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How macrophytes can improve the ecological assessment of rivers in a multiple-pressure context: development of a new diagnostic tool

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ABSTRACT

To achieve the European Union's water policy objective of "good" ecological status for all water bodies, a number of biotic indices have been developed, some of which are based on macrophyte communities. Despite the initial targets, 60% of water bodies still fail to achieve this status. One of the numerous reasons for this is the difficulty of accurately identifying the real causes of the degradation of water bodies, especially in a context of multiple pressures, so that truly effective restoration measures can be taken. To disentangle the effects of these co-occurring anthropogenic pressures on rivers, we have developed a diagnostic tool based on random forest models to predict the probability of impairment of river macrophyte communities. Thirteen categories of pressure on water quality, including diffuse contaminations by micropollutants (such as Polycyclic Aromatic Hydrocarbons and pesticides), and seven categories of pressure linked to hydromorphological alterations, were taken into account. By modifying local environments, each individual pressure induces changes in the taxonomic composition of macrophyte assemblages and in the adaptations selected within these assemblages. Based on a large set of taxonomic and functional metrics, this diagnostic approach has been illustrated in its ability (i) to monitor these changes at the river reach scale, but also (ii) to highlight clear spatial or temporal trends in the evolution of anthropogenic pressures on the scale of mainland France. The proposed models, based on macrophytes, are ecologically relevant and are promising for identifying water quality and habitat degradation from springs to lowland rivers. We hope that this progress will lead to a better understanding of the causes of river degradation, which will consistently help river managers to make better conservation or restoration decisions.

1. Introduction

Bioassessment is broadly implemented by government and national agencies in an effort to measure how species communities respond to anthropogenic stressors, e.g., the European Union Water Framework Directive (WFD; [European Council, 2000](#)). The aim of these bioassessment programmes is to support the implementation of EU water policy objective of "good" ecological status for all water bodies. To achieve this objective, it is necessary to (i) efficiently assess the ecological status of water bodies and (ii) identify as precisely as possible the main causes of their degradation in order to implement, when necessary, appropriate management and restoration programmes.

Methods based on biotic indices are essential bioassessment tools for

evaluating the ecological status of water bodies and are traditionally derived from the tolerance and sensitivity of taxa to anthropogenic disturbance ([Moog et al., 2018](#)). Using biological communities to measure ecological quality is more stable than using physico-chemical variables alone, given the ability of biological communities to integrate environmental changes over variable time steps depending on the Biological Quality Element (BQE) selected ([Hering et al., 2010](#)). In Europe, implementation of the WFD has required the development of freshwater biomonitoring indices based on the main BQEs (i.e., fishes, benthic invertebrates, phytobenthos, phytoplankton and macrophytes), which has given rise to a flourishing scientific literature. As early as 2012, [Birk et al. \(2012\)](#) identified around three hundred European bioassessment methods based on these BQEs.

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BQEs are commonly considered complementary (Caroni et al., 2013). Differences in their life history strategies imply different responses of the main biological communities to environmental gradients and anthropogenic disturbances (Johnson et al., 2006). For example, Hering et al. (2006) emphasized the ability of diatom communities to detect the effects of water eutrophication better than other groups of biological organisms (Kelly et al., 1995; Rott et al., 1999). Other authors have highlighted the greater impact of organic pollution on macroinvertebrate assemblages through oxygen depletion (Armitage et al., 1983; Statzner et al., 2001; Zelinka and Marvan, 1961). Fish communities have been especially used as indicators of habitat degradation (Gorman and Karr, 1978), river continuum disruption (Northcote et al., 1998) and flow regulation (Bain et al., 1988). Macrophyte communities have been considered to be good indicators of long-lasting habitat changes (Trempe and Kohler, 1995; Westlake, 1975) and have demonstrated their ability to highlight eutrophication (Hilton et al., 2006; O'Hare et al., 2018) and the general degradation of watercourses (Abou-Hamdan et al., 2005; O'Hare et al., 2006). The ability of the different BQEs to detect anthropogenic disturbances is, of course, not limited to these examples.

Macrophyte-based indices are also relevant for assessing many other physico-chemical impairments of water bodies, as highlighted by the scientific literature on nutrient enrichment (particularly nitrogen and phosphorus, e.g., Dodkins et al., 2005; Gebler et al., 2017; Hauray et al., 2008, 2006; Onaindia et al., 2005; Szoszkiewicz et al., 2020, 2017), water contamination by metallic elements (e.g., Fawzy et al., 2012) or even acidification (e.g., Thiébaud and Muller, 1999; Trempe and Kohler, 1995). Macrophyte communities have also proved effective in detecting habitat degradation, including the risk of substrate clogging (e.g., Dodkins et al., 2005) and alteration of riparian vegetation (e.g., Ferreira et al., 2005), or, conversely, for studying the beneficial effects of hydromorphological restoration (e.g., Lorenz et al., 2012). These capabilities led Hering et al. (2006) to recommend the use of macrophyte-based assessment methods in the context of rivers subject to multiple pressures, while advising more extensive use of criteria based on functional community structure in these methods. Despite their usefulness in bioassessment programmes, macrophyte-based assessment methods remained relatively scarce for a long time compared to other BQEs (Birk et al., 2012; Hering et al., 2006). This scarcity was partly due to a certain historical disaffection with aquatic plants on the part of botanists. However, the interest of aquatic ecologists in this BQE has been considerably stimulated by the methodological requirements of the WFD, leading to a rapid development of macrophyte-based bioassessment methods in all European states over the last 20 years.

Basically, many biotic indices have limitations, whatever the BQE used, which prevent them from really going beyond the step of assessing the overall ecological status of water bodies, and therefore identifying the causes of water body degradation in order to implement appropriate management programmes. Although the ecological status of water bodies has been assessed for all EU member states, our poor ability to identify the precise causes of water body degradation could be one of the reasons why only 40% of water bodies were in at least "good" ecological status in Europe in 2018, a far cry from the targets announced in the early 2000s (European Environment Agency, 2018). The main limitation to overcome is that many of the biotic indices usually used do not identify specific risks of degradation. If eutrophication is the pressure best covered by the assessment methods, there is little evidence that assessment methods reliably detect the effects of hydromorphological alterations, toxic pollution or acidification. In fact, out of total of 423 European assessment methods identified in Poikane et al. (2020), only 40 have demonstrated pressure-specific relationships for hydromorphological alterations, eight for toxic contamination and two for acidification. A diagnostic capability should be available for these pressures, and its absence makes it more difficult to disentangle the respective effects of co-occurring anthropogenic pressures. This greatly complicates the choice of the most appropriate restoration measures for

a given water body. Making progress in this area requires additional collective research efforts (Poikane et al., 2020).

To overcome these limitations, robust diagnostic approaches have recently been developed for streams and rivers (Dézerald et al., 2020; Larras et al., 2017; Mondy and Usseglio-Polatera, 2013) and applied on large spatial scale (Alric et al., 2021). They are able to identify individually the different pressures involved in the impairment of biological communities. The differences in sensitivity to the different pressure categories among BQEs are clearly reflected in the results of the different diagnostic tools, with each BQE providing original ecological information that complements the information given by the other BQEs (Alric et al., 2021; Meyer et al., 2022). Insofar as none of these tools are based on aquatic plants and taking into account the recommendation of Hering et al. (2006), macrophytes seem an ideal candidate for progress in the development of ecological diagnostic tools adapted to rivers subject to multiple pressures.

Diagnostic approaches are based on the fundamental notion that the local environment is a selective force (acting at different nested scales, from reach to ecoregion) that shapes community composition through environmental filtering and/or biotic interactions favouring the species best adapted to the characteristics of a given river reach (Southwood, 1988, 1977; Townsend, 1989; Townsend and Hildrew, 1994), despite dispersal processes. Species capable of occurring in this reach share common functional traits, i.e., according to McGill et al. (2006) and Violle et al. (2007), "morphological, physiological or phenological features that can be measured at the individual level and used comparatively across species", stable at large spatial (Archambault et al., 2005; Charvet et al., 2000; Statzner et al., 2001) and temporal scales (Bêche et al., 2006; Bêche and Resh, 2007). Therefore, by modifying local environments, anthropogenic disturbances can induce changes not only in taxonomic assemblages, but also in the traits selected within these assemblages leading to trait combinations that are often specific to a given pressure category (Desrosiers et al., 2019). The aim of diagnostic approaches is therefore to track these changes in taxonomic structure and trait combinations, using a large set of metrics exploring different features of the biotic assemblages.

In this study, we evaluated the ability of macrophyte communities to (i) identify specific risks of river degradation, and (ii) disentangle the effects of co-occurring pressures acting in a multi-pressure context, especially focusing on hydromorphological alterations and diffuse contaminations by various micropollutants, such as Polycyclic Aromatic Hydrocarbons (PAHs) and pesticides. The final objective was to develop a diagnostic tool, based on a machine learning method, able to identify the contribution of each individual pressure category acting significantly on macrophyte communities in rivers, which could be used by scientists and river managers.

2. Material & methods

2.1. Macrophyte and pressure datasets

This study was based on two sets of complementary data gathering information, for more than 15,000 sampling events, on (i) the taxonomic and functional structure of macrophyte communities (i.e., the macrophyte dataset) and (ii) the chemical and hydromorphological pressure levels occurring in the sites sampled (i.e., the pressure dataset). These two datasets were extracted from the national database "Naiades" (<http://www.naiades.eaufrance.fr>, accessed on 17 April 2023) and included data from 3085 sampling sites on streams and rivers in mainland France (Fig. 1). The data come from the monitoring programmes implemented in the WFD monitoring networks, for the campaigns between 2005 and 2020.

2.1.1. The macrophyte datasets

The sampling method used is the standardized protocol agreed for France, i.e., the Macrophyte Biological Index for Rivers (IBMR; NF

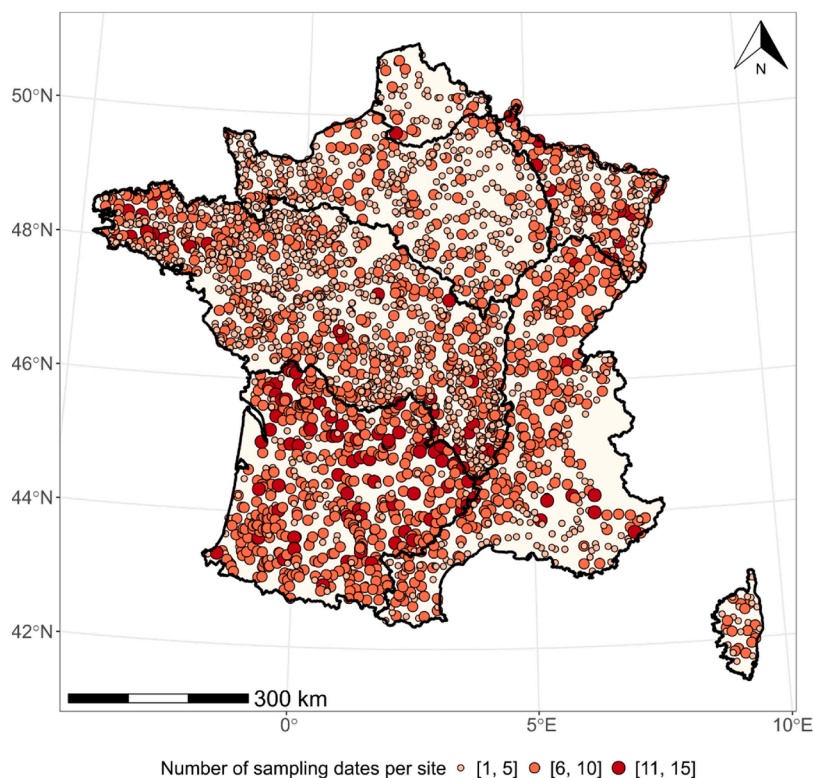


Fig. 1. Map of the sampling sites selected in this study. The size and the colour of the dots indicate the number of sampling events available per site over the period 2005–2020. The black lines correspond to the boundaries of the basins managed by the six French water agencies.

T90–395, AFNOR, 2003; Haury et al., 2006). At the date considered, the database contained 15,099 macrophyte community records (site x sampling date), providing a taxonomic list and the cover (percentage of the total record area) of all macrophyte taxa (including macroscopic algae, cyanobacteria, colonies of heterotrophic micro-organisms, bryophytes, pteridophytes, lichens and spermatophytes) encountered on the site over a 100 m stretch. This dataset is referred below as the “macrophyte coverage dataset” (MCD).

The second macrophyte dataset, referred as the macrophyte trait dataset (MTD), contained information on the traits of macrophyte taxa. Descriptive information related to physiology, morphology, phenology, life history strategy and ecological preferences of organisms was gathered from literature and previous authors’ original works (Loriot et al., 2014) to build this dataset (32 traits, Table 1). Traits were resolved into a number of modalities (Table 1) and were fuzzy-coded to capture the full biological and ecological variability of attributes – i.e., the variability among individuals and populations – of each taxon (see Chevenet et al., 1994; Usseglio-Polatera et al., 2000). The MTD was then combined with the MCD to calculate numerous metrics at the community level for each sampling event (see § 2.2.1).

2.1.2. The pressure dataset

Environmental features were described for each macrophyte sampling event following the approach developed by Mondy and Usseglio-Polatera (2013). Twenty pressure categories (Table 2) were defined including 13 categories describing impairments of water quality (hereafter referred to as “WQ”) based on 190 individual parameters, and seven categories describing habitat degradation, related to land use or hydromorphological alterations, at various spatial scales (hereafter referred to as “HD”) based on ten individual parameters (for details, see supplementary materials S1 and S2 of Alric et al. (2021)).

The values of the individual parameters of WQ pressures for each sampling event were calculated by averaging all the chemical measures available for the site in the national database “Naiades” ([\[des.eaufrance.fr/\]\(https://naia.des.eaufrance.fr/\), accessed on 17 April 2023\) during one year before the macrophyte sampling date. The pressure level, for each individual parameter describing a given WQ pressure category, was assessed using the classification of the French water quality system \(i.e., SEQ-Eau V2.0, Oudin and Maupas, 2003\) into five quality classes \(high/good/moderate/poor/bad\). Then, the level of each WQ pressure category \(when defined by several individual parameters\), was allocated according to the “one out-all out” aggregation rule \(Gottardo et al., 2011\), by assigning to each sampling event the worst quality class assigned to all the individual parameters taken into account to characterize this pressure category.](https://naia</p>
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The individual parameters describing the HD pressures were assessed only once for the whole period studied (Alric et al., 2021; Dézerald et al., 2020; Larras et al., 2017; Mondy and Usseglio-Polatera, 2013). The classification of individual parameters – and HD pressures – covered four quality classes (high/good/moderate/poor). Corresponding class boundaries have been detailed in Mondy et al. (2012). As with WQ pressures, the level of each HD pressure category (when defined by several individual parameters) was assigned according to the “one out-all out” aggregation rule.

2.2. Data analyses

2.2.1. Metrics

The macrophyte coverage dataset (MCD) and the macrophyte trait dataset (MTD) were used to calculate 518 metrics (complete list provided in supplementary material S2) at the community level for each sampling event. These metrics will be used as explanatory variables in the development of the diagnostic tool. They provide information about the taxonomic and functional composition or structure of macrophyte assemblages:

- 5 metrics describe the taxonomic structure of assemblages, using species richness (S), Shannon-Weaver diversity (H’; Shannon, 1948),

Table 1

Traits and their modalities used to calculate macrophyte-based metrics, at the community level, relative to (A) physiology, morphology, phenology and life history strategy, (B) ecological preferences related to water physico-chemical composition and (C) ecological preferences related to habitat characteristics (including hydromorphology). The fuzzy-coding description of traits for each taxon is presented in supplementary material S1 for traits relative to physiology, morphology, phenology and life history strategy. The information can be found in Loriot et al. (2014) for traits relative to ecological preferences. No. modalities = number of modalities per trait.

A - Physiology, morphology, phenology and life history strategy		No. modalities
Beginning of flowering	March; April; May; June; July (or later); no flower	6
End of flowering	May; June; July; August; September; October (or later); no flower	7
Duration of flowering	≤ 2 months; 3 months; 4 months; 5 months; 6 months; ≥ 7 months; no flower	7
Main season of flowering	spring (early, mid or late); summer (early, mid or late); autumn (early and mid); no flower	8
Flower colors	white; blue; yellow; brown; pink; green; no flowers	7
Clonal growth form	corrus; planlet; root (lato sensu); Thallus; Tuber; colony (for unicellulars)	6
Dispersal agent	hydrochory; meteochoy (anemochory); zoo- and anthro-choy; autochory	4
Dispersal unit type	fruit or seed; spore; vegetative (accident); vegetative (propagule)	4
Life form	attached (emergent, floating leaves or submerged); free (floating or submerged); stem (ascending, prostrate or erect)	8
Storage organ	rhizome; succulent; tuber; no storage organ	4
Reproduction type	sexual; asexual	2
B - Ecological preferences (physico-chemistry)		No. modalities
Ca ([Ca], mg.L ⁻¹)	≤ 10;]10 – 25];]25 – 50];]50 – 85]; > 85	5
Organic carbon ([C], mg.L ⁻¹)	≤ 1;]1 – 3];]3 – 5];]5 – 7]; > 7	5
Conductivity (µS at 25 °C)	≤ 50;]50 – 150];]150 – 300];]300 – 500]; > 500	5
BDO5 ([O ₂], mg.L ⁻¹)	≤ 1;]1 – 2];]2 – 3];]3 – 5]; > 5	5
Mg ²⁺ ([Mg], mg.L ⁻¹)	≤ 2;]2 – 4];]4 – 8];]8 – 12]; > 12	5
Suspended particles (mg.L ⁻¹)	≤ 5;]5 – 7];]7 – 14];]14 – 28]; > 28	5
NH ₄ ⁺ ([NH ₄], mg.L ⁻¹)	≤ 0.05;]0.05 – 0.07];]0.07 – 0.10];]0.10 – 0.15]; > 0.15	5
NO ₃ ⁻ ([NO ₃], mg.L ⁻¹)	≤ 3;]3 – 6];]6 – 12];]12 – 24]; > 24	5
PO ₄ ³⁻ ([PO ₄], mg.L ⁻¹)	≤ 0.025;]0.025 – 0.050];]0.050 – 0.100];]0.100 – 0.200]; > 0.200	5
pH	≤ 7.2;]7.2 – 7.5];]7.5 – 7.8];]7.8 – 8.0]; > 8.0	5
Temperature (°C)	≤ 12.5;]12.5 – 14.0];]14.0 – 15.5];]15.5 – 17.0]; > 17.0	5
C - Ecological preferences (habitat/hydromorphology)		No. modalities
Elevation (m)	≤ 150;]150 – 300];]300 – 600];]600 – 1000]; > 1000	5
Depth (index [0.05 – 1.00], from shallowest to deepest*)	≤ 0.2;]0.2 – 0.4];]0.4 – 0.6];]0.6 – 0.8]; > 0.8	5
Current velocity (index [0.025 – 1.000], from slowest to fastest*)	≤ 0.1;]0.1 – 0.25];]0.25 – 0.4];]0.4 – 0.5]; > 0.5	5
Light (index [0.2 – 1.0], from the shadiest to the brightest*)	≤ 0.40;]0.40 – 0.53];]0.53 – 0.66];]0.66 – 0.80]; > 0.80	5
QAQ5 ([0 – 0.671], indicator of annual hydrological stability*)	≤ 0.07;]0.07 – 0.12];]0.12 – 0.22];]0.22 – 0.39]; > 0.39	5
Strahler rank ([1 – 8])	≤ 1;]1 – 2];]2 – 3];]3 – 5]; > 5	5
Bed Calibre Index (index [-8 – 9], from coarse to fine substrates*)	≤ -7;]-7 – -6];]-6 – -3.5];]-3.5 – -1.5]; > -1.5	5
Fine substrate coverage (index [0 – 100])	≤ 0%;]0% – 25%];]25% – 50%];]50 – 75%]; > 75%	5
Coarse substrate coverage (index [0 – 100])	≤ 0%;]0% – 25%];]25% – 50%];]50 – 75%]; > 75%	5
Organic substrate coverage (index [0 – 100])	≤ 0%;]0% – 25%];]25% – 50%];]50 – 75%]; > 75%	5

* see Loriot et al. (2014) for details.

Table 2

Codes and labels of water quality (WQ) and habitat degradation (HD) pressure categories investigated in this study.

Code	WQ pressure category	Code	HD pressure category
WQ1	Organic matter	HD1	Transportation facilities (at reach level)
WQ2	Nitrogen compounds (excl. nitrates)	HD2	Riverine vegetation
WQ3	Nitrates	HD3	Urbanization (at reach level)
WQ4	Phosphorous compounds	HD4	Clogging risk
WQ5	Suspended particles	HD5	Hydrological instability
WQ6	Acidification	HD6	Straightening (at reach level)
WQ7	Mineral micropollutants	HD7	Catchment anthropization
WQ8	Pesticides (all types included)		
WQ9	Herbicides		
WQ10	Fungicides		
WQ11	Insecticides		
WQ12	PAH		
WQ13	(other) Organic micropollutants		

Simpson dominance (Simpson, 1949), Pielou evenness (Pielou, 1966) and Hill diversity (Hill, 1973);

- 111 metrics are based on the relative abundance of different taxonomic groups: 102 families and nine groups of higher taxonomic level, i.e., algae, liverworts, mosses, heterotrophs, lichens, phanerogams (helophytes, hydrophytes and others) and pteridophytes;

- 28 metrics are based on the relative abundance of species according to their growth form (e.g., Batrachid, Bryid, Ceratophyllid, Charid, Elodeid, Graminoid, Isoetid, Lemnid) adapted from the classification of growth forms by Wiegleb (Wiegleb, 1991; Wiegleb et al., 2014);
- 4 metrics are based on the relative abundance of invasive and alien species;
- 167 metrics describe the relative use of individual trait modalities (for the 32 traits defined in Table 1) by taxa in macrophyte assemblages (e.g., the proportion of taxa with flowering beginning in March);
- 32 metrics (one per trait, Table 1) describe the diversity of macrophyte assemblages in terms of trait category preferences using the Rao index (Rao, 1982);
- 32 metrics (one per trait, Table 1) describe the level of specialization of macrophyte assemblages in terms of biological features or ecological preferences at the community level using the community specialization index (CSI; Mondy and Usseglio-Polatera, 2014);
- 128 metrics are based on the degree of overlap of trait profiles within macrophyte assemblages using the indices of Pianka and Czekanowski (Czekanowski, 1932; Gotelli et al., 2015; Pianka, 1974);
- 11 metrics are derived from the French Macrophyte Biological Index for Rivers (IBMR; Haury et al., 2006) and its primary components (e.g., based on the species scores and coefficients of ecological amplitude).

2.2.2. Random forest models

We developed Random Forest (RF) models, a particular type of

machine learning method able to investigate complex and potentially non-linear relationships (Breiman, 2001). RF have been successfully applied to many different research areas such as air quality modelling (Choubin et al., 2020), flood susceptibility assessment (Hosseini et al., 2020), groundwater nitrate modelling (Rahmati et al., 2019), and stream impairment modelling under a multi-pressure scenario (Desrosiers et al., 2019; Dézerald et al., 2020; Larras et al., 2017; Mondy and Usseglio-Polatera, 2013). Here, we parametrized the RF models following the recently published setting parameters to maximize both reliability and performance of models (i.e., 30 random forests per pressure category, 1500 classification trees per forest, random selection of 25 biological metrics at each node in individual trees; Alric et al., 2021). Each pressure-specific model was built taking the pressure level (“low” versus “significant”) as the response variable and the 518 biological metrics as the predictive variables. Each model provided, for each sampling event, a mean impairment probability (IP) calculated over all the trees defining the thirty forests (i.e., 45,000 trees). The “low” pressure level corresponded to the “high” and “good” quality classes for the corresponding pressure category, described in § 2.1.2. The “significant” pressure level therefore corresponded to (i) the “moderate”, “poor” and “bad” quality classes for pressure categories related to water quality, and (ii) the “moderate” and “poor” quality classes for pressure categories related to hydromorphology and land use.

Each model was built on a learning dataset (75% of available sampling events) and then tested on a test dataset (25% of sampling events). These two subsets corresponded to independent subsamples of the MCD dataset, on which metrics have been calculated. In order to minimize potential temporal correlations among sampling events within sites, the sampling events corresponding to a given site were randomly distributed in either the learning subset or the test subset. Model performance was then assessed through cross-validation of the learning and testing subsets by calculating the Area Under the ROC Curve (AUC; ROC = Receiver Operating Characteristic; Fawcett, 2006; Fielding and Bell, 1997). The AUC assesses the ability of a fitted model to differentiate between pressure levels (“low” versus “significant”). We evaluated the models’ performance with both the test (AUC_{TEST}) and learning (AUC_{LEARN}) data subsets to (i) minimize prediction errors in the test data subset and (ii) avoid overfitting in the learning data subset. AUC values range from 0.00 to 1.00 with 1.00 indicating perfect discrimination of the two pressure levels, 0.50 indicating that the model is unable to separate “low” from “significant” pressure levels, and 0.00 indicating a systematic error in the model’s ecological diagnostic. The values have been interpreted in accordance with Heikkinen et al. (2012), Swets (1988), with values include in the ranges]0.60–0.70],]0.70–0.80],]0.80–0.90] and]0.90–1.00], respectively considered as corresponding to “poor”, “fair”, “good” and “excellent” predictive performance. Only models with both AUC_{TEST} and AUC_{LEARN} values > 0.70 were selected for the final diagnostic tool.

To describe the accuracy of the selected RF models, other metrics of performance, including sensitivity, specificity and prevalence were also calculated for each model at different impairment probability thresholds to classify the sampling events into “positive” (i.e., “significant” pressure level) and “negative” (i.e., “low” pressure level) categories. Sensitivity measures the ability of the models to correctly identify all positive sampling events (i.e., the number of correctly classified true positives divided by the number of all sampling events that should have been identified as positive; sensitivity = TP / (TP + FN), with TP and FN the numbers of true positives and false negatives, respectively). Specificity measures the ability of the models to correctly identify all negative sampling events (i.e., the number of correctly classified true negatives divided by the number of all the samples that should have been identified as negative; specificity = TN / (TN + FP), with TN and FP the numbers of true negatives and false positives, respectively). The positive and negative predictive values (PPV and NPV) were also computed from sensitivity, specificity and prevalence (the last one being the number of positive cases in the entire dataset). PPV and NPV measure respectively

the proportions of positive and negative results provided by a model that are true positive and true negative results, taking into account the prevalence of true positives in the dataset (Eqs. (1) & (2)). The ideal value of all these metrics is 1, and the worst possible value would be zero. The PPV and NPV have been used to help river managers interpret the information produced by RF models more easily.

$$PPV = \frac{\text{sensitivity} * \text{prevalence}}{\text{sensitivity} * \text{prevalence} + (1 - \text{specificity}) * (1 - \text{prevalence})} \quad (\text{Eq. 1})$$

$$NPV = \frac{\text{specificity} * (1 - \text{prevalence})}{\text{specificity} * (1 - \text{prevalence}) + (1 - \text{sensitivity}) * \text{prevalence}} \quad (\text{Eq. 2})$$

2.2.3. Metric importance and ecological validation

Five methods (i.e., “anova.test”, “auc”, “chi.squared”, “information.gain” and “kruskal.test”; see Bischl et al., 2016) were used to evaluate the importance of each individual metric in the macrophyte assemblage response to each pressure category. These methods have enabled us to rank the metrics according to their contribution to each model. In order to summarize the results provided by these different methods, we first rescaled the results given by each method in the interval [0–1], then averaged them, for each metric, over all the methods taken into account (Congalton, 1991). The mean scaled rank obtained was taken as the final measure of the metric importance in RF models. The relationships between the values of the 10 most important metrics (for each model) and impairment probabilities (IPs) were investigated using non-parametric Spearman correlations in order to validate the ecological significance of models’ results.

The IPs returned by all the models were also compared with the quality classes allocated to sampling events based on environmental information (see § 2.1.2), using boxplots. Differences between boxplots were tested with Kruskal-Wallis tests followed by Dunn post-hoc tests (both with a p-value threshold of 0.01). We also explored the temporal distribution pattern of the significant impairment probability values returned by the different models for mainland France as a whole over the period 2012–2020, using Friedman tests followed by Sign post-hoc tests.

2.2.4. R packages and statistical test significance

All data analyses were performed with R 4.1.2 (R Core Team, 2021) using the *ecodiag* package (<https://github.com/CedricMondy/ecodiag>) for developing RF models, the *caret* package for calculating the sensitivity, specificity and other related parameters (Kuhn, 2008), the *mlr* package for evaluating the importance of each individual metric (Bischl et al., 2016) and the *vegan* package for the calculation of numerous taxonomic and trait metrics (Oksanen et al., 2018). All the statistical tests were performed with a p-value threshold of 0.01.

3. Results

3.1. Model performances

All the WQ and HD models had AUC_{LEARN} values and AUC_{TEST} values higher than 0.70 (Table 3), indicating at least a “fair” predictive model performance. WQ models related to nutrient enrichment, PAH, suspended particles and organic matter (WQ 1–5 and 12) and HD models related to transportation facilities (HD1), clogging risk (HD4), hydrological instability (HD5) and catchment anthropization (HD7) exhibited the best performances with “good” to “excellent” predictive performances. The differences between AUC_{LEARN} values and AUC_{TEST} values were relatively low, indicating no model overfitting on the learning dataset, which would have led to a model that cannot make accurate predictions from any data other than the training data.

As the RF models provide IPs of sampling events by different pressure categories, it is also interesting to examine the consequence of applying

Table 3

Number of sampling events (SEs) characterized, proportion of sampling events with a “low” vs. “significant” pressure level, AUC_{TEST} and AUC_{LEARN} values calculated for each of the RF models built for 20 pressure categories (full labels in Table 2) linked to water quality (WQ) or habitat degradation (HD). Colours denote model predictive performances: blue = “excellent”, green = “good” and yellow/orange = “fair”.

Code	SEs	% “low” / “signif.”	AUC _{LEARN}	AUC _{TEST}
WQ1	14878	93.2% / 6.8%	0.87	0.87
WQ2	14866	98.9% / 1.1%	0.88	0.89
WQ3	14868	54.4% / 45.6%	0.89	0.89
WQ4	14869	96.4% / 3.6%	0.87	0.86
WQ5	14866	99.1% / 0.9%	0.83	0.87
WQ6	14880	96.7% / 3.3%	0.72	0.75
WQ7	8566	56.6% / 43.4%	0.74	0.75
WQ8	10285	11.4% / 88.6%	0.80	0.78
WQ9	10285	50.7% / 49.3%	0.75	0.75
WQ10	8911	18.4% / 81.6%	0.71	0.71
WQ11	10230	23.1% / 76.9%	0.76	0.75
WQ12	8997	93.0% / 7.0%	0.85	0.84
WQ13	9972	40.1% / 59.9%	0.74	0.74
HD1	15115	78.6% / 21.4%	0.89	0.89
HD2	15111	62.6% / 37.4%	0.79	0.79
HD3	15115	75.0% / 25.0%	0.77	0.78
HD4	12374	60.5% / 39.5%	0.82	0.81
HD5	11958	70.9% / 29.1%	0.80	0.82
HD6	15103	29.7% / 70.3%	0.74	0.74
HD7	11031	10.9% / 89.1%	0.96	0.96

different threshold values for classifying sampling events as positive (i.e., significantly impaired) or negative (i.e., not significantly impaired). Fig. 2 shows the responses of the positive (dashed lines) and negative (full lines) predictive values to variations in the selected threshold value, for all the RF models. The response patterns of PPV and NPV are quite

different depending on the RF model considered, and depend at least to a certain extent on the balance between the proportions of sampling events with “low” versus “significant” pressure levels (for each category of pressure) in the datasets.

These response patterns can also provide a “reading grid” to help

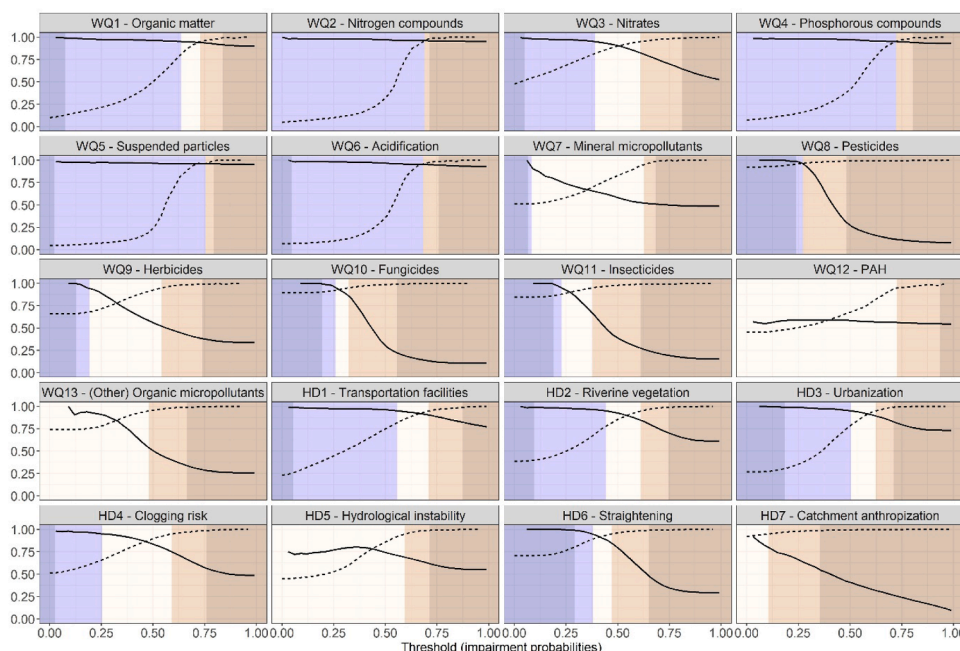


Fig. 2. Relationships between positive (PPV, dashed lines) and negative (NPV, full lines) predictive values and thresholds (impairment probabilities) used to classify sampling events as “positive” (i.e., a “significant” pressure level) and “negative” (i.e., a “low” pressure level) by the RF models built for 20 pressure categories related to water quality (WQ) and habitat degradation (HD). Five ranges of impairment probabilities have been defined as (i) most likely low (dark purple area), (ii) probably low (light purple area), (iii) uncertain (off-white area), (iv) probably significant (light brown area) or (v) most likely significant (dark brown area). See the text (§ 3.1) for further details.

river managers interpret the results produced by RF models. For each model, we have defined five ranges of impairment probabilities for which the pressure level can be considered as (i) most likely low (dark purple area on Fig. 2), (ii) probably low (light purple area), (iii) uncertain (off-white area), (iv) probably significant (light brown area) or (v) most likely significant (dark brown area). The first class corresponds to the IPs for which the NPV ≥ 0.95 . The second class corresponds to NPV values included in the interval $[0.90 - 0.95[$. The third class is bounded between the IPs for which NPV < 0.90 and PPV > 0.90 , respectively. The fourth class corresponds to PPV values included in the interval $[0.90 - 0.95[$. Finally, the fifth class corresponds to the IPs for which the PPV ≥ 0.95 . As the environmental cost of not detecting a potentially impaired situation seems to us to be greater than classifying a situation with a “low” pressure level as a false positive, we have defined that in the event of a conflict between PPV and NPV, i.e., when both values are greater than 0.90 for example, the PPV is favoured.

3.2. Metric contributions to models and ecological validation

Among the 10 metrics most contributing to at least one model, 67.6% described the relative use of a trait modality by the macrophyte assemblages (65.7% of them described ecological preferences and only 1.9% were related to physiological, morphological, phenological or life history traits). The level of trait specialization of macrophyte assemblages was taken into account by 13.3% of the most contributory metrics and 6.7% of them were related to the IBMR index and its primary components (e.g., the species scores and coefficients of ecological amplitude, see Haury et al., 2006). Metrics based on (i) the degree of overlap of trait profiles within macrophyte assemblages (i.e., Pianka and Czekanowski indices), (ii) the relative abundance of taxonomic groups or groups of macrophytes with similar growth forms, (iii) the taxonomic structure of assemblages (i.e., species richness, diversity, evenness and dominance) and (iv) the diversity of ecological preferences within assemblages (i.e., based on Rao index) corresponded respectively to 6.2%,

4.3%, 1.4% and 0.5% of the total number of most contributing metrics. These metrics were listed in supplementary material S2.

The investigation of the relationships between raw metric values and IPs, using Spearman correlations, revealed highly significant, often non-linear, relationships (Fig. 3A–F), as for example between the IPs provided by the model “nitrates” and the proportion of macrophytes exhibiting an affinity for the lowest nitrate concentration range (≤ 3 mg. L⁻¹ Fig. 3A) or, between the IPs returned by the model “transportation facilities” and the community specialization index (CSI) scores for Strahler rank preferences (Fig. 3E). The relationships between the raw values of the 10 most important metrics contributing to each RF model and the probabilities of impairment provided by this model are available in supplementary material S3.

The IPs returned by all the models were also compared with the quality classes assigned to the sampling events on the basis of environmental information (see § 2.1.2), with boxplots (Fig. 4A) and comparison graphs (Fig. 4B). Only the results of the “nitrate” model have been presented in Fig. 4 (see supplementary material S4 for the results of the other models). For all the models, there was a clear and statistically significant difference (Kruskal-Wallis test followed by Dunn’s post-hoc test, both with a p-value < 0.01 , see supplementary material S4) between the distributions of IP values in the “low” (high and good quality classes) and “significant” (moderate, poor and bad quality classes) pressure levels.

The correspondence between pressure levels (“low” versus “significant”, based on environmental information) and IP classes varied according to the model considered. The impairment probability range for the “uncertain” class varied greatly depending on the model considered. Some models (e.g., WQ12 -PAH and WQ13 - Organic micropollutants) fail to clearly identify “low” pressure conditions. In the case of the “nitrates” model (Fig. 4B), more than 80% of the macrophyte sampling events exhibited a good match between the environmental characterization and the IP class assigned. However, for 16% of the sampling events, the model was unable to clearly identify the level of pressure

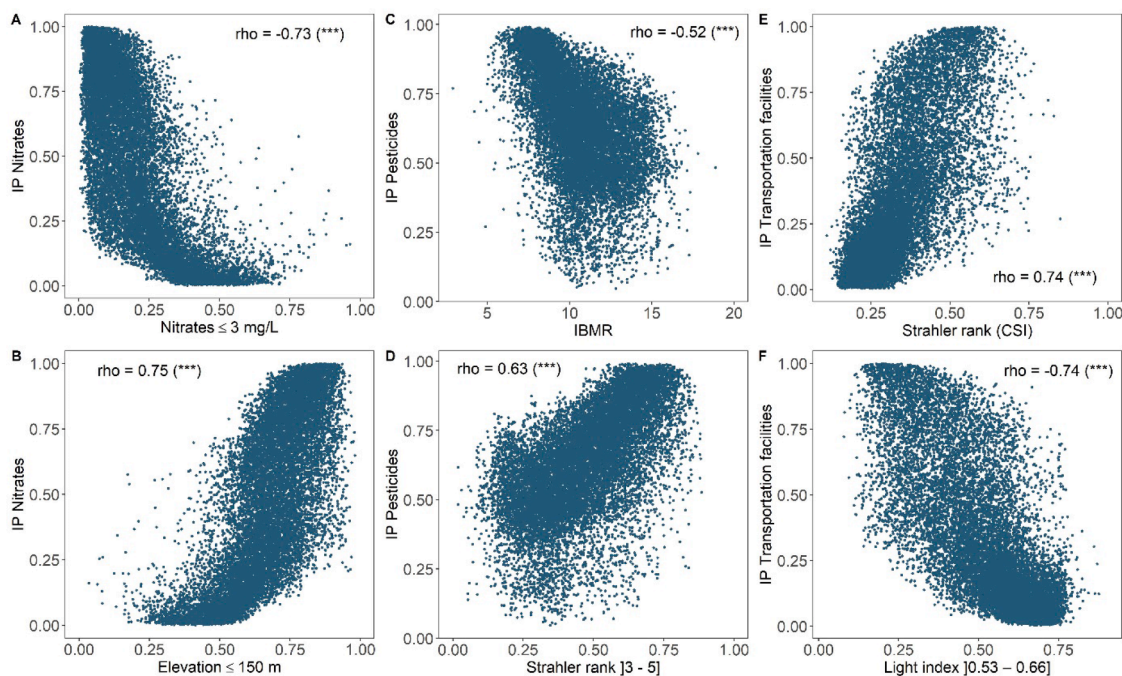


Fig. 3. Examples of relationships, over 15,099 macrophyte sampling events (SEs), between the impairment probabilities (IPs) provided by the “nitrates” model and the taxon preferences in macrophyte assemblages for (A) the lowest range of nitrate concentration (≤ 3 mg.L⁻¹), and (B) the lowest range of elevation (≤ 150 m); between the IPs provided by the “pesticides” model and (C) the IBMR scores, and (D) the intermediate values of “Strahler rank” ([3 – 5]); between the IPs provided by the “transportation facilities” model and (E) the community specialization index (CSI) scores for Strahler rank preferences, and (F) the intermediate values of the “light index” ([0.53 – 0.66]). The relationships were tested using Spearman’s coefficient, whose rho value and the significance of the relationship are indicated for each model (***) = p-value < 0.01). Each point corresponds to an SE.

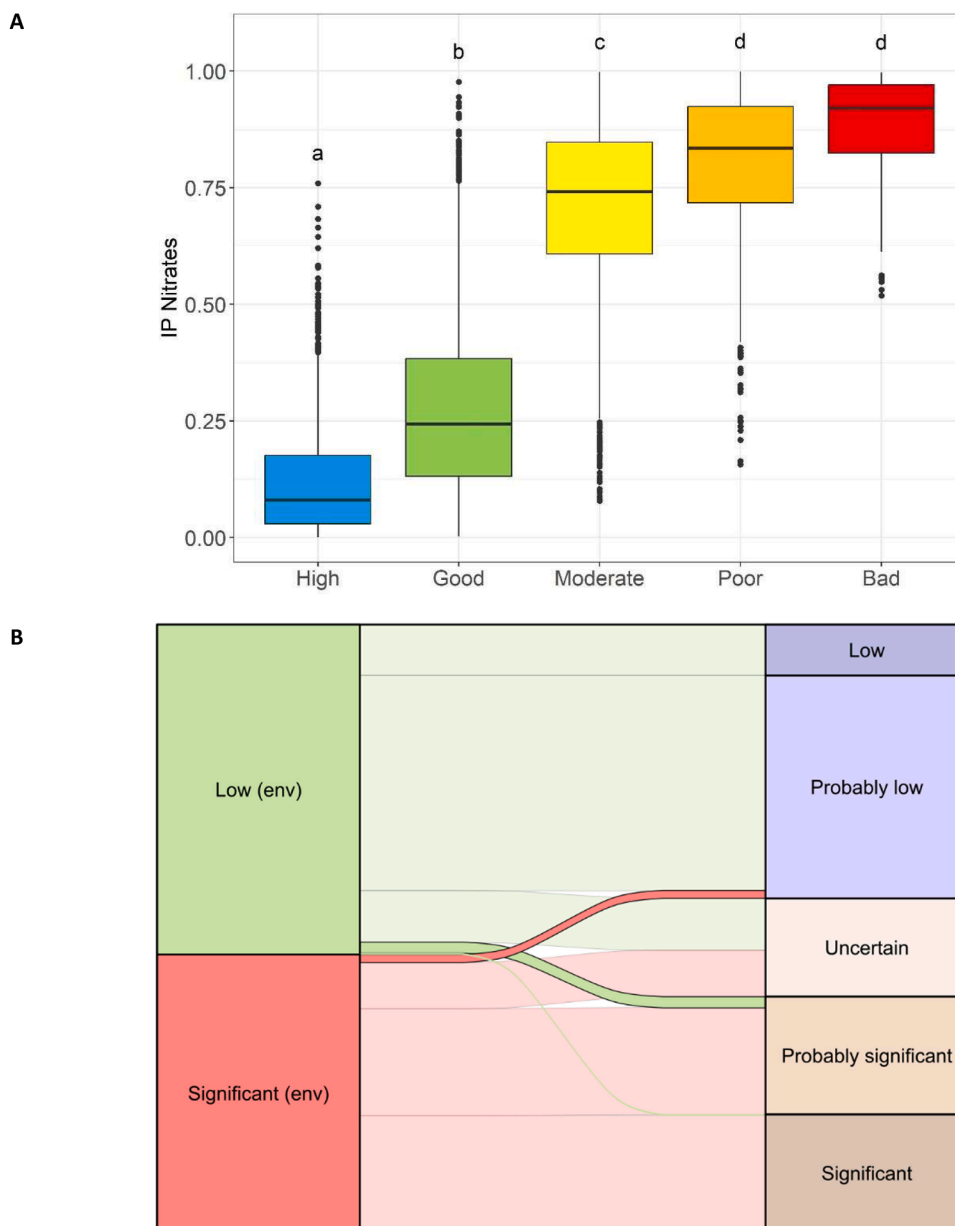


Fig. 4. Comparison between environmental information and impairment probabilities (IPs). (A) Distribution of IP values (with box-plots) in the five quality classes for the “nitrates” model. Different letters indicate significant differences (p-value < 0.01) between quality classes according to a Kruskal-Wallis test followed, if necessary, by a Dunn’s post-hoc test. (B) Correspondence (with a comparison graph) between the two pressure levels (“low” and “significant”) and the five IP classes (“most likely low”, “probably low”, “uncertain”, “probably significant” and “most likely significant”). The left-hand column corresponds to the pressure levels determined on the basis of environmental characteristics, and the right-hand column corresponds to classes assigned on the basis of the IPs returned by the model (in this case “nitrates”). The bands between the two columns show the correspondence between these two classifications, and their heights are proportional to the number of macrophyte samples corresponding to these status classes. Misclassification cases are highlighted by a more intense colour: green when the environmental classification is “low” and red when it is “significant”.

(“uncertain” IP class). The IP class was incorrectly assigned in 3.3% of cases (2% of samples were wrongly classified as significantly impaired and 1.3% were wrongly classified as unimpaired).

4. Discussion

The aim of diagnostic approaches is to track changes in the taxonomic structure and trait combinations of biotic communities subjected to anthropogenic disturbances, in order to identify more specifically the main causes of river degradation for which traditional tools (such as biotic indices) provide only an overall assessment. By using a wide range of metrics exploring different aspects of the trait and taxonomic

structure of macrophyte assemblages, we have provided concrete evidence of the ability of macrophyte communities to effectively identify multiple risks of river alteration and been able to build effective models to identify the specific causes of river degradation and disentangle the effects of co-occurring pressures.

4.1. A practical diagnostic tool for river managers: impairment probabilities across spatial and temporal scales

What is the probability of impairment (IP) required to classify with a high degree of confidence, a sampling event as being significantly impacted - or not - by a given pressure category? To this end, positive

and negative predictive values (PPV and NPV) have been calculated to provide a “reading grid” to help managers interpret the results of the diagnostic tool. On the basis of the PPV and NPV, we have defined five classes of IPs: (i) “most likely low”, (ii) “probably low”, (iii) “uncertain”, (iv) “probably significant” and (v) “most likely significant”. Possible uses for the tool are illustrated below at different spatial and temporal scales.

4.1.1. Local scale, positive and negative predictive values

Based on radar diagrams, Fig. 5 illustrates the diagnostic tool outputs for a given stream reach in mainland France. The results for the stream “La Légère” at S^t Faziol illustrate the diagnostic tool’s ability to distinguish the significant nature of the different categories of pressure in a context of multiple pressures. The RF models highlight the “probably significant” or “most likely significant” effect of nitrogen and phosphorous compounds (WQ2 to WQ4; see the corresponding black arrows in Fig. 5A), organic matter (WQ1) and, to a lesser extent, pesticides as a whole (WQ8), insecticides (WQ11), metals (WQ7) and other organic micropollutants (WQ13). These pressures considered as significant via macrophyte-based metrics are consistent with the direct description of both water quality and land use at the sampling site (see the outer circle in Fig. 5A & B): 87.8% of the upstream catchment area was occupied by agriculture, former mining activities (i.e., silver mine active until the early 20th century) and long known industrial activities (e.g., chemical industry for 150 years covering 2.7% of the upstream catchment). The description of the water quality of this river reach confirms these results, since it was classified as “moderate” to “bad” for all the pressures mentioned (Fig. 5A). The low IP obtained by the “herbicides” model (WQ9; IP = 0.17) is consistent with the environmental characterization of the sampling event for this pressure category (i.e., “good” quality). The diagnostic tool is therefore capable of clearly assessing biological responses of different intensities, even within the same “family” of pressures (pesticides, in this case). The same applies to hydro-morphological pressures, since the diagnostic tool is able to distinguish the low effect of “urbanization” at the river reach level (HD3; IP = 0.27 and “good” quality based on environmental criteria) from the strong effect of “anthropization” at the catchment level (HD7; IP = 0.97 and “poor” quality, Fig. 5B).

A very high IP returned by a model does not systematically mean that the corresponding pressure has led to strong deterioration of the site (leading to a “bad” quality), but only that the diagnostic tool has detected a very clear signal of significant deterioration (leading, at best,

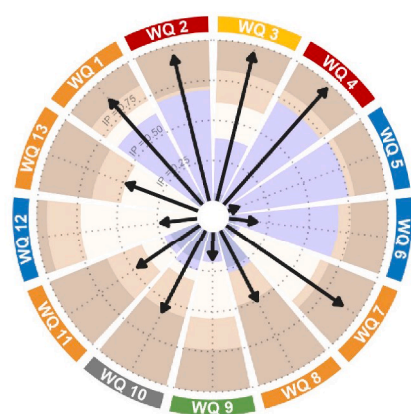
to a “moderate” quality) for the pressure category in question. This is clearly illustrated for the site studied in Fig. 5, by the high IP value obtained for nitrates (WQ3, IP = 0.95) even though the pressure level observed at this site is only “moderate” (Fig. 5A) and the medium IP value obtained for pesticides (WQ8, IP = 0.50) even though the pesticide concentration observed corresponds to the “poor” quality class.

4.1.2. Large spatial scale and identification of regional trends

We explored the spatial distribution of the significant impairment probability (IP) values returned by the models over the whole mainland France. The IPs of four models were illustrated in Fig. 6, in which we crossed the impairment probability class assigned to each of the 15,099 sampling events of the database (among the five impairment probability classes defined in § 3.1) with the quality class assigned, independently, on the basis of the environmental information (see supplementary material S5 for the other models). We used the five IP classes to split sites, according to their IP value for a given pressure category, on five distinct maps corresponding to sites exhibiting (i) “most likely low”, (ii) “probably low”, (iii) “uncertain”, (iv) “probably significant” and (v) “most likely significant” pressure levels. The colours of the dots indicate the quality classes assigned to the sites based on environmental information (see § 2.1.2 and the legend of Fig. 6). The main objective was to describe the possibilities for cartographic illustration of the results of the diagnostic tool.

Quite different spatial distribution patterns of impairment probability values were observed according to pressure categories. For all pressure categories, there is a high degree of consistency between the probability of significant impairment class (which defines the location of sites on one of the five maps, for a given pressure category) and the environmental quality class (which defines the colour in which the site is represented on a given map) for the pressure category in question (Fig. 6A–D). Several pressure categories, such as nitrates (Fig. 6A) or herbicides (Fig. 6B), show clearly regionalized spatial distribution patterns, linked to differences in land use in these regions. The highest IPs provided by the nitrate model are mainly located in the north-west of France, where intensive agricultural activities are more prevalent than in the south-east, where agricultural practices are more diversified (fruit trees, vineyards, etc.). The spatial distribution of IP values provided by the herbicide model is reversed, with high impairment probabilities mainly in the south-east of France. The large-scale pattern of site distribution could also be linked to the geological substrate, with the low probabilities provided by the clogging risk model (Fig. 6C) being mainly

A - Water quality pressures



B - Habitat degradation pressures

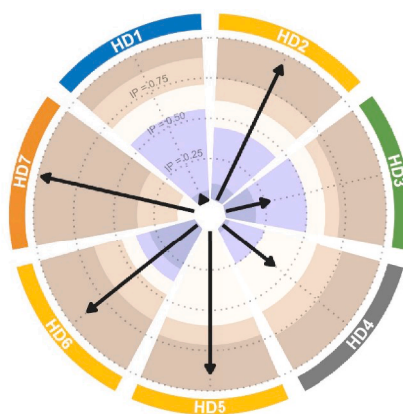


Fig. 5. Radar diagrams representing the results provided by the macrophyte-based diagnostic tool for the stream reach “La Légère” at S^t Faziol (sampled on the 2012–07–08; coord. 46°12′09.4″ N, 00°08′49.9″ W). The end of each arrow indicates the impairment probability value (IP) provided by each RF model for the stream reach studied. Concentric dotted lines correspond to IPs of 0.25, 0.50, 0.75 and 1.00. The background colour corresponds to the IP classes defined § 3.1 (with PPV and NPV): dark purple = “most likely low”, light purple = “probably low”, white = “uncertain”, light brown = “probably significant” and dark brown = “most likely significant”. The colours of labels in the outer circle denote the quality classes allocated to sampling events based on environmental information (see § 2.1.2): blue = “high”, green = “good”, yellow = “moderate”, orange = “poor”, red = “bad” and grey = “unknown”. See Table 2 for the full labels of pressure categories.

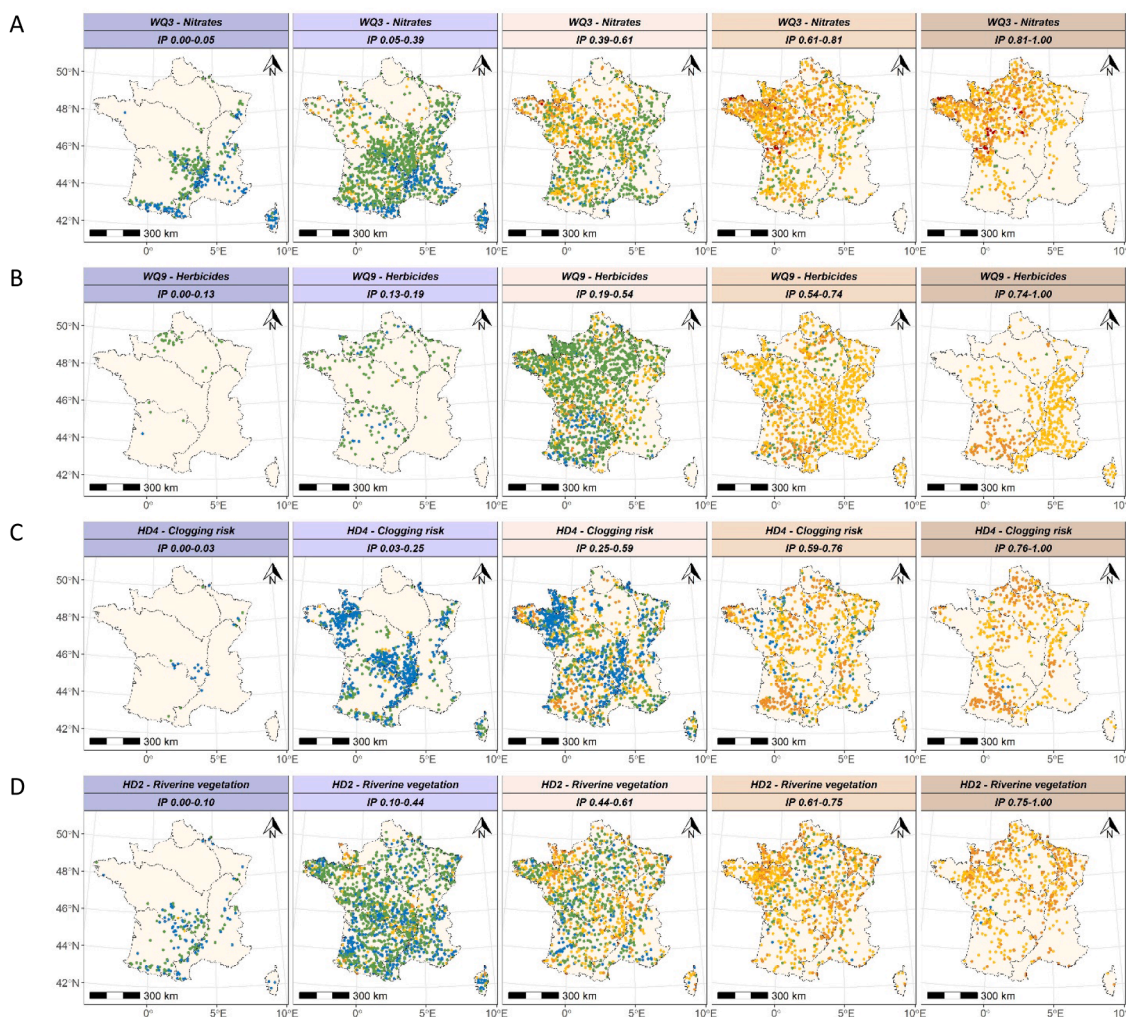


Fig. 6. Spatial distribution of impairment probabilities provided by the (A) “nitrates”, (B) “herbicides”, (C) “clogging risk” and (D) “riverine vegetation” RF models. Based on the impairment probability ranges defined § 3.1 (with PPV and NPV), we have split the IPs provided by each model on five distinct maps corresponding to sites exhibiting (i) “most likely low”, (ii) “probably low”, (iii) “uncertain”, (iv) “probably significant” and (v) “most likely significant” pressure levels (respectively from left to right). The colours of the dots denote the quality classes allocated to sampling events based on environmental information (see § 2.1.2): blue = “high”, green = “good”, yellow = “moderate”, orange = “poor” and red = “bad”.

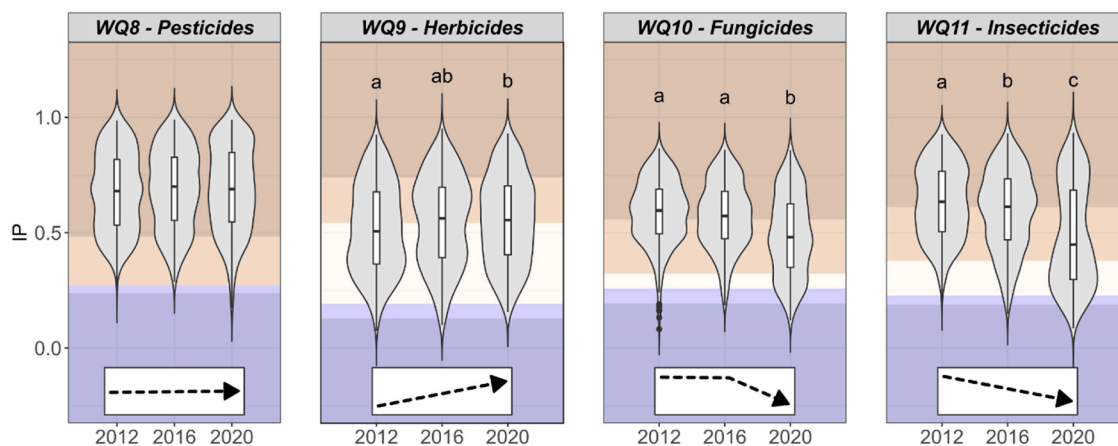


Fig. 7. Temporal changes of impairment probabilities (IPs, with violin-plots) generated by the four pesticide models (WQ8 to 11), at the 471 sites sampled in 2012, 2016 and 2020. The Friedman tests had p-values of 0.17 for the “pesticides” model (WQ8) and close to zero for the three others models (p-value < 10^{-5} , WQ9 to 11). The significant Friedman tests were followed by Sign post-hoc tests (with p-value threshold fixed at 0.01). Different letters indicate significant differences between years. The dotted arrow at the bottom of the violin-plot summarizes the temporal trends in the probabilities of impairment over the study period.

observed in crystalline massifs (e.g., the Central, Armorican and Vosges massifs). Finally, the IPs provided by some models do not seem to be organized spatially on a large scale. This is the case, for example, for the riverine vegetation model (Fig. 6D).

4.1.3. Impairment probabilities across temporal scale

We also investigated broad temporal patterns in river degradation in mainland France, by examining changes in the distribution of the impairment probabilities at 471 sites sampled in 2012, 2016 and 2020 (see Fig. 7 and supplementary material S6). None of the seven habitat degradation pressure categories showed a significant trend during this period (Friedman tests with a p-value threshold fixed at 0.01). However, among the 13 water quality pressure categories, nine categories presented a significant decreasing risk of river impairment (Friedman tests followed by Sign post-hoc tests, both with p-value threshold fixed at 0.01). This decrease was expected for certain pressure categories, such as nitrates, other nitrogen compounds and phosphorus compounds, given the recent reduction in nutrient pollution in freshwater ecosystems as a result of numerous EU environmental policies aimed at preventing nutrient contamination of freshwater (Dézerald et al., 2020; European Environment Agency, 2018; Flourey et al., 2013; Latfi et al., 2017). Two water quality pressure categories, “acidification” and “herbicides”, exhibited a significant increasing risk of river impairment, although in the case of acidification this did not lead to downgrading to the “significantly impaired” category. The results of the four pesticide models (i.e., “pesticides” as a whole, “herbicides”, “fungicides” and “insecticides”; Fig. 7) are interesting. The IPs provided by the “pesticides” model did not change significantly over the period studied. This lack of a significant temporal trend can perhaps be explained by opposite trends when examining the models specific to each category of pesticides. While the pressure exerted by fungicides and insecticides decreased significantly over the period studied, the pressure exerted by herbicides tended to increase.

An important advantage of the diagnostic approach is that various combinations of spatial and temporal scales can be studied in order to significantly help stakeholders and managers to better identify the causes of river degradation. Although it is not possible to define the level of impact of each type of pressure for each sampling event (if the value of the impairment probability falls into the class “uncertain” pressure level), the use of the five impairment probability classes represents an important advance in terms of decision support so that stakeholders and managers can propose and implement appropriate management measures.

4.2. Ecological interpretation

The relationship between the impairment probabilities provided by the RF models and the values of the metrics characterizing the macrophyte communities was explored for the 10 most contributing metrics to each RF model (see supplementary material S3). Metrics based on the relative abundance of taxonomic groups or groups of macrophytes with similar growth forms as well as descriptors of the taxonomic structure of macrophyte assemblages (species richness, diversity, evenness and dominance) make a proportionally small contribution to the construction of RF models (only 5–6% of the 10 most contributing metrics in each model). Most of the high contributing metrics are derived from the species traits of macrophytes. However, there is still an imbalance in the use of metrics between those derived from traits related to physiology, morphology, phenology and life history strategy and those describing ecological preferences, which are much more widely used. This result contrasts with those obtained when studying communities of benthic invertebrates or fishes (Dézerald et al., 2020; Mondy and Usseglio-Polatera, 2013). This can be partly explained by the absence of certain relevant “biological” traits, such as leaf area and shoot height (even if partially taken into account by growth forms) or carbon-nitrogen ratio, due to the lack of information available in the

literature for many species.

Even if traits have been resolved into a number of modalities and were fuzzy-coded to capture the potential variability of biological/ecological attributes among individuals and populations of each taxon, this approach does not allow to evaluate the role of intraspecific trait variability in community assembly at a given site (Jung et al., 2014, 2010; Violle et al., 2012). A number of studies have addressed this issue for the assembly of aquatic macrophyte communities (Fu et al., 2014; Liu et al., 2021), particularly in the context of ecological assessment (Fu et al., 2020; Rao et al., 2022), with promising results. Fu et al. (2020) showed that between 13 and 60% of the variability in the four traits tested was within species. They concluded that intraspecific variability in traits may be important in determining the assembly processes of aquatic macrophyte communities and that it may be useful for conservation to look beyond species to the individual level. The habitat is probably more restrictive for aquatic plants (e.g., due to hydraulic and lighting constraints) than for terrestrial plants, which probably explains their greater intraspecific morphological or phenological variability. However, this intraspecific approach is very difficult to implement on large spatial and temporal scales, as it requires individual measurements for each observed assemblage. In this respect, basing the diagnostic tool on “potential” fuzzy coded traits seems to be a very good compromise for a routine use, making it possible to compensate for the lack of consideration of intra-specific variability, by including a greater number of traits for a larger number of species (without the need to measure these traits).

4.3. Reliability of the diagnosis-based approach

The macrophyte trait dataset (MTD) is relatively exceptional compared to what can be found in the literature. We were able to collect information on 167 modalities of 32 traits describing characteristics linked to the physiology, morphology, phenology, life history strategy and ecological preferences of more than 400 macrophyte taxa (mainly species) with a very high completion rate. Studies looking at the assembly of aquatic macrophyte communities according to their traits are still relatively rare compared to studies on communities of benthic macroinvertebrates or fishes for example. They have focused either on a limited number of traits (e.g., Göthe et al., 2017) or on a limited number of species (e.g., Fu et al., 2014; Liu et al., 2021; Rao et al., 2022). There are a few examples of trait databases for plants that include both a large number of species and a large number of traits, but these databases often exhibit a relatively low completion rate, particularly for aquatic species (e.g., Kattge et al., 2011).

When we look for studies using these trait approaches for macrophyte communities in an ecological assessment context, the number of publications decreases even further. One example is the development of the new Danish Stream Plant Index (Baattrup-Pedersen et al., 2017), which uses 18 modalities of eight traits in its calculation but focuses on a few pressures (urban, agriculture, nitrate, phosphorous and three hydromorphological pressure categories). Unlike most of the previous macrophyte-based studies investigating ecological assessment, we have considered a broader set of pressure categories linked to water quality ($n = 13$), focusing especially on diffuse contaminations by various families of micropollutants, such as herbicides, fungicides, insecticides, metals or PAHs, and also linked to habitat degradation ($n = 7$).

All the produced RF models exhibited at least “fair” performances without overfitting on the learning dataset. Eleven RF models even exceeded the “fair” level of performance, with “good” performance for ten of them, and even “excellent” performance for the “catchment anthropization” model. The reasons why we did not achieve a good or excellent level of performance for all the RF models certainly differ from a model to another. For the “acidification” model, the number of sampling events available for each of the two pressure levels (“low” and “significant”) was certainly too skewed. In fact, 96.7% of our sampling events were classified as “low” pressure level and 3.3% as “significant”

pressure level, with only 0.2% in the “bad” quality class. In this case, the pressure gradient was certainly not wide enough to be efficiently discriminated by the RF model. For organic micropollutant models (WQ 8, 9, 10, 11 and 13), measurement of the individual parameters describing these pressures is certainly not frequent enough and has a detection/quantification limit that is not always sufficient to characterize the sampling events reliably. For the mineral micropollutant model (WQ 7), the pressure level classification is based on the inherent toxicity of each element. Determining toxicity thresholds is, however, not straightforward. Metal toxicity can indeed be modulated by numerous environmental factors. In particular, the cationic, anionic and organic matter content of water strongly influence the bioavailability of metals (Di Toro et al., 2001; Laderrière et al., 2021, 2020; Mebane et al., 2020). Metal uptake and toxicity decrease with water hardness, while the role of pH is more complex as it influences metal complexation. Based on measured concentrations of cations, anions and DOC as well as pH, and thermodynamic databases (i.e., the thermodynamic binding constants of each element), models such as the Windermere humic–aqueous model VII (WHAM, Lofts and Tipping, 2011) could be used to estimate free metal ion concentrations, enabling a better estimate of the toxicity of metals present in sampling events. Although this approach is not easy to use for large-scale studies, as it requires both a lot of time and data that are not always available, it would be interesting to explore whether it could provide a better description of the real intensity of the impact of metal contamination, and therefore enable better calibration of the “mineral micropollutants” model. Models assessing the impact of pressures linked to land use or hydromorphological alterations with “fair” performance may suffer from the fact that these pressures have only been characterized once for the whole period studied due to the limited field information available. In addition, not all categories of pressure act on the same timescale (e.g., catchment structuring, hydrology, chemical flows, climatic conditions). Consequently, the relationship between environmental parameters and the structure of aquatic vegetation on a given site and at a given date may be affected by a certain variability/uncertainty.

The large number of metrics used (518) to build the RF models could be a source of concern, as too many variables can sometimes introduce noise into the data and produce unstable results. This does not seem to be a problem here as we have tested the reliability of our models’ performance by repeating their construction 50 times by randomly changing the data in the training and test subsets, as well as the selection of metrics at each node in the individual trees (see § 2.2.2). The results did not reveal any significant differences in the level of performance of the simulated models compared with the model included in the diagnostic tool (on the basis of the training or test AUC), for the 20 pressure categories (see supplementary material S7).

4.4. Disentangling the effects of co-occurring pressures acting in a multi-pressure context

There is a real need to be able to identify the specific risks of degradation for a given river reach, and to disentangle the effects of co-occurring pressures acting in a context of multi-pressures in order to select the most appropriate restoration measures. By calculating the average number of pressure categories having a significant impact on the macrophyte communities sampled, we found that these communities were on average significantly affected by more than seven pressure categories (7.16 ± 2.02 pressure categories whose probability of impairment is classified as “probably significant” or “most likely significant”, 7.94 ± 1.56 pressure categories whose probability of impairment is classified as “probably low” or “most likely low”, and 4.90 ± 1.42 pressure categories considered as “uncertain”). Only one sampling event (among the 15,000 used in this study) was not affected by any of the pressures studied and over 90% of the sampling events were affected by at least five pressure categories, which is consistent with other studies (Dézerald et al., 2020). This statement is all the more worrying as it does

not take into account a number of emerging pollutants such as lithium (Choi et al., 2019), pharmaceutical substances and contrast agents from domestic and hospital effluents (Jones et al., 2004), microplastics (Issac and Kandasubramanian, 2021) or PFAS (Domingo and Nadal, 2019).

Different categories of anthropogenic pressure induce different changes in selected traits within macrophyte assemblages, leading to trait combinations that are often specific to a given pressure category (Desrosiers et al., 2019), and thus offering a great opportunity to differentiate the effects of pressure categories acting simultaneously. This specificity has been highlighted by the 10 metrics most contributing to each of the 20 RF models. In average, only less than one metric of a given model (0.82 ± 1.65) was indeed shared with one of the other 19 models, and 111 metrics (over the 200 potential metrics, i.e., 10 metrics \times 20 RF models) belonged to the 10 most contributing metrics for at least one model. This fact makes the RF method robust for clearly assessing biological responses of different intensities, even within the same group of pressures (e.g., pesticides).

5. Conclusion

The aim of the diagnostic approach is to supplement the information provided by assessment methods based on biotic indices, by identifying the categories of pressure that have a major impact on macrophyte communities, particularly in situations of multiple pressures. Each category of anthropogenic pressure induces changes in the structure and taxonomic and functional composition of the macrophyte assemblages, leading to original combinations of species and bio/ecological traits depending on the nature and intensity of the anthropogenic constraint. This set of signals, sometimes weak, analysed by random forests, is robustly converted into a probability of significant impact, the value of which we have tried to show in the ecological diagnosis of watercourses at different scales of observation. The macrophyte-based models are indeed ecologically relevant and exhibit promising efficiency for the diagnostic of both water quality alteration and habitat degradation. These models are also capable of diagnosing various water bodies, from headwaters to large lowland rivers. Merging the information provided by models based on different biological quality elements (benthic macroinvertebrates, benthic diatoms, fishes and macrophytes) should pave the way for an even more effective understanding of the causes of river degradation, which will consistently help stakeholders and water managers in the decision-making process relating to river restoration.

CRedit authorship contribution statement

Thibault Leboucher: Software, Data curation, Visualization, Formal analysis, Conceptualization, Writing – original draft, Methodology. **Jérémy Bacon:** Data curation, Writing – review & editing, Formal analysis. **Christian Chauvin:** Writing – review & editing, Data curation, Funding acquisition. **Elise Billoir:** Methodology, Writing – review & editing. **Maud Pierre:** Data curation. **Augustine Fulcrand:** Data curation. **Bastien Bourillon:** Data curation, Writing – review & editing. **Philippe Usseglio-Polatera:** Methodology, Conceptualization, Writing – original draft, Data curation, Funding acquisition.

Declaration of competing 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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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at [doi:10.1016/j.watres.2025.124173](https://doi.org/10.1016/j.watres.2025.124173).

Data availability

<http://www.naiades.eaufrance.fr> (Taxonomic and environmental data can be extracted at . Biological trait data are provided in Table S1. Ecological trait data are in Loriot et al. (2014).)

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