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PRIMARY RESEARCH PAPER

A new trophic index (TIM_2S) to evaluate trophic alteration of small shallow lakes: a predictive reference-based approach

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Abstract Small shallow lakes (SSLs) have great conservation value and support numerous ecosystem services. However, these small ecosystems are faced with many threats, including eutrophication, which tends to shift biodiverse SSLs to a turbid state dominated by phytoplankton. The ecological quality of SSLs still remains poorly evaluated because of the lack of adapted tools. We propose a new trophic index—TIM_2S—based on the tolerance range of 245 macrophyte species to total phosphorus. As a single trophic index can favour oligotrophic ecosystems and their associated species to the detriment of more eutrophic but rare species, we converted TIM_2S into a predictive reference-based model. Then, we compared TIM_2S with five existing trophic indices in their efficiency to discriminate trophic levels and disentangle eight anthropogenic or internal pressures. TIM_2S was the only index strongly correlated with total phosphorus and able to discriminate trophic levels. Most existing trophic indices are expert-based, and reflect community alteration rather than eutrophication. These expert-based indices are also dependent on numerous environmental factors, highlighting the need for robust predictive models to evaluate ecological statuses accurately. TIM_2S is Water Framework Directive-compatible and can be used widely in Europe to evaluate the trophic status and trophic alterations of SSLs.

Keywords Aquatic macrophytes · Bioassessment · Eutrophication

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Introduction

Small shallow lakes (SSLs) are the most abundant lake types (Meerhoff & Jeppesen, 2010) and provide numerous ecosystem and social services, such as nutrient retention (Hilt et al., 2017) or carbon storage (Gilbert et al., 2021). They have a great conservation value because they harbor a rich and original biodiversity (Williams et al., 2004). SSLs face many threats, including climate change, pollution due to agricultural intensification and land artificialization, and inappropriate management (Indermuehle et al., 2008). Among these threats, eutrophication is one of the most common causes of water quality degradation (Le Moal et al., 2019) and aquatic biodiversity alteration (Schindler, 2006). Eutrophication can cause SSLs to shift to a turbid state (Scheffer et al., 1993; Meerhoff et al., 2022), with dramatic losses in ecosystem services and biodiversity for fish, birds, macrophytes and invertebrates (Hilt et al., 2017). As a consequence, monitoring trophic alteration of SSLs is essential for preserving the biodiversity and ecosystem services they provide (Williams et al., 2004).

Macrophytes are sensitive to the trophic level of water bodies (Hootsmans & Vermaat, 1991; Thiébaut & Muller, 1999; O'Hare et al., 2018). At the community level, aquatic plants have been used as indicators of eutrophication of freshwater ecosystems for many years (Carbiener et al., 1990; Holmes et al., 1999; Thiébaut & Muller, 1999). Numerous indices based on the trophic profile of macrophyte species have been developed in Europe to evaluate the trophic status of rivers (Holmes et al., 1999; Schneider & Melzer, 2003; Haury et al., 2006) or large lakes (Stelzer et al., 2005; Seo et al., 2014). However, SSLs—less than 50 ha in size—are rarely included in monitoring programs (Biggs et al., 2017). Only two trophic indices have been developed for SSLs in Europe: the macrophyte nutrient index for ponds (M-NIP) was developed in Switzerland for SSLs with surface areas from 6 m^2 to 9.62 ha (Sager & Lachavanne, 2009); the trophic ranking score (Palmer, 1992) and its reference-based version included in the predictive system for multimetrics (PSYM) index developed in Great Britain for SSLs with surfaces below 5 ha (Biggs et al., 2000). Their application at a larger geographical scale or to larger SSLs has not been tested. Numerous trophic indices for lakes in Europe concerned only standing waters with surface area > 50 ha (Table 1). Most of them exclude helophytes as indicator species, potentially crucial for the accuracy of a trophic index, especially for the smallest SSLs, with low floristic richness (Labat et al., 2021). Most existing trophic indices transposable to SSLs are expert-based (e.g. IBML for WFD French lakes), or community-based (e.g. TRS, PLEX), and could be uncorrelated with trophic levels as observed in other waterbodies like rivers (Demars et al., 2012).

As a consequence, numerous SSLs lack an adequate indicator to assess their trophic level. Moreover, existing trophic indices such as M-NIP are not reference-based or are specific to water types (Hering et al., 2010). Interpretation of these indices implies that eutrophic waters are the result of an anthropogenic eutrophication, although eutrophic waters can be the natural trophic status of SSLs in lowlands due to long water resident times and low geographic relief (Borics et al., 2013). These eutrophic waterbodies can shelter patrimonial or rare species (e.g., *Ranunculus peltatus baudoti* and *Butomus umbellatus*) (Rosset et al., 2014). This highlights the need to maintain various trophic conditions in an SSL network for biological conservation (Rosset et al., 2014). Different trophic conditions could be taken into account in a reference-based trophic index, considering eutrophic waters resulting from a natural process in a "good" status.

Our goals were to propose (1) new trophic profiles for macrophytes of standing waters, widely applicable at the European scale, and (2) a new trophic index for SSLs computed from these new trophic profiles. To reach these goals, we compared the performance of this new trophic index with the performances of other existing trophic indices. The aims were (2a) to discriminate trophic levels, and (2b) to identify sources of eutrophication pressures or plant community alterations. Our first hypothesis was that the new index would be more correlated than the other indices with the trophic levels of SSLs. Our second hypothesis was that the new reference-based index would better discriminate the impacted sites and the pressure they underwent than the other indices did.

Materials and methods

The main steps of the design are summarized in Fig. 1, from data collection to the construction of the reference-based index and the identification of its ecological quality class boundaries.

Development of a new trophic profile for plants and its associated trophic index

Trophic levels of lakes and ponds are usually assessed from nutrient concentrations in water [total phosphorus (TP)], water transparency or chlorophyll a (Vollenweider & Kerekes, 1982; Søndergaard et al., 2005). Phosphorus is a key factor for aquatic plants in freshwaters, and it is easy to assess from TP concentrations (Correll, 1999). TP is highly correlated with water transparency and chlorophyll a (Qin et al., 2012), and covaries with nitrogen (Håkanson, 2012).

Table 1 Main plant indices used to evaluate trophic alterations in European standing waters

Index	Conception							Ecosystem	Area (number of climatic region)	Weakness	Strength
	Species nutrient affinity	Expert-based	Community-based	Species ecological amplitude	Abundance/coverage	Helophytes included	Reference-based				
M-NIP (Sager & Lachavanne, 2009)	X			X	X	X		Alpine ponds and small lakes < 10 ha	Switzerland (2)	Only two climatic regions Mostly calcareous geology SSLs > 10 ha not concerned	Adapted to SSLs Helophytes included Correlated with TP
TRS (Palmer, 1992)			X			X	X	Ponds, lakes and canals	Great Britain (1)	Only one climatic region Community-level	Adapted to SSLs, with a wide range of surface area Helophytes included
PLEX (TRS derivative) (Duigan et al., 2007)			X				(X)	Ponds and lakes	Great Britain (1)	Only one climatic region Community-level Helophytes excluded	Adapted to SSLs, with a wide range of surface area
IBML (Boutry et al., 2013)		X		X	X	X	X	Lakes and shallow lakes > 5 ha (usually > 50 ha)	France (4)	Expert-based Not fitted for SSLs < 5 ha	Four climatic regions Helophytes included
RI (Stelzer et al., 2005)		X			X		X	Lakes and shallow lakes > 50 ha	Germany (2)	Not fitted for SSLs Only two climatic regions Not based on nutrient	
ESMI (Ciecierska & Kolada, 2014)					X		X	Lowland alkaline lakes > 50 ha	Poland (1)	Not fitted for SSLs Only one climatic region Not based on nutrient-	Potentially fitted to evaluate stable equilibria status

Table 1 (continued)

Index	Conception							Ecosystem	Area (number of climatic region)	Weakness	Strength
	Species nutrient affinity	Expert-based	Community-based	Species ecological amplitude	Abundance/coverage	Helophytes included	Reference-based				
ICM_{LM} (European Commission. Joint Research Centre. Institute for environment and Sustainability & Poikane, 2009)	X				(x)			European lakes > 50 ha	North of Europe (3?)	Not fitted for SSLs Helophytes excluded	At least three climatic regions Correlated with TP
Plant Trophic Score (Free et al., 2006)	X						X	Lakes > 50 ha	Ireland (1)	Not fitted for SSLs Only one climatic region Helophytes excluded	Based on nutrient
Tic (Penning et al., 2008)				X			X	Lakes > 50 ha	Norway (3)	Not fitted for SSLs Helophytes excluded Limited number of indicator species	Three climatic regions

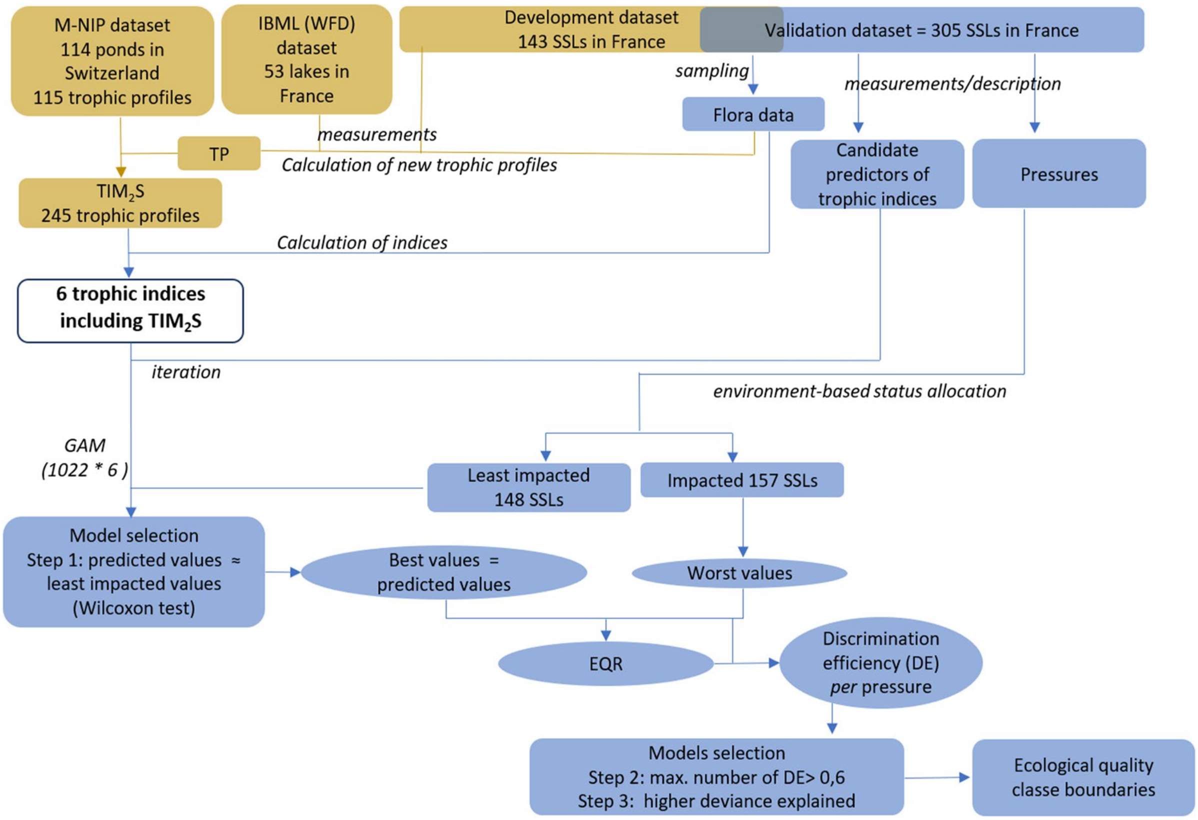

Fig. 1 Flow diagram showing the main steps of the construction of TIM_2S and the efficiency test assessing the ability of all trophic indices to discriminate anthropogenic pressures and define ecological quality class boundaries

Trophic profiles are addressed in the literature for species living in lakes and rivers (Landolt, 1977; Melzer, 1988; Bornette et al., 1994; Robach et al., 1996; Eglin et al., 1997; Holmes et al., 1999; Willby et al., 2000; Thiébaut, 2008). They represent the nutrient ranges within which plant species can be found. We followed the methodology developed for the M-NIP to build our new trophic index. However, M-NIP is built for Alpine ponds and small Swiss lakes (6 to 96,200m^2, and ranged along an altitudinal gradient from 210 to 2757 m above sea level a.s.l.).

To build a database from the trophic profiles of macrophyte species found in western European standing waters, we selected floristic and TP data from four climatic regions (Alpine, Atlantic, Continental, Mediterranean): 143 SSLs sampled with the S_3m method (Labat et al., 2022) in France, 114 ponds from the M-NIP database (Sager & Lachavanne, 2009) sampled with the IBEM method in Switzerland (Indermuehle et al., 2010), and 53 lakes sampled with the protocol of the "indice biologique macrophyte lacustre" (IBML) (AFNOR, 2010) in France. Surface area of the standing waters were from 1 m^2 to 58.3 km^2, and covered an altitude range from 3 to 3340 m above sea level. Principal characteristic of SSLs sampled with the S_3m method were summarized in Table 2. Mediterranean region was less sampled, because it was difficult to find SSLs with aquatic plants and water during the vegetation period.

TP was measured in winter, when biological activity is at its minimum intensity and the concentration of nutrients in their inorganic form tends to be highest (Linton & Goulder, 2000). Water samples were taken in the euphotic zone near the deepest point of each SSL, using a sampling bottle (approx. 20 cm under the water surface for ponds and SSLs, and an integrated sampling for lakes). Samples were stored in a cooler and were analyzed in less than 24 h. TP was measured by spectroscopy by an accredited laboratory (AFNOR, 2009), or using the ascorbate acid/

Table 2 Main features of the tested SSLs (mean and standard deviation in brackets, except for number of SSLs) of SSLs from development and validation dataset. A.s.l. = above sea level

Climatic region	Number of SSLs	Surface area (m^2)	Mean depth (m)	Elevation (m a.s.l.)	TP (mg/L)
Development dataset					
Alpine	19	1916 (±42,978)	1.66 (±2.00)	1647 (±726)	0.049 (±0.082)
Atlantic	75	16,088 (±44,041)	1.09 (±1.44)	82 (±99)	0.038 (±0.067)
Continental	44	44,240 (±81,473)	1.61 (±1.58)	502 (±347)	0.076 (±0.208)
Mediterranean	5	24,434 (±48,884)	1.60 (±0.65)	438 (±319)	0.014 (±0.005)
Validation dataset					
Alpine	45	10,812 (±30,789)	1.09 (±1.53)	1895 (±612)	
Atlantic	160	18,683 (±86,706)	1.10 (±1.72)	79 (±89)	
Continental	94	39,684 (±72,314)	1.31 (±1.26)	515 (±374)	
Mediterranean	6	20,409 (±44,822)	1.83 (±0.81)	491 (±313)	

molybdenum blue method for M-NIP data (APHA et al., 1998). Then, TP values were converted into trophic categories: oligotrophic (0–10 µg/l TP), mesotrophic (10–35 µg/l), eutrophic (35–100 µg/l), or hypertrophic (> 100 µg/l) according to OECD threshold values (Vollenweider & Kerekes, 1982).

Trophic profiles were defined based on two metrics, i.e., the indicator value (*IV*) and the ecological tolerance of each species.

To do so, we followed a procedure similar to that of Schneider & Melzer (2003) for rivers and by Sager & Lachavanne (2009) for SSLs. Species present in less than three SSLs were excluded.

The *IV* was calculated for each species using weighted averaging (Eq. (1)):

$$IV_{\mathrm{a}} = \frac{\sum_{i=1}^{n} O_{ai} \times T_i}{\sum_{i=1}^{n} O_{ai}}, \tag{1}$$

where IV_{a} is the indicator value of species *a*, O_{ai} the number of occurrences of species *a* in trophic category *i*, and T_i the value of trophic category *i* (from 1 = oligotrophic to 4 = hypertrophic, according to the thresholds defined by Vollenweider & Kerekes (1982)).

In order to express the ecological tolerance of a species to phosphorus, we calculated the root-mean-square-deviation weighted by the number of occurrences in each nutrient category.

Tolerance t_{a} was calculated for each species using the root-mean-square-deviation weighted by the number of occurrences of the species in each trophic category (Eq. (2)), and converted according to Table 3:

$$t_{\mathrm{a}} = \sqrt{\frac{\sum_{i=1}^{n} \left(T_i - IV_{\mathrm{a}}\right)^2 \times O_{\mathrm{ai}}}{\sum_{i=1}^{n} O_{\mathrm{ai}}}}, \tag{2}$$

where t_{a} is the tolerance of species *a*, T_i is the value of nutrient category *i* (from 1 = oligotrophic to 4 = hypertrophic), IV_{a} is the indicator value of species *a*, and O_{ai} is the number of occurrences of species *a* in trophic category *i*. Then, t_{a} and IV_{a} computed with our dataset were weighted by t_{a} and IV_{a} obtained by Sager & Lachavanne (2009) according to the number of observations of each plant species in respective datasets.

The new index—called "trophic index for macrophytes of small shallow lakes" (TIM_2S; Eq. (3)) corresponded to the formula of M-NIP (Sager & Lachavanne, 2009) or of TIM (Schneider and Melzer,

Table 3 Correspondence between the ecological tolerance range and the weighting factors attributed to each indicator species

Tolerance *ta*	Weighting factor *W*
0–0.2	16
0.2–0.4	8
0.4–0.6	4
0.6–0.8	2
> 0.8	1

2003), and was also the term used in the saprobic index of Zelinka & Marvan (1961):

$$TIM_2S = \frac{\sum_{i=1}^{n} IV_a \times W_{pa} \times Q_a}{\sum_{i=1}^{n} W_{pa} \times Q_a}, \quad (3)$$

where TIM_2S is the new trophic index for SSLs, IV_a is the indicator value of species a, W_{pa} is the weighting factor corresponding to the ecological amplitude of species a (Table 3), Q_a the quantity of plant species *a* in the SSL, corresponding to the cubed abundance class scale of species *a*.

Performance of the new trophic index for French SSLs

To evaluate the performance of TIM_2s, six candidate indices were compared with the S_3m protocol in 305 SSLs sampled in France. These indices (Table 1) were TIM_2S, M-NIP, the plant lake ecotype index for Great Britain lakes (PLEX; Duigan et al., 2007), the trophic ranking score (TRS; Palmer, 1992), the French macrophyte index for Water Framework Directive (WFD) lakes (IBML; Boutry et al., 2013), and the intercalibration common metric for European WFD lakes (ICM_{LM}; Hellsten et al., 2014; Kolada et al., 2014). PLEX and TRS are community-based indices, with scores corresponding to community types, and are sensitive to trophic alteration (Duigan et al., 2007) or TP reduction (Gunn et al., 2013). PLEX is an update of TRS, excluding helophytes and redefining plant scores through a more precise typology. M-NIP is a TP-based trophic index, with profiles defined from TP concentrations, including ecological amplitude of species. The trophic profiles and ecological amplitudes of IBML are expert-based. ICM_{LM} was developed for WFD intercalibration exercises, and propose a weighted-mean TP score from a wide set of WFD lakes, excluding helophytes, and do not considered ecological amplitude of plant species (European Commission. Joint Research Centre. Institute for environment and Sustainability & Poikane, 2009; Kolada et al., 2014).

Study area and field survey

Fieldwork was performed from 2013 to 2021. The SSLs differed by their geology (calcareous to siliceous), water supply (rainfall, groundwater, river flow), surface area (1 m^2 to 41.4 ha), mean depth (0.05 to 13 m), elevation (2 to 3340 m above sea level) and climatic region (Alpine, Mediterranean, Continental, Atlantic). They were man-made or natural.

Aquatic macrophytes and riparian vegetation were surveyed according to the S_3m protocol (Labat et al., 2022) during the vegetation growth period (mostly in summer, except for the Mediterranean area, monitored in spring). Vegetation abundance was assessed using a five-class abundance scale (class 1: a few individuals; 2: isolated small patches; 3: numerous small patches; 4: large discontinuous patches; 5: large continuous patches). Plants at the outer edge (including plants growing to the highest water mark) and the shallow part of each site were inventoried by walking or wading in a zig-zag pattern, whereas deeper water zones were point-sampled from a boat, with a grapnel or a rake, following a zig-zag pattern. This sampling method is more representative and less time consuming than quadrat sampling strategies for SSLs (Labat et al., 2022). Taxa such as Characeae, *Callitriche* and mosses were kept in alcohol or dried for identification in the laboratory. All hydrophytes and riparian vegetation (spermatophytes, bryophytes, and Characeae, excluding other algae) were identified at the species level when possible.

Correlation of the six indices with TP, and efficiency in discriminating trophic categories

We examined Spearman correlations between TP and each index, and we classified them into four trophic categories (oligotrophic to hypertrophic). These trophic categories were based on the thresholds of TP concentrations defined by Vollenweider & Kerekes (1982). Differences between the intervals of index values by trophic categories were checked with Wilcoxon tests and expressed with box plots. These analyses were conducted in the 146 SSLs sampled with the S_3m protocol and with TP data ("Development of a new trophic profile for plants and its associated trophic index" section).

Performance of the six indices in discriminating pressures using reference-based models

We tested the ability of each trophic index to discriminate anthropogenic pressures using reference-based models, following the methodology summarized in Fig. 1.

The WFD recommends two strategies to develop a reference-based index. We followed system B, which predicts the reference values of an index directly from a large set of environmental predictors and index values obtained in a wide dataset of least impacted ecosystems (Heiskanen et al., 2004). We expected system B to be more adapted to SSLs because (1) regionalization is bound to be very complex for small ecosystems because macrophyte communities partly depend on local conditions (e.g., geological singularities), (2) regionalization tends to oversimplify certain environmental factors identified as determinants of macrophyte communities because it uses intervals (e.g., elevation; (Labat et al., 2021), and (3) regionalization is adapted to one country, whereas we aimed at a reference-based index widely applicable in neighboring countries.

To predict the reference values of each index, we had to (1) identify the least impacted sites within a large dataset, and (2) identify the environmental predictors of each index in least impacted conditions so as to develop predictive models.

Identification of the least impacted sites First, we separated the least impacted SSLs from the impacted SSLs according to the presence of eight pressures likely to influence trophic levels or trophic indices. Three categories of pressures were considered: surrounding land use, external pollution inputs, and biotic sources of disturbances.

Surrounding land uses were (1) % of fertilized meadows or intensive grazing, (2) crops, and (3) urbanization. They were computed with GIS analyses from Corine Land Cover (2018), and a circular buffer zone with a 50 m-based radius weighted by the surface area of each SSL in m^2 ($r = 50 + \sqrt{\text{surface area}}$) was applied: a 1 m^2 pond corresponded to a 51 m radius buffer, a 1 ha pond to a 150 m radius buffer, and 10 ha to a 366 m radius buffer. This roughly corresponded to the efficient buffer zone for the detection of land use effects on plant communities in wetlands of equivalent surface areas (Houlahan et al., 2006). Fertilized meadows or intensive grazing were differentiated from other meadows by expert advice by globally homogenized plant communities in the surrounding meadows. Urban SSLs are more impacted than rural ponds by a cocktail of driving factors such as artificial substrates, a simple shoreline outline, low water quality, isolation, and exotic species (Oertli & Parris, 2019). The TP concentration tends to be higher in urbanized catchments than in agricultural ones, whereas the nitrogen concentration tends to be higher in agricultural catchments (Duan et al., 2012; Matej-Lukowicz et al., 2020). The water quality of SSLs is indeed largely dependent on different agricultural land uses (meadows/crops) (Zębek & Szymańska, 2017), with a greater influence of intensive agriculture (Halina et al., 2005; Céréghino et al., 2007). Meadows should not be neglected because they can be at least a source of nutrients through pasture (Ruggiero et al., 2004) and long-term inputs of inorganic fertilizers and manure (Jennings et al., 2003).

External pollution inputs corresponded to (4) the presence of a sewage treatment plant in the watershed or a polluted stream feeding the SSL, according to river WFD evaluation or, if lacking, expert decision.

Direct or indirect biotic sources of disturbances were: (5) livestock pressure, with visual degradations on banks or plants, (6) visible alteration of the littoral zone by waterbirds, (7) the presence of exotic species: muskrats, coypu or bioturbators crayfish, and (8) the presence of cyprinids. Livestock can affect macrophyte communities through organic pollution, nutrient loads, sediment resuspension, trampling, and increased soil salinity and drying (Kutschker et al., 2014). Fish, waterbirds, muskrats and coypu can modify plant communities by grazing on them (Prigioni et al., 2005; Wood et al., 2012; Phillips et al., 2016; Gethöffe & Siebert, 2020); eutrophication can also be increased by the presence of bread of human origin (Turner & Ruhl, 2007) or dejections (Scherer et al., 1995). Fish—especially cyprinids—can affect macrophyte communities and nutrient cycling through sediment resuspension and zooplankton feeding (Moss et al., 1997); trophic levels can also be increased by angling baits (Arlinghaus & Mehner, 2003). Bioturbators exotic crayfish such as *Procambarus* and *Faxonius immunis* destroy aquatic vegetation when they proliferate (Rodríguez-Pérez et al., 2016; Herrmann et al., 2018; Hossain et al., 2020), and generate bioturbation likely to increase nutrient concentrations in water (Gao et al., 2021), or may influence trophic indices by eliminating significant indicator species.

Candidate environmental predictors of the indices To test the ability of the six trophic indices to disentangle trophic pressures in a reference-based predictive model, we selected eleven candidate environmental predictors suspected to influence trophic levels in least impacted contexts. These environmental predictors were collected during the macrophyte survey or computed/measured through GIS analyses. They included three spatial predictors: (1) distance from the source (DIS), (2) distance from the nearest river (DNR), and (3) distance from the coast (DC). DIS is a simple proxy of watershed size because the watersheds of SSLs are often difficult to delimit. When the SSL was outside a river floodplain, it was very small and DIS = 0. When the SSL was itself a source, DIS = the length of the longest distance from the banks to the SSL outlet. When the SSL was a river impoundment, DIS = the length from the source of the river to the SLL outlet. Where the SSL was in a river floodplain, DIS = the length from the source to the perpendicular formed by the line between the river and the SSL. DNR is a proxy of river connectivity, whereas DC is a proxy of marine influence, including sea sprays and sea water intrusions in freshwater groundwater.

Other factors were (4) elevation, (5) geological typology (siliceous = 1; calcareous = 2) according to a cross-analysis of sylvoecoregions (Cavaignac, 2009), the IPR+ database (Marzin et al., 2016), and the "European typology for rivers and lakes" database (Lyche Solheim et al., 2019), (6) shading (% of surrounding vegetation), (7) mean depth, and (8) surface area. Finally, climatic conditions were considered with (9) mean annual temperature, (10) annual temperature amplitude, and (11) mean precipitations, extracted from the French National Institute for Agronomic and Environmental Research (INRAE) reanalysis (Marzin et al., 2016) of the SAFRAN/France database for the years 2010–2016 (Vidal et al., 2010).

Computation and selection of the reference-based models To predict reference values in least impacted conditions, we used a generalized additive model (GAM; (Hastie & Tibshirani, 1999)) between trophic indices and the eleven candidate predictors from the least impacted SSLs. Candidate predictors were assessed for normality and homoscedasticity using Shapiro–Wilk tests and by examining histograms, and transformations were applied when appropriate. The significant predictors were identified according to the REML method combined with null space penalization (Marra & Wood, 2011). All combinations of the ten quantitative candidate predictors associated with geological typology (1022 combinations) were tested.

Each trophic index *Ti* was expressed in ecological quality ratio (EQR) following Hering et al. (2006), Eq. (4):

$$Ti_{\mathrm{EQR}} = \frac{\mathrm{Obs} - \mathrm{Worst}}{\mathrm{Best} - \mathrm{Worst}} \tag{4}$$

With Obs = real computed value of the selected trophic index, Worst = minimum value (IBML) or maximum value (all other indices) computed for the 305 sites for each index, Best = predicted value of the trophic index according to the GAM model.

To select the best predictive model for each index, we applied a 3-step validation:

(1) Good prediction of reference values: the model-predicted values were not significantly different than the index values obtained in least impacted conditions, according to Wilcoxon test.
(2) Discrimination efficiency: each $\mathrm{Ti}_{\mathrm{EQR}}$ discriminated as many pressures as possible according to discrimination efficiency ($\mathrm{DE} \geq 0.6$) (Ofenböck et al., 2004). DE is the proportion of impacted SSLs with lower EQR values than the first quartile of the distribution of the least impacted SSLs.
(3) Explanatory power: the model explained the highest deviance.

This 3-step validation avoided biases induced by the inevitable spatial heterogeneity of the least impacted SSLs in strongly anthropized areas.

Finally, we used Pearson correlation test on reference-based indices to test correlations between the indices and the intercalibration metric $\mathrm{ICM}_{\mathrm{LM}}$ in order to determine the WFD-compatibility of each candidate index.

Ecological quality class boundaries

As recommended by the WFD, we defined ecological class boundaries according to the distribution of the $\mathrm{TIM_2S_{EQR}}$ values in the least impacted conditions. The "high-good" and "good-moderate" boundaries corresponded to the 75th and 25th percentiles, respectively. Then, the "moderate-poor" and "poor bad" boundaries corresponded to a division of the 0

Table 4 Results of the Spearman correlation test between the six indices and total phosphorus (TP)

	TIM_2S	M-NIP	PLEX	TRS	ICM_{LM}	IBML	TP
TIM_2S	1	0.60	0.55	0.47	0.60	− 0.61	0.62
M-NIP		1	0.33	0.40	0.32	− 0.48	0.24
PLEX			1	0.90	0.82	− 0.81	0.10
TRS				1	0.74	− 0.82	0.07
ICM_{LM}					1	− 0.72	0.19
IBML						1	− 0.22
TP							1

TIM2S Trophic Index for Macrophytes of Small Shallow lakes, *M-NIP* Macrophyte Nutrient Index for Ponds, *PLEX* Plant Lake Ecotype index, *TRS* Tropic Ranking Score, *ICMLM* Inter-Calibration Metric for Lake Macrophytes, *IBML* Indice Biologique Macrophytes Lacustres

to good-moderate boundaries into three equal classes (Mondy et al., 2012).

Results

TIM_2S: new trophic profiles for macrophytes of west European standing waters

TIM_2S trophic profiles are provided in Table S1. Two hundred and fifty-one indicator species were included.

Correlation between the seven trophic indices and trophic levels

TIM_2S was the index with the highest correlation with TP (Table 4). IBML, TRS, PLEX and ICM_{LM} were strongly correlated with each other ($r > 0.7$ or < -0.7) (Table 4). Only TIM_2S discriminated trophic categories from oligotrophic to eutrophic ones, but failed to significantly discriminate eutrophic SSLs from hyper-eutrophic ones (Fig. 2).

Discrimination efficiency of the six indices in reference-based models

Table 5 summarizes the main results obtained from the 1022 combinations of environmental predictors after the 3-step selection of the best predictive models.

Each trophic index was predicted by different combinations of environmental predictors. Shade was a determinant of all indices except M-NIP. Elevation was a strong predictor for TIM_2S, M-NIP and TRS ($F = 2.83$, 7.28 and 14.99, respectively), whereas geology was a very strong predictor for PLEX, TRS, ICM_{LM} and IBML ($t = 8.18$, 7.34, 6.84 and − 5.38, respectively). Climate, surface area and spatial predictors (DIS, DC and DNR) also influenced certain indices. TIM_2S characterized the largest number of SSLs (99%), followed by IBML and TRS (97%). M-NIP was not computed for 45% of the SSLs. IBML was the index with the higher mean DE (0.593), discriminating 6 pressures, followed by TRS (mean DE = 0.596) and TIM_2S (mean DE = 0.571), discriminating 5 pressures. No index discriminated SSLs concerned by fertilized meadows or livestock. Only PLEX, TRS and IBML discriminated urbanized landscapes.

WFD compatibility: correlation of the indices with reference-based ICMLM

The Pearson correlation analysis of the reference-based indices highlighted that TIM_2S_{EQR}, ICM_{LMEQR} and $IBML_{EQR}$ were highly correlated with each other ($P > 0.6$, Table 6).

Ecological quality boundaries of the most discriminant indices

The calculated values of the ‘high–good’, ‘good–moderate’, ‘moderate–poor’ and ‘poor–bad’ boundaries for the reference-based TIM_2S_{EQR} were 1, 0.795, 0.530 and 0.265, respectively. The four most discriminant indices ($IBML_{EQR}$, TRS_{EQR}, TIM_2S_{EQR} and ICM_{LMEQR}) were clearly efficient in discriminating the least impacted sites (sites with 0 pressure

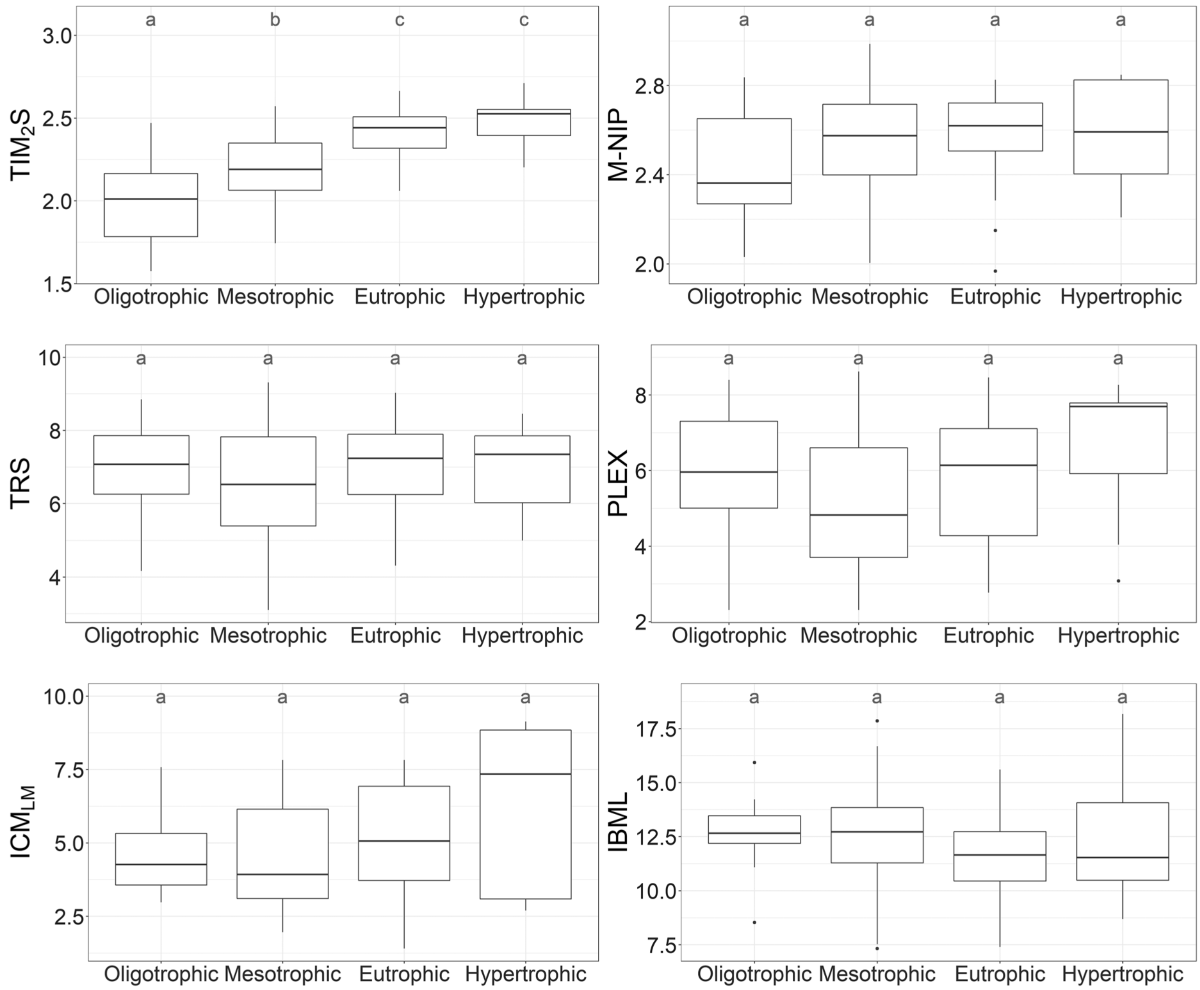

Fig. 2 Box-plots of the six trophic indices per trophic category based on TP (OECD TP trophic scale). Small letters (a, b, c) correspond to significantly different groups according to Wilcoxon tests

identified) from those with high pressure levels (the sites with at least four pressures identified, Fig. 3).

Discussion

Most of the trophic macrophyte profiles used for freshwater trophic indices are expert-based. As discussed by Demars et al. (2012) for rivers, these profiles tend to confound other chemical, physical and spatial factors with nutrients. IBML and ICM_{LM} were strongly correlated with the community-based indices TRS and PLEX, and these four indices were weakly correlated with TP. These results confirm that these four indices highlight community alterations induced by a large panel of pressures more than eutrophication. The lowest correlation between TP and M-NIP can be explained by the dominant calcareous geology substratum in Switzerland, resulting in the absence of trophic profiles for common indicator species typical of siliceous SSLs such as *Potamogeton polygonifolius* or *Isolepis fluitans*. As a consequence, we did not compute M-NIP for numerous SSLs from France. TIM_2S was the only index with a strong correlation with TP. Our first hypothesis was validated.

M-NIP and PLEX discriminated pressures least. These bad results can be explained by (1) the lack of numerous indicator species of siliceous SSLs for M-NIP, (2) the consideration of only hydrophyte species for PLEX. Very small SSLs harbor a very low

Table 5 Discrimination efficiencies (DE), *F* values and *t* values of the best GAM models, GAM statistics and % of SSLs with a calculable index for each index

		TIM_2S	M-NIP	PLEX	TRS	ICM_{LM}	IBML
DE	Fertilized meadows	0.3182	0.3235	0.2857	0.4773	0.2973	0.4090
	Crops	**0.6923**	0.5294	**0.7368**	**0.7692**	**0.7894**	**0.8462**
	Exotic species	**0.6970**	**0.6250**	**0.7000**	0.4063	**0.5384**	**0.6061**
	Waterbirds	**0.6364**	0.3333	**0.6000**	**0.6364**	**0.7273**	**0.8182**
	Livestock	0.3429	0.5263	0.2631	0.3125	0.3478	0.1176
	Cyprinids	**0.6923**	**0.6667**	0.3333	**0.6923**	0.5833	**0.6154**
	External pollution inputs	**0.6944**	0.4231	**0.6667**	**0.6111**	**0.7667**	**0.6667**
	Urbanization	0.5000	0.4167	**0.6250**	**0.6111**	0.4375	**0.6667**
Mean DE		0.571	0.481	0.526	0.565	0.561	0.593
GAM *F* values or *t* values	DIS			3.62**		11.11***	5.65***
	Elevation	2.83**	7.28***		14.99***		
	Shade	4.52***		3.42**	3.28**	24.41***	6.82***
	Surface area	2.13*				1.99**	
	Geology			8.18***	7.34***	6.84***	-5.38***
	T° amplitude			4.79***	13.09***	3.03**	
	Precipitation			2.08*			
	DC			2.06**			
	DNR					1.90*	3.09**
GAM statistics	R^2-adjusted	0.24	0.28	0.67	0.58	0.61	0.45
	Deviance	27%	34%	69%	60%	64%	47%
% SSLs with index value		99%	55%	73%	97%	80%	97%

Bolded values correspond to Discrimination Efficiency (DE) ≥ 0.6

Significance: ***P < 0.001, **P < 0.01, *P < 0.05

TIM2S Trophic Index for Macrophytes of Small Shallow lakes, *M-NIP* Macrophyte Nutrient Index for Ponds, *PLEX* Plant Lake Ecotype index, *TRS* Tropic Ranking Score, *ICMLM* Inter-Calibration Metric for Lake Macrophytes, *IBML* Indice Biologique Macrophytes Lacustres. *F* values concerned all predictors except geology (*t* values). *DIS* distance from the source, *DNR* distance from the nearest river, *DC* distance to the coast

Table 6 Results of the Pearson correlation test comparing the EQRs of the six indices

	TIM_2S_{EQR}	$M\text{-}NIP_{EQR}$	$PLEX_{EQR}$	TRS_{EQR}	ICM_{LMEQR}	$IBML_{EQR}$
TIM_2S_{EQR}	1	0.66	0.02	0.51	0.65	0.63
$M\text{-}NIP_{EQR}$		1	0.03	0.55	0.39	0.51
$PLEX_{EQR}$			1	0.01	0.00	0.05
TRS_{EQR}				1	0.68	0.64
ICM_{LMEQR}					1	0.63
$IBML_{EQR}$						1

TIM2S Trophic Index for Macrophytes of Small Shallow lakes, *M-NIP* Macrophyte Nutrient Index for Ponds, *PLEX* Plant Lake Ecotype index, *TRS* Tropic Ranking Score, *ICMLM* Inter-Calibration Metric for Lake Macrophytes, *IBML* Indice Biologique Macrophytes Lacustres

floristic richness (Hassall et al., 2011; Labat et al., 2021). Therefore, including all taxa (helophytes, bryophytes and hydrophytes) in the calculation of the index can be determining for a robust evaluation of trophic levels whatever the SSL size or shading likely to reduce floristic richness. Even if some species or groups of species were not directly sensitive to the nutrient status, they may have disappeared due to the

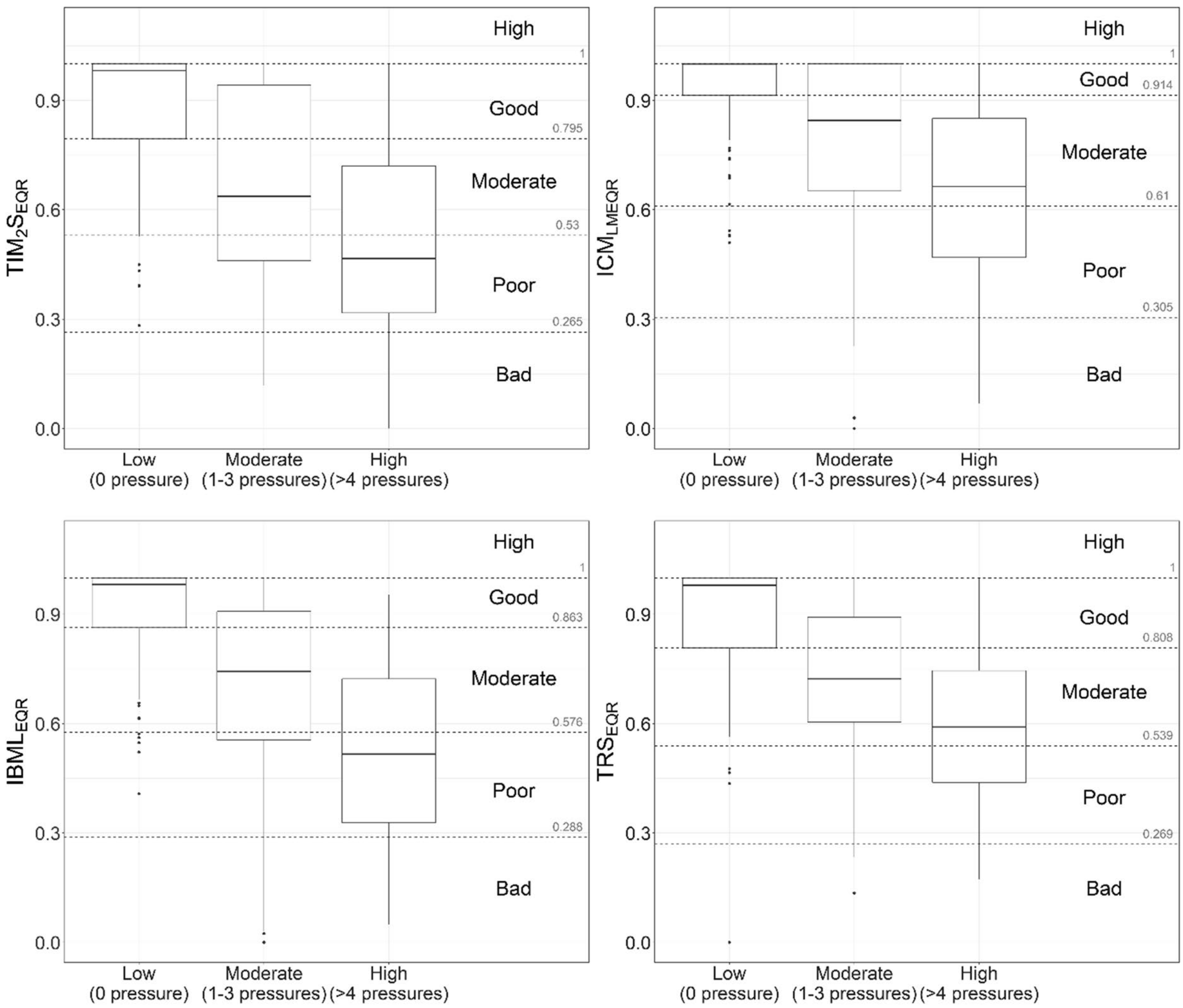

Fig. 3 Boxplot representing the best reference-based indices and their ecological quality boundaries according to the 143 least impacted SSLs, the 115 SSLs with 1–3 pressures and the 23 SSLs with more than 4 pressures

development of more competitive taxa in eutrophic conditions, e.g., Bryophyta vs. higher plants (Bergamini & Pauli, 2001). TIM_2S was computable in 99% of the SSLs. A strictly TP-based trophic index can be applied in larger geographical areas than community-based trophic indices can be. TIM_2S was only influenced by elevation, shade and surface area, whereas community-based indices were also influenced by determining factors of community composition such as climate, mineralization (geology and DIS) or connectivity (DIS and DNR) (Labat et al., 2021). Consequently, the accuracy of these community-based indices is more dependent on environmental factors than strict trophic indices are. For example, IBML reference values for the WFD are predicted according to four meta-types depending on elevation and alkalinity (Boutry et al., 2013), whereas in our study it was influenced by the complex interaction of six environmental predictors such as temperature amplitude, lake size or connectivity.

Finally, the predictive models provided four reference-based indices with high discrimination efficiency and sensitive to major sources of eutrophication [crops, external pollution inputs (Carpenter, 2005) and waterbirds (Boros et al., 2021)]. TIM_2S_{EQR} was also sensitive to the bioturbators crayfish, coypu and cyprinids, but failed to strongly discriminate urbanization effects, in contrast to

$IBML_{EQR}$ and TRS_{EQR}. Therefore, our second hypothesis is partially validated. The lower efficiency of TIM_2S_{EQR} in disentangling urbanization effects can be explained by interactions between urbanized areas and the natural areas that frequently surround urban SSLs and can play a buffer role for nutrients (Patenaude et al., 2015). As community-based indices, $IBML_{EQR}$ and TRS_{EQR} can better disentangle other urbanization effects, e.g., exotic species (Ehrenfeld, 2008) or anthropogenic trampling (Pescott & Stewart, 2014). Finally, TIM_2S_{EQR} was the only index well correlated with nutrient levels. With various pressures with DE > 0.6, it could be included in new multimetric indices.

In this study, we propose objective nutrient profiles derived from TP for a large number of plants, a new trophic index (TIM_2S) and its reference-based index. Numerous macrophyte trophic indices are still not reference-based because it is hard to find references or least impacted conditions (Garcia et al., 2003; Demars et al., 2012). The reference-based indices for the large lakes of the WFD are inevitably under human influence in most European countries because they belong to large watersheds and supply numerous services requiring paleolimnological investigations or retrospective analyses (Hutorowicz, 2020). On the other hand, SSLs in least impacted conditions are easier to find because they belong to smaller catchment areas, sometimes free of human activities. Reference-based indices developed especially for SSLs can indeed be more reliable because of more robust reference data. Including some least impacted SSLs in WFD indices could be useful to increase their efficiency, despite possible differences in their functioning (Padisák & Reynolds, 2003).

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Data availability The data presented in this study are available on request from the corresponding author. The data are not publicly available due to private funding.

Declarations

Conflict of interest The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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