion of taxa where no data were available. This was due to the relatively poor coverage of Mediterranean taxa in the ASTERICS database, particularly in lakes with a high proportion of endemic taxa such as lake Ohrid (Albrecht and Wilke, 2008). Therefore, we calculated taxon density, and selected the average score per taxon index (ASPT) (Armitage et al., 1983) and the percentage of Ephemeroptera, Plecoptera and Trichoptera (EPT%), as they are commonly used and may respond to catchment degradation, lakeshore modifications, and a combination of trophic status and morphological alterations (McGoff and Sandin, 2012; Timm and Mols, 2012; Urbanic et al., 2012; Poikane et al., 2016). The ASPT uses presence/absence information of indicator taxa in a sample, operates mainly at the taxonomical level of family, and may be used across different water body types without modifications (Sandin and Hering, 2004). It ranges from 1 to 10, with high values indicating oligotrophic, unpolluted habitats. The EPT% operates on the relative abundance of EPT taxa in a sample, which may respond in a different manner to the ASPT.

2.3.3. Macrophytes

Submerged macrophytes, that is angiosperms and charophytes, were surveyed at the same time as the diatom samples were collected (Table 1). We used belt transects of approximately 10 m width – perpendicularly to the shoreline – from the upper littoral to the lower vegetation limit. Emergent macrophytes were generally not abundant at our study sites. Primary floras and identification guides were Casper and Krausch (1980, 1981) and Krause (1997). Each transect was divided into depth zones: 0–1 m, 1–2 m, 2–4 m, and >4 m depth. Species occurrence was registered in each transect and each depth zone, and the abundance of each species was estimated according to a five-degree scale (1 = very rare, 2 = infrequent, 3 = common, 4 = frequent, 5 = abundant, predominant). In order to ensure comparability with the hydrochemistry, diatom and macroinvertebrate results, only the macrophyte data from shallow water, i.e. from a depth between zero and two meters, were used for further analysis. This also ensured comparability of the macrophyte vegetation among lakes, because extensive macrophyte beds below 4 m water depth occurred only in one of our study lakes (Lake Ohrid).

As an approximation for the biomass of macrophytes, we calculated the sum of the cubed abundances of all species. We did so because they better reflect relative values than the five-degree scale used for estimation in the field (Melzer, 1999). The Balkan macrophyte index (BMI) was calculated as described in Schneider et al. (2020). The BMI was chosen as a metric because it reflects nutrient supply and is applicable to lakes in the Balkan area. The BMI ranges from 1 to 5, with high values indicating nutrient pollution.

In some lakes, particularly in lake Ohrid, large quantities of the benthic green alga *Cladophora* sp. were observed, and its abundance was estimated in the same way as macrophyte abundance. In order to approximate the biomass of macrophytes and benthic algae, the cubed abundances of *Cladophora* sp. were added to the cubed abundances of all macrophyte species.

2.4. Estimation of site-specific nutrient input from the nearshore surroundings

The CORINE (Coordination of Information on the Environment) Land Cover map covers all Europe and classifies areas into 44 landcover classes, grouped in a three-level hierarchy (EEA (European Environment Agency), 2007). It has last been updated in 2012 (EEA (European Environment Agency), 2014). In each lake, we registered CORINE land use from the same six sites from where the water, sediment and biological samples were taken (instead of doing entire lake catchments) in order to have the same level of uncertainty as for the biological and chemical variables. For each site, we used GIS to delineate a 10-ha trapezoid that stretched 500 m inland from the shore of the lake. 500 m was chosen based on the topography of the adjacent land: we aimed at standardizing the area while avoiding including valleys with an orientation parallel to the shoreline (since nutrients from such areas would not be transported towards the site). The width of the trapezoid was 100 m at the shoreline, and 300 m at the far end. These widths were chosen so that no adjacent trapezoids would overlap. For each trapezoid, we calculated the areas of different land cover classes using CORINE. Phosphorus export coefficients for each land use category were taken from Smith et al. (2005). Smith et al. (2005) provide – to our knowledge – the most comprehensive overview over phosphorus export coefficients from different CORINE land use categories that are relevant at our study lakes. Although the absolute values of export coefficients for land use categories vary among studies (see e.g. Johnes et al., 1996; Pasztaleniec and Kutyła, 2015; Palviainen et al., 2016), the relative ranking is the same (urban > agriculture > grassland > forest) and within each class they are in the same order of magnitude. For each trapezoid, the phosphorus export (in kg/year) was calculated by summarizing the products of land cover area (in each land use class) with their export coefficients. Note that we use the land cover in the nearshore surroundings to approximate only the nutrient load to the littoral, and that our method is not appropriate for calculating total loads to the entire lake.

2.5. Data analysis

In order to provide results which are relevant for whole lake ecological status assessment according to the WFD, results from the six sites in each lake were averaged, and averages were used to reflect the overall condition of each lake. Averaging is commonly used to calculate whole lake assessments from transect- or site-specific data (e.g. Pall and Moser, 2009; Zervas et al., 2018). Data were visually inspected and transformed when necessary to improve normality and homoscedasticity. Data were visually checked for hump-shaped relationships, and none occurred. We therefore used Pearson correlations to test the strength of linear relationships. Results were accepted as significant at $p < 0.05$, but given that we only have few data points, we also carefully interpreted p-values < 0.1. Because each analysis represented a separate hypothesis, and because we were interested in detecting meaningful patterns instead of searching for individual significance, there was no need to adjust α for multiple testing (Perneger, 1998).

3. Results

3.1. Summary statistics

Complete results, including within-lake variation of all measured variables, are given in Tables S1, S2 and S3 in the Appendix. Summary results are presented in Table 2. Briefly, the immediate surroundings of lake Ohrid were characterized by a mixture of urban and agricultural land cover classes, as well as natural vegetation. The surroundings of lake Prespa were characterized by a mixture of agriculture and natural vegetation, while the surroundings of lake Sava were mainly urban, with some natural vegetation. In contrast, lakes Lura, Crno and Biogradsko were surrounded by natural vegetation, mainly forests. The runoff calculated from the CORINE landuse data ranged from 0.2 kg/ha (Biogradsko) to 1.6 kg/ha (Ohrid). Water total phosphorus concentrations (average values for samples taken in spring and autumn at 6 sites per lake) ranged from 7 (Ohrid) to 25 μg P/l (Prespa), and NO_3^- concentrations from 9 (Prespa) to 178 μg N/l (Biogradsko). Sediment TP content ranged from 241 (Lura) to 1487 mg/kg dry weight (Biogradsko).

The average number of diatom taxa per site ranged from 36 (Sava) to 90 (Crno). The diatom assemblages of lake Ohrid were dominated by taxa within the genus *Gomphonema* Ehrenberg (*G. paratergestinum* Levkov, Mitic-Kopanja & E.Reichardt, *G. prespanense* Levkov, Mitic-Kopanja & E.Reichardt, *G. pumilum* (Grunow) E.Reichardt & Lange-Bertalot and *G.* aff. *micropumilum* E.Reichardt) together with *Nitzschia dissipata* (Kützing) Rabenhorst. The diatom assemblages of lake Prespa were diverse, with a general prevalence of *G. paratergestinum* and *Pantocsekiella ocellata* (Pantocsek) K.T.Kiss & Ács. *Cyclotella cretica* var. *cyclopuncta* (Håkansson & J.R.Carter) R.Schmidt and *Encyonopsis microcephala* (Grunow) Krammer were recorded as the most dominant taxa in lake Lura. In lake Crno, *Achnanthidium minutissimum* (Kützing) Czarnecki was the most abundant taxon at all sampling sites, while lake Biogradsko was dominated by *Denticula tenuis* Kützing and *E. microcephala*. The diatom assemblages of Lake Sava were dominated by *A. minutissimum* and *E. microcephala*.

The average number of invertebrate taxa (average values for samples taken in spring and autumn in both 0.5 and 2 m water depth at 6 sites per lake) ranged from 0 (Crno) to 10 (Ohrid). The number of macroinvertebrate individuals ranged from 0 (Crno) to 3158 individuals per m^2 (Ohrid). Lake Ohrid had diverse macroinvertebrate assemblages and dominating species differed among sites. The most prevalent taxa were the freshwater amphipod *Gammarus roeselii* Gervais, and the snails *Theodoxus fluviatilis* Linnaeus and *Chilopyrgula sturanyi* Brusina. Also in lake Prespa, macroinvertebrate assemblages were diverse, and the following species reached high abundances at several sites: the amphipod *Gammarus triacanthus* ssp. *prespensis* Karaman S. & G (which according to Karaman and Pinkster (1977) is a form of *Gammarus roeselii*), the mussel *Dreissena presbensis* Kobelt, the oligochaetes *Potamothrix hammoniensis* Michaelsen and *Limnodrilus hoffmeisteri* Claparede, and the chironomid *Chironomus plumosus* Linnaeus. The dominating species in lake Sava were the snails *Valvata piscinalis* O.F.Müller, *Esperiana acicularis* Férussac and *Esperiana esperi* Férussac. The mayfly *Ephemera Danica* Müller, the snail *Radix* Montfort sp. and the caddisfly *Limnephilus* Leach sp. dominated in lake Biogradsko. We found only few macroinvertebrate individuals in lake Crno, and these were the water boatman *Micronecta scholtzi* Fieber, the oligochaete *Nais barbata* Müller and the chironomid *Chironomus plumosus*.

The average number of macrophyte taxa per transect ranged from 0 (Lura) to 11 (Ohrid). Lakes Ohrid, Prespa and Sava had high biomasses of macrophytes, lakes Biogradsko and Crno had little macrophytes, while lake Lura was practically devoid of macrophytes (except a single shoot of *Myriophyllum spicatum* L. at one site). The littoral vegetation

Table 2
Summary statistics for the analyzed lakes; averages calculated from 6 sites in each lake, standard deviations are given in brackets; see methods for calculation of macrophyte abundances; no autumn macroinvertebrate samples were taken in lake Lura in 2 m depth, and no macroinvertebrates were found in lake Crno in 0.5 m water depth. A reliable calculation of the Balkan macrophyte index requires a certain minimum occurrence of macrophyte species; therefore, no reliable BMI could be calculated in lakes Lura and Crno. BMI = Balkan macrophyte index, ASPT = average score per taxon, EPT = Ephemeroptera-Plecoptera-Trichoptera; IPS, TI, SI, TDI and TDIL are diatom indices (see methods for details).

	Lake	Ohrid	Prespa	Lura	Biogradsko	Crno	Sava
Chemistry and runoff	sed_TP [mg/kg]	469(409)	407(142)	241(117)	1487(680)	1113(1061)	283(108)
	water_TP [μg/l]	7(1.6)	25(2.6)	12(4.3)	18(2.6)	16(3.9)	11(7.2)
	water_NO3 [μg/l]	34(11)	9(4)	97(15)	178(14)	109(12)	142(58)
	Runoff [kg/ha*year]	1.6(1.6)	1.1(1)	0.3(0.05)	0.2(0.03)	0.4(0.01)	0.8(0.4)
Macrophytes	Abundance macrophytes and Cladophora (0–2 m)	384 (95)	230(145)	0.1(0.4)	16(28)	16(22)	265(118)
	Species number	11(2)	8(3)	0.2(0.4)	2(2)	1(1)	8(2)
	BMI (reliable values)	3.03(0.2)	3.72(0.1)		2.35(0.2)		3.23(0.18)
	BMI (only data from 0–2 m)	3.54(0.33)	3.35(0.25)	3(−)	3.54(1.36)	3(0)	3.35(0.15)
Macroinvertebrates	Abundance [ind/m^2] _spring_0.5	1733(1581)	1900(1273)	275(66)	421(142)	0(0)	32(18)
	Abundance [ind/m^2] _autumn_0.5	2192(2227)	1529(1037)	300(138)	650(278)	33(81)	114(81)
	Abundance [ind/m^2] _spring_2	3158(1682)	1008(681)	108(681)	238(147)	0(0)	1146(735)
	Abundance [ind/m^2]_autumn_2	938(699)	1079(959)		225(76)	8(20)	1074(870)
	Number of Taxa_spring_0.5	10(3)	10(4)	4(1)	3(2)	0(0)	6(3)
	Number of Taxa_autumn_0.5	10(3)	9(4)	3(1)	5(2)	1(1)	11(3)
	Number of Taxa_spring_2	13(4)	7(2)	3(1)	3(2)	0(0)	6(1)
	Number of Taxa_autumn_2	9(3)	5(2)		2(1)	0.2(0.4)	8(2)
	ASPT_spring_0.5	4.41(0.7)	3.78(0.7)	3.33(0.3)	5.56(1.8)		2.61(1)
	ASPT_autumn_0.5	4.41(0.7)	3.78(0.7)	3.49(0.9)	6.54(1.3)	2.67(−)	3.59(0.6)
	ASPT_spring_2	3.46(0.5)	2.81(0.7)	2.22(1.3)	5.63(2.3)		2.90(0.3)
	ASPT_autumn_2	3.44(0.7)	2.78(1.1)		5(2.3)	1(−)	3.5(0.5)
	EPT-Taxa [%]_spring_0.5	3(4)	4(5)	19(27)	62(23)		0(0)
	EPT-Taxa [%]_autumn_0.5	2(3)	9(17)	19(22)	63(14)	0(−)	19(26)
	EPT-Taxa [%]_spring_2	0.2(0.5)	0.3(0.7)	0(0)	48(43)		0(0)
	EPT-Taxa [%]_autumn_2	4(8)	1(2)		39(38)	0(−)	0(0)
Diatoms	Species number	67(19)	60(29)	73(13)	60(8)	90(9)	36(9)
	IPS	15.5(1)	14.9(0.4)	18.2(0.4)	17.1(0.3)	18.0(0.6)	16.3(1.2)
	TI	10.5(2)	9.6(1)	15.2(0.5)	15.0(0.5)	13.0(1)	13.1(2.6)
	SI	15.4(0.9)	15.6(1.1)	18.5(0.3)	18.5(0.2)	16.9(0.6)	17.5(0.8)
	TDI	6.5(2.8)	5.9(0.7)	16.7(0.7)	17.7(0.9)	14.0(1)	15.5(1.2)
	TDIL	10.7(1.2)	11.9(0.8)	14.8(0.4)	14.9(0.3)	13.9(1.1)	13.6(1.2)

of Lake Ohrid was dominated by charophytes together with different *Potamogeton* species, *Elodea canadensis* Rich. & Michx. and *Myriophyllum spicatum*. In lake Prespa, *Ceratophyllum demersum* L. dominated, together with *Myriophyllum spicatum*, *Stuckenia pectinata* (L.) Börner and *Potamogeton perfoliatus* L. The littoral vegetation of lake Biogradsko was dominated by charophytes, and that of lake Crno by *Myriophyllum spicatum*. In lake Sava, *Myriopyllum spicatum* dominated together with *Najas minor* All., *Potamogeton pectinatus* and *P. pusillus L.*

The reasons for the observed low abundances of macroinvertebrates in lake Crno and of macrophytes in lake Lura are unclear. They may possibly be related to water level fluctuations. However, in all instances we found enough littoral diatoms in 0.5 m water depth, indicating that this depth was below the water surface for at least several weeks before sampling.

3.2. Testing hypothesis 1: runoff explains biomass of benthic primary producers

We found a higher abundance of macrophytes and *Cladophora* in lakes with a higher estimated phosphorus runoff from the adjacent land (Table 3, Fig. 2a). Note that the correlation between runoff and macrophytes alone, as well as with *Cladophora* sp. alone, was marginally significant ($p < 0.1$; Table 3), and only the sum of both correlated significantly with runoff. Lakes with a higher phosphorus runoff from the adjacent land did not have higher water column nutrient concentrations. Instead, runoff correlated negatively with water nitrate concentrations in autumn, while a negative correlation with autumn ammonium concentrations was marginally significant (Table 3). No other correlations occurred between runoff and water or sediment chemistry. We also found a higher density of macroinvertebrates in lakes with a higher phosphorus runoff from the adjacent land (although the relationship in 0.5 m depth was only marginally significant).

3.3. Testing hypothesis 2: high benthic primary producer biomass is related to low dissolved nutrient concentrations

There were no relationships between the abundance of macrophytes and benthic algae and measured sediment TP, water littoral BOD, total phosphorus or total nitrogen concentrations (Table 4). However, in lakes with a high abundance of benthic primary producers, nitrate (Fig. 2b) and ammonium concentrations (if we accept $p = 0.055$; Table 3) were low, but only in autumn, not in spring (Table 3). In lakes with a higher abundance of macrophytes and benthic algae there also occurred a higher density of macroinvertebrates, but only in 2, not in 0.5 m depth.

Table 3
Pearson correlation coefficients between phosphorus runoff from the adjacent land (estimated from CORINE; log-transformed) and sediment TP content, water nutrient concentrations, estimated abundance of macrophytes and benthic algae (between 0 and 2 m water depth, algae represented by *Cladophora* sp.), and density of macroinvertebrates; TP = total phosphorus, TN = total nitrogen, BOD = biochemical oxygen demand after 5 days, MI = macroinvertebrates.

	Pearson r	p
sediment_TP_autumn	−0.47	0.347
water_TN_spring	−0.17	0.741
water_TN_autumn	−0.03	0.951
water_TP_spring	0.13	0.804
water_TP_autumn	−0.29	0.580
water_BOD_spring	0.43	0.397
water_BOD_autumn	−0.01	0.984
water_NO3_spring	−0.39	0.449
water_NO3_autumn	**−0.89**	**0.018**
water_NH4_spring	0.06	0.914
water_NH4_autumn	*−0.78*	*0.069*
abundance macrophytes	*0.73*	*0.097*
abundance *Cladophora*	*0.75*	*0.087*
abundance macrophytes plus *Cladophora*	**0.96**	**0.002**
MI density_0.5 m [ind/m^2]	*0.77*	*0.074*
MI density_2m [ind/m^2]	**0.94**	**0.006**

Significant relationships are marked in bold, marginally significant relationships are marked in italics.

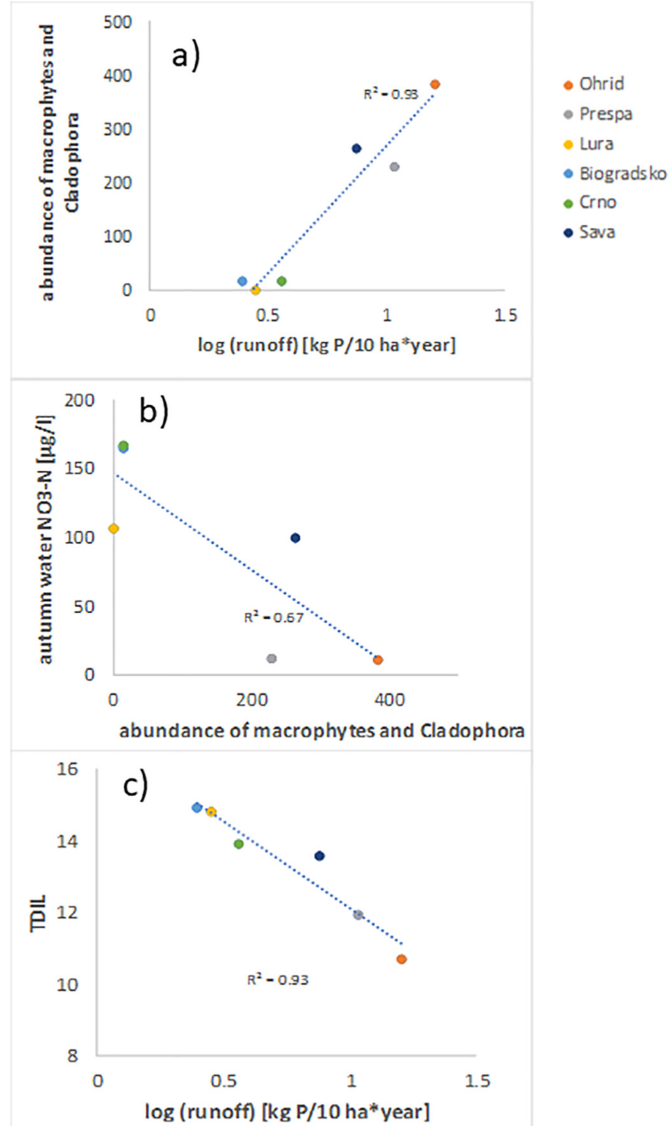

Fig. 2. Estimated runoff from the adjacent land plotted against the abundance of macrophytes and benthic algae (algae represented by *Cladophora* sp.) (a), and the diatom index TDIL (c); estimated abundance of macrophytes and benthic algae (algae represented by *Cladophora* sp.) plotted against autumn littoral water nitrate concentrations (b); note that log(runoff) is given per 10 ha in order to avoid negative numbers; note that lakes Biogradsko and Crno almost lie on top of each other in panel (b).

3.4. Testing hypothesis 3: WFD indices will reflect runoff more closely than measured water nutrient concentrations

Neither the macrophyte index BMI, nor the macroinvertebrate index ASPT or the proportion of EPT taxa were related to estimated phosphorus runoff from the adjacent land or any of the measured water or sediment chemical parameters (Table 5). Four of five diatom indices were related to autumn water nitrate concentrations, indicating that lakes with higher nitrate concentrations had higher, i.e. more “oligotrophic”, diatom indices. However, all diatom indices were negatively correlated with runoff, indicating that lower, i.e. more “eutrophic” diatom indices occurred in lakes with higher phosphorus runoff from the adjacent

Table 4
Pearson correlation coefficients between estimated abundance of macrophytes and benthic algae (algae represented by *Cladophora* sp.) and sediment TP content as well as water nutrient concentrations in the littoral and the density of macroinvertebrates; TP = total phosphorus, TN = total nitrogen, BOD = biochemical oxygen demand after 5 days, MI = macroinvertebrates.

	Pearson r	p
sediment_TP_autumn	−0.43	0.392
water_TN_spring	−0.16	0.756
water_TN_autumn	−0.17	0.753
water_TP_spring	0.00	0.994
water_TP_autumn	−0.44	0.377
water_BOD_spring	0.34	0.516
water_BOD_autumn	0.03	0.950
water_NO3_spring	−0.14	0.789
water_NO3_autumn	**−0.82**	**0.046**
water_NH4_spring	0.23	0.663
water_NH4_autumn	*−0.80*	*0.055*
MI density_0.5 m [ind/m^2]	0.69	0.129
MI density_2m [ind/m^2]	**0.98**	**0.000**

Significant relationships are marked in bold, marginally significant relationships are marked in italics.

land (see Fig. 2c for the TDIL index). Although all diatom indices indeed were significantly correlated with runoff, the correlation with the TDIL was closest, suggesting that the TDIL may be a good metric for assessing nutrient enrichment in lakes of the Western Balkans.

4. Discussion

In principle, an increased intensity of agriculture and urbanization in the nearshore surroundings should lead to an increased supply of nutrients to the lake littoral (Fig. 3). This should initially lead to increased littoral water nutrient concentrations (Fig. 3). However, from the littoral water, nutrients will be removed either by incorporation into the biomass of benthic organisms, by sedimentation to the littoral sediment (from where they still are available to aquatic macrophytes; Barko and Smart, 1981), or by transport to the pelagic and profundal of the lake (Fig. 3). Pelagic water chemistry, therefore, may underestimate nutrient availability to plants and algae in the littoral (Lambert et al., 2008). However, for lake status assessment according to the WFD, pelagic water chemistry, measured above the deepest point of a lake, generally is used (e.g. Dudley et al., 2013). This may lead to mismatches between a “surprisingly eutrophic” littoral, and low pelagic water nutrient concentrations, as observed e.g. in lake Ohrid (Schneider et al., 2014; Vermaat et al., 2020). In our project, we measured littoral water nutrient concentrations, because we expected littoral water chemistry to be more relevant for littoral organisms such as macrophytes, diatoms and macroinvertebrates, since they may have access to land-derived nutrients before they are diluted in the open water (see e.g. Lambert et al., 2008; Kelly et al., 2018). Indeed, we measured slightly higher TP concentrations in the littoral of lake Ohrid (7 μg/l, Table 2) than what was reported for the upper pelagic in 2013/2014 (5 μg/l; Veljanoska-Sarafiloska et al., 2019).

However, despite using littoral instead of pelagic water chemistry, none of the tested metrics was meaningfully correlated with any of the measured water chemical parameters (Table 5; note that eutrophic diatom assemblages were associated with low autumn nitrate concentrations; these correlations therefore would not be interpreted as meaningful). Such an absence of meaningful correlations between water chemistry and biological metrics is generally interpreted as a sign of poorly performing metrics (Carvalho et al., 2019). However, lakes with diatom communities indicating nutrient rich conditions also had a higher abundance of benthic primary producers, which was then associated with lower concentrations of nitrate and ammonium (ammonium: marginally significant), but only in autumn, not in spring (Table 4). This was anticipated (hypothesis 2) and can easily be explained with primary producers taking up nitrate and ammonium and incorporating it into biomass. Since this mainly occurs during the summer growth season, spring nutrient concentrations were unaffected. It is also likely that benthic primary producers contributed indirectly to the decrease in nitrate concentrations by providing surface area for denitrifying bacteria (Weisner et al., 1994). Unfortunately, we did not measure soluble reactive phosphorus (SRP), but we assume that SRP would have given similar results to nitrate and ammonium. Total phosphorus and total nitrogen concentrations, as well as BOD, were unaffected, probably because particulate nutrients and BOD are not

Table 5
Pearson correlation coefficients between phosphorus runoff from the adjacent land (calculated from CORINE landcover; log-transformed), sediment and water chemistry, with macrophyte, macroinvertebrate and diatom indices commonly used for ecological status assessment; BMI = Balkan macrophyte index (reliable values for the entire littoral, and separately for depth 0–2 m), ASPT = average score per taxon, EPT = proportion of Ephemeroptera, Plecoptera and Trichoptera; IPS, TI, SI, TDI and TDIL are diatom indices (see methods for further details), TP = total phosphorus, TN = total nitrogen, BOD = biochemical oxygen demand after 5 days.

	BMI (reliable values)	BMI0–2	ASPT_0.5	ASPT_2	EPT_0.5	EPT_2	IPS	Rott_TI	Rott_SI	TDI	TDIL
sed_TP_autumn	−0.89	0.24	0.53	0.36	0.60	0.77	0.26	0.30	0.24	0.35	0.36
	p = .110	p = .644	p = .283	p = .477	p = .212	p = .072	p = .615	p = .568	p = .647	p = .501	p = .484
water_TN_spring	−0.12	0.05	−0.07	0.02	0.12	0.25	0.01	0.01	0.06	0.23	0.25
	p = .884	p = .928	p = .890	p = .977	p = .828	p = .630	p = .988	p = .979	p = .916	p = .654	p = .629
water_TN_autumn	−0.20	0.23	0.39	0.11	0.21	0.43	−0.18	−0.32	−0.35	−0.32	−0.16
	p = .801	p = .660	p = .441	p = .830	p = .696	p = .399	p = .726	p = .537	p = .498	p = .537	p = .767
water_TP_spring	0.29	0.31	0.30	0.22	0.19	0.31	−0.52	−0.46	−0.35	−0.39	−0.18
	p = .707	p = .544	p = .564	p = .680	p = .724	p = .557	p = .293	p = .354	p = .492	p = .443	p = .740
water_TP_autumn	0.27	−0.19	0.01	−0.09	0.19	0.20	−0.07	−0.10	0.03	−0.02	0.26
	p = .728	p = .713	p = .981	p = .864	p = .713	p = .709	p = .901	p = .857	p = .960	p = .968	p = .612
water_BOD_spring	0.81	0.31	−0.06	−0.03	−0.24	−0.02	−0.57	−0.65	−0.61	−0.48	−0.42
	p = .191	p = .556	p = .915	p = .951	p = .647	p = .975	p = .238	p = .160	p = .199	p = .331	p = .411
water_BOD_autumn	0.06	0.47	0.39	0.56	0.47	0.47	−0.44	−0.08	0.09	0.05	0.13
	p = .944	p = .347	p = .445	p = .252	p = .349	p = .346	p = .385	p = .874	p = .869	p = .923	p = .799
water_NO3_spring	−0.72	0.37	0.43	0.64	0.66	0.62	0.23	0.62	0.65	0.71	0.52
	p = .281	p = .470	p = .398	p = .168	p = .154	p = .186	p = .660	p = .190	p = .166	p = .114	p = .285
water_NO3_autumn	−0.78	−0.39	0.05	−0.02	0.50	0.47	**0.83**	**0.82**	0.78	**0.88**	**0.88**
	p = .220	p = .449	p = .931	p = .969	p = .317	p = .342	**p = .039**	**p = .046**	p = .070	**p = .021**	**p = .021**
water_NH4_spring_1	0.10	0.03	−0.31	0.08	−0.08	−0.19	0.00	0.23	0.30	0.39	0.21
	p = .899	p = .961	p = .549	p = .885	p = .885	p = .725	p = 1.00	p = .662	p = .558	p = .446	p = .694
water_NH4_autumn_1	−0.81	−0.36	0.08	−0.12	0.40	0.47	0.70	0.55	0.48	0.59	0.67
	p = .192	p = .480	p = .876	p = .820	p = .436	p = .347	p = .120	p = .256	p = .332	p = .215	p = .144
log(runoff)	0.73	0.47	−0.14	−0.02	−0.62	−0.50	**−0.87**	**−0.90**	**−0.89**	**−0.88**	**−0.96**
	p = .265	p = .342	p = .792	p = .974	p = .191	p = .316	**p = .025**	**p = .014**	**p = .017**	**p = .022**	**p = .002**

Significant relationships are marked in bold.

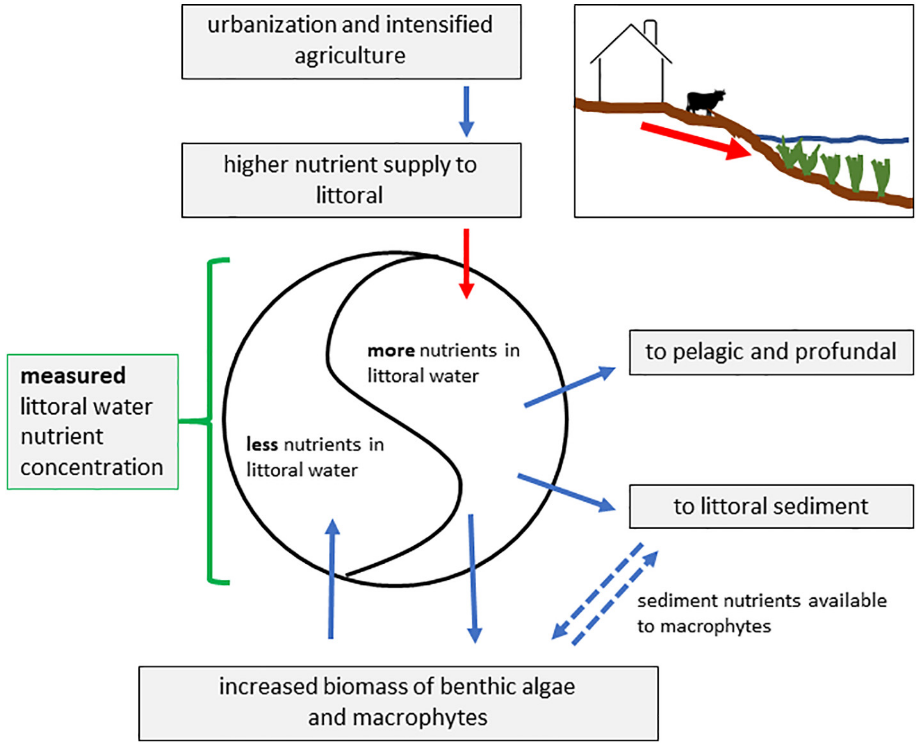

Fig. 3. Conceptual model of the fate of nutrients reaching the littoral from the adjacent land.

immediately available to primary producers, and because disturbance of the littoral by e.g. waves and boating may affect particle concentrations. The absence of a correlation between benthic primary producer abundance and water total phosphorus and total nitrogen concentrations also indicated that sedimentation of nutrient rich particles in dense macrophyte patches (Sand-Jensen, 1998) was not in all cases outweighed by their supply and resuspension. This is likely because we only looked at the shallow littoral (up to 2 m depth), where dense macrophyte cover is not continuous (own observations). In this upper littoral, resuspension of particles by e.g. wave action may have played a role. Also, resuspension of sediment in the shallow littoral strongly depends on weather conditions, such that day-to-day variation may have masked the effect of sedimentation in dense macrophyte patches.

Water nutrient concentrations are always the result of supplied minus removed nutrients (Fig. 3), and an increase in concentrations occurs where supply exceeds removal. In lakes where nutrients are efficiently retained in benthic habitats, both pelagic and littoral water nutrient concentrations therefore are a poor descriptor of the stressor "nutrient enrichment". We therefore approximated nutrient enrichment by calculating phosphorus runoff from CORINE land cover and found that commonly used diatom indices targeting nutrient enrichment were significantly and meaningfully correlated with runoff, but not with littoral water chemistry (Table 5). This indicates that CORINE land cover may be more useful for the characterization of nutrient enrichment in the littoral of lakes than water chemistry, and that using phosphorus runoff estimated from CORINE land cover, instead of water chemistry, as an approximation for the stressor "nutrient enrichment" may improve stressor-response relationships for benthic diatoms (hypothesis 3). We are aware of the fact that the absolute values of export coefficients for different land use categories vary among different studies (see e.g. Johnes et al., 1996; Pasztaleniec and Kutyła, 2015; Palviainen et al., 2016). However, their relative ranking generally is the same (urban > agriculture > grassland > forest). We are therefore confident that using different export coefficients – if it had been possible - may have affected the strength of the observed correlations, but likely would not have changed the overall result.

However, macrophyte and macroinvertebrate WFD indices were not related to runoff either (Table 5). For macrophytes, this changed when looking at a different response parameter. All primary producers need nutrients to grow. Increased nutrient input should therefore initially lead to increased primary producer biomass. This is consistent with Lambert et al. (2008), who found that littoral periphyton biomass increased with increasing development of the immediate lake surroundings, and with the results of our study, where lakes with a higher runoff from the adjacent land also had higher total benthic primary producer abundances (hypothesis 1; Table 3). Together with the observation that the macrophyte index BMI was unrelated to runoff, this may indicate that increased nutrient supply has led to increased growth of benthic primary producers (i.e. affected an ecosystem function) but has not (yet) significantly changed species composition (i.e. ecosystem structure) towards species typically occurring in nutrient enriched conditions. Metrics based on species composition that ignore benthic primary producer growth, such as the BMI and most macrophyte indices used for assessment according to the WFD (e.g. Schaumburg et al., 2004; Penning et al., 2008), cannot capture such comparatively "early" signs of nutrient enrichment. Similar observations have been made e.g. in Norway, where mass development of *Juncus bulbosus* L., a species preferring nutrient poor conditions, is described from rivers and lakes with low nutrient concentrations and "oligotrophic" macrophyte vegetation (Schneider et al., 2013; Moe et al., 2013). We therefore argue that growth of benthic primary producers should be incorporated into WFD assessment systems, in order to capture comparatively "early" responses to nutrient enrichment. We are aware that the abundance of primary producers used here may not necessarily represent annual growth, since a mix of annual and perennial plants may occur. However, most species which typically occurred in the shallow littoral of our study lakes were annual. While the simple method used in our study does not give absolute values of biomass in g/m^2, it has the advantage that no additional measurements are necessary in the field. These calculations can therefore be done on existing data, offering the possibility to test our hypothesis on existing datasets across Europe. Note that, in larger datasets containing severely polluted lakes, the relationship between macrophyte biomass and nutrient enrichment is expected to be hump-shaped because biomass production of especially charophytes is negatively affected by nutrient addition (Bakker et al., 2010) and light limitation generally constrains macrophyte growth in eutrophic lakes (Egertson et al., 2004). Consequently, low biomass of benthic primary producers may not be used as unanimous indicator for low nutrient enrichment.

Similarly, macroinvertebrate densities were also related to estimated phosphorus runoff (significantly in 2 m, marginally significant in 0.5 m depth; Table 3), while the ASPT and EPT% metrics were unrelated to runoff and water chemistry (Table 5). The relationship between runoff and macroinvertebrate density is likely to be indirect, since macroinvertebrates, depending on organic material for food, should not directly benefit from an increased supply of phosphorus. Indeed, macroinvertebrate densities were also related to the abundance of benthic primary producers (Table 4), at least at 2 m water depth, where there is less disturbance by wave action than at 0.5 m. This may indicate that an increased abundance of benthic primary producers, possibly caused by increased nutrient supply, provides more food and habitat for macroinvertebrates (Cyr and Downing, 1988). Consistent with our results, Timm and Mols (2012) could not find meaningful correlations between macroinvertebrate metrics (including the ASPT and EPT) and lake total phosphorus concentrations or the percentage of "green" shorelines (i.e. forests, bogs, shrubbery, natural meadows). Trophic effects on benthic invertebrates may be masked by local habitat heterogeneity (McGoff and Sandin, 2012). Therefore, littoral macroinvertebrates remain a challenging group for the assessment of nutrient enrichment in lakes.

Knowledge of littoral ecosystems is important, for example when deciding if more beaches, more restaurants, hotels or marinas should be permitted at a lake shore, possibly posing a threat to littoral ecosystem functioning. However, current littoral assessment metrics are often "mistrusted" because of poor "stressor – response" relationships (Carvalho et al., 2019). Among our study lakes, littoral diatoms indicated more nutrient-enriched conditions in lake Ohrid than in lakes Sava and Prespa, even though lake Ohrid has long been "famous" for its clear water and low water nutrient concentrations (Matzinger et al., 2007), while lakes Prespa and Sava have a well-known history of eutrophication (Matzinger et al., 2006; Jovanović et al., 2017). Our results indicate that "eutrophic" littoral diatom indices should be taken seriously, even if pelagic or littoral water nutrient concentrations are low. In such lakes, the littoral is likely to significantly contribute to nutrient translocation, as indicated by the high abundance of benthic primary producers. Nutrient enrichment primarily leads to increased growth of primary producers, and in a second step, via immigration and competition, to changes in species composition (Schneider et al., 2016). This means that macrophyte growth reacts faster to nutrient enrichment than structural metrics based on macrophyte species composition. Similarly, diatom species composition reacts faster to nutrient enrichment than macrophyte species composition (Schneider et al., 2012). We therefore argue that both increased macrophyte biomasses and "eutrophic" diatom indices may be interpreted as "early warning" signals for nutrient enrichment in the littoral of lakes, even where water nutrient concentrations are low. This is consistent with Lambert et al. (2008), who found that periphyton biomass in lakes in Canada increased with lake recreational development but was not related with open water phosphorus concentrations. Lambert et al. (2008) concluded that periphyton biomass may be a tool for early detection of lake perturbation.

We are aware that our results are based on six lakes only, and that we only have correlative, not causal evidence. Many issues need to be examined using larger datasets, e.g. the importance of the extent of the littoral, a possible role of emergent littoral vegetation, the interaction between littoral and pelagic nutrient cycling, a possible role of macrophyte traits, the size and shape of the area from where land use may affect the lake littoral, and many more. Consistent datasets such as ours, using the same methods at the same sites for many parameters in a considerable number of sites per lake, are rare, probably because it takes a substantial amount of time and effort to collect such data. Despite the limitations of our data, our results are plausible. In order to collect more evidence, we suggest that our results are put to the test using large trans-national datasets, for example the WISE WFD database hosted by the European Environment Agency. Such data will have less consistency of methods than our data, but this may be outweighed by the greater number of lakes.

CRediT authorship contribution statement

Susanne C. Schneider: Conceptualization, Formal analysis, Investigation, Writing - original draft, Project administration, Funding acquisition. **Vera Biberdžić:** Investigation, Writing - review & editing. **Vjola Braho:** Investigation, Writing - review & editing. **Biljana Budzakoska Gjoreska:** Investigation, Writing - review & editing. **Magdalena Cara:** Investigation, Writing - review & editing. **Zamira Dana:** Investigation, Writing - review & editing. **Pavle Đurašković:** Investigation, Writing - review & editing. **Tor Erik Eriksen:** Investigation, Writing - review & editing. **Dag Hjermann:** Software, Formal analysis, Writing - review & editing. **Alma Imeri:** Investigation, Writing - review & editing. **Katarina Jovanović:** Investigation, Writing - review & editing. **Jelena Krizmanić:** Investigation, Writing - review & editing. **Lirika Kupe:** Investigation, Writing - review & editing. **Tatjana Loshkoska:** Investigation, Writing - review & editing. **Joanna Lynn Kemp:** Formal analysis, Writing - review & editing. **Aleksandra Marković:** Investigation, Writing - review & editing. **Suzana Patceva:** Investigation, Writing - review & editing. **Jelena Rakočević:** Investigation, Writing - review & editing. **Katarina Stojanović:** Investigation, Writing - review & editing. **Marina Talevska:** Investigation, Writing - review & editing. **Sonja Trajanovska:** Investigation, Writing - review & editing. **Sasho Trajanovski:** Investigation, Writing - review & editing. **Elizabeta Veljanoska-Sarafiloska:** Investigation, Writing - review & editing. **Danijela Vidaković:** Investigation, Writing - review & editing. **Konstantin Zdraveski:** Investigation, Writing - review & editing. **Ivana Živić:** Investigation, Writing - review & editing. **Jan E. Vermaat:** Conceptualization, Investigation, Writing - review & editing.

Declaration of competing interest

We confirm that this manuscript has not been published elsewhere and is not under consideration by another journal. All authors have approved the manuscript, do not have any conflict of interest and agree with its submission to Science of the Total Environment.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2020.141193.

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