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Ecosystems monitoring powered by environmental genomics: A review of current strategies with an implementation roadmap

Tristan Cordier¹  | Laura Alonso-Sáez² | Laure Apothéloz-Perret-Gentil¹  |
 Eva Aylagas³  | David A. Bohan⁴ | Agnès Bouchez⁵ | Anthony Chariton⁶  |
 Simon Creer⁷ | Larissa Frühe⁸ | François Keck⁵  | Nigel Keeley⁹ | Olivier Laroche⁹ |
 Florian Leese^{10,11}  | Xavier Pochon^{12,13} | Thorsten Stoeck⁸  | Jan Pawlowski^{1,14,15} |
 Anders Lanzén^{2,16} 

¹Department of Genetics and Evolution, Science III, University of Geneva, Geneva, Switzerland

²AZTI, Marine Research, Basque Research and Technology Alliance (BRTA), Spain

³Red Sea Research Center (RSRC), Biological and Environmental Sciences and Engineering (BESE), King Abdullah University of Science and Technology (KAUST), Thuwal, Saudi Arabia

⁴Agroécologie, INRAE, University of Bourgogne, University Bourgogne Franche-Comté, Dijon, France

⁵UMR CARTELE, INRA, USMB, Thonon-les-Bains, France

⁶Department of Biological Sciences, Macquarie University, Sydney, NSW, Australia

⁷School of Natural Sciences, Bangor University, Gwynedd, UK

⁸Department of Ecology, Technische Universität Kaiserslautern, Kaiserslautern, Germany

⁹Benthic Resources and Processes Group, Institute of Marine Research, Tromsø, Norway

¹⁰Aquatic Ecosystem Research, Faculty of Biology, University of Duisburg-Essen, Essen, Germany

¹¹Centre for Water and Environmental Research (ZWU), University of Duisburg-Essen, Essen, Germany

¹²Coastal & Freshwater Group, Cawthron Institute, Nelson, New Zealand

¹³Institute of Marine Science, University of Auckland, Warkworth, New Zealand

¹⁴ID-Gene Ecodiagnostics, Geneva, Switzerland

¹⁵Institute of Oceanology, Polish Academy of Sciences, Sopot, Poland

¹⁶Basque Foundation for Science, IKERBASQUE, Bilbao, Spain

Correspondence

Tristan Cordier, Department of Genetics and Evolution, University of Geneva, Science III, Geneva, Switzerland.
 Email: tristan.cordier@gmail.com

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Abstract

A decade after environmental scientists integrated high-throughput sequencing technologies in their toolbox, the genomics-based monitoring of anthropogenic impacts on the biodiversity and functioning of ecosystems is yet to be implemented by regulatory frameworks. Despite the broadly acknowledged potential of environmental genomics to this end, technical limitations and conceptual issues still stand in the way of its broad application by end-users. In addition, the multiplicity of potential implementation strategies may contribute to a perception that the routine application of this methodology is premature or “in development”, hence restraining regulators from binding these tools into legal frameworks. Here, we review recent implementations of

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environmental genomics-based methods, applied to the biomonitoring of ecosystems. By taking a general overview, without narrowing our perspective to particular habitats or groups of organisms, this paper aims to compare, review and discuss the strengths and limitations of four general implementation strategies of environmental genomics for monitoring: (a) Taxonomy-based analyses focused on identification of known bioindicators or described taxa; (b) De novo bioindicator analyses; (c) Structural community metrics including inferred ecological networks; and (d) Functional community metrics (metagenomics or metatranscriptomics). We emphasise the utility of the three latter strategies to integrate meiofauna and microorganisms that are not traditionally utilised in biomonitoring because of difficult taxonomic identification. Finally, we propose a roadmap for the implementation of environmental genomics into routine monitoring programmes that leverage recent analytical advancements, while pointing out current limitations and future research needs.

KEYWORDS

biodiversity, biomonitoring, ecosystem management, environmental DNA, implementation strategy, metabarcoding

1 | THE NEED FOR BROAD SCALE ECOSYSTEM MONITORING STRATEGIES

Biodiversity drives the fundamental processes of ecosystems and provides invaluable services on which we depend. Anthropogenic, detrimental impacts on ecosystems, including accelerating climate change, are unprecedented (Waters et al., 2016) and have led to a decline of biodiversity across the globe (Butchart et al., 2010; Cardinale et al., 2012; Hughes et al., 2018). Recent reports stress that one out of the 8 million known species are presently at risk of extinction (IPBES, 2019). This threatens ecosystem function(ing) and services. Therefore, the urgent challenge is now to build a set of efficient tools to enhance our capacity to predict or detect early warnings of critical ecological shifts efficiently, in order to forecast the direction of such shifts and their impacts on ecosystem functions and services (Barnosky et al., 2012; Carpenter et al., 2011; Ratajczak et al., 2018).

Because our societies aim to reach a trade-off between socioeconomic development and ecosystems sustainability (UN A/RES/70/1, 2015), regulatory frameworks have been established worldwide for the sustainable development of industries within environmental constraints (Niemeijer, 2002; Grizzetti, Lanzanova, Liqueste, Reynaud, & Cardoso, 2015). Such regulatory systems have been incorporated into various national and international directives, especially for aquatic ecosystems (e.g., the Water Framework Directive, WFD, Directive 2000/60/EC and Marine Strategy Framework Directive, MSFD, Directive 2008/56/EC in Europe, the Clean Water Act of the US Environmental Protection Agency in the USA, as well as the United Nations Convention on the Law of the Sea, UNCLOS). The backbone of such monitoring programmes is the biological component of ecosystems, as a measure of ecosystem "health" or "integrity" (Karr, 1999). This biological component is often referred to as

the Biological Quality Elements in those regulations (BQEs, Borja et al., 2013; Hering et al., 2018). Most monitoring strategies implemented in regulations rely on the bioindication principle (autecology, Box 1), i.e., significant correlations between the occurrence of specific organisms and a set of environmental variables. Although chemical and hydrological monitoring techniques provide an environmental quality snapshot, biological indicators convey a cumulative time-integrated measure as their occurrence is the product of their local adaptation and their responses to ecosystem variations and/or disturbances across an extended period of time (Birk et al., 2012; Carignan & Villard, 2002; Lear, Dopheide, Ancion, & Lewis, 2011).

2 | THE LIMITS OF CURRENTLY IMPLEMENTED ECOSYSTEM MONITORING STRATEGIES

Traditionally, morphologically distinguishable invertebrates have been used as bioindicators in both aquatic and terrestrial ecosystems (Bongers & Ferris, 1999; Gerlach, Samways, & Pryke, 2013; Hodkinson & Jackson, 2005; Reynoldson & Metcalfe-Smith, 1992). Fishes, amphibians, macrophytes, phytoplankton and diatoms are also routinely used in aquatic ecosystems (Birk et al., 2012). Various biotic indices (BIs) have been formalized, based on the predictable responses of bioindicator species to environmental disturbances (autecological value) in marine (Borja, Franco, & Pérez, 2000; Maurer, Nguyen, Robertson, & Gerlinger, 1999; Rygg & Norling, 2013), freshwater (Kelly, Penny, & Whitton, 1995; Prygiel & Coste, 2000; Stark, 1998) and terrestrial (Marull, Pino, Mallarach, & Cordobilla, 2007; Urzelai, Hernández, & Pastor, 2000) ecosystems. Almost half of the monitoring methodologies currently used in Europe rely on such BIs (Birk et al., 2012). However, for environments

BOX 1 Glossary of terms used in this paper

- **Implementation strategy:** Refers to the way environmental genomics data is produced and analysed in an ecosystem monitoring context. It includes the choice of all the molecular biology steps, i.e., targeted molecules (DNA vs. RNA), metabarcoding (amplicon sequencing) versus metagenomics or metatranscriptomics (shotgun sequencing), and the computational biology steps (analytical approach), i.e., focusing on the taxonomically assigned sequences or considering all the sequences, the use of compositional turnovers (beta-diversity), structural metrics (alpha or phylogenetic diversity and ecological network properties) or functional metrics (functional genes or transcripts diversity).
- **Environmental genomics:** Suite of molecular tools to sample, process and analyse nucleic acids from an environmental sample (soil, water, sediment, faeces)
- **Environmental DNA/RNA:** Nucleic acids present in an environmental sample. It encompasses the DNA/RNA within living multi- or unicellular organisms, dead or decaying as well as extracellular material.
- **Metabarcoding:** A molecular workflow to simultaneously study the diversity of PCR-selected organisms from environmental samples using high-throughput sequencing. This is equivalent to amplicon sequencing of a taxonomic marker.
- **Metagenomics:** Shotgun sequencing of the genomic DNA isolated from an environmental sample. There is no PCR selection of particular taxonomic group and include coding as well as non-coding genomic material.
- **Metatranscriptomics:** Shotgun sequencing of retrotranscribed RNA isolated from an environmental sample. As for metagenomics, there is no PCR selection but includes only transcribed RNA (mRNA, rRNA), supposedly functional.
- **Bioindicator:** A taxon, marker sequence, gene or transcript that is used as an indicator of the ecological status of an environment.
- **Autecological value:** Ecological knowledge about the distribution and abundance of particular species obtained by studying interactions of individual organisms with their environments.
- **Biotic indices:** Continuous or discrete variables that measure the level of disturbance of an environment based on the composition and relative abundance of bioindicator taxa (or OTUs/ASVs). Around half of the existing monitoring programmes rely on biotic indices (BIs). The BIs usually includes several ordered discrete classes, usually from "poor" to "high" ecological status.
- **Ecological network:** Representation of statistically inferred biotic interactions through spatial or temporal co-occurrence or co-exclusion. Taxa (nodes) are connected by pairwise links (edges). Network ecology aims to understand how these network properties are linked to the functioning of ecosystems.

or geographical regions for which no BI has been calibrated, ecological assessments rely instead on biodiversity measures of "charismatic" groups such as fishes (Pont et al., 2006), amphibians (Welsh & Ollivier, 1998) and insects (Basset et al., 2004).

Morphology-based methodologies require the collection and identification of hundreds to thousands of specimens per sample, which is a slow, labour-intensive process. These limitations seriously hamper our capacity to scale up biomonitoring and satisfy the increasing demand for environmental monitoring programmes in a timely fashion that allows informed ecosystem management (Baird & Hajibabaei, 2012). Moreover, this conventional morphology-based approach is compromised by several other shortcomings: (a) it focuses only on morphologically identifiable biodiversity, ignoring the inconspicuous meiofaunal and microbial domains, which are known to include powerful bioindicators; (b) cryptic diversity remains unrecognized (morphologically indistinguishable look-alikes with differing tolerance to disturbances); and (c) variation in species life stages, damaged specimens and misidentifications caused by decreasing taxonomic expertise worldwide may lead to variable and noisy species' inventories, and by extension, to uncertain ecological assessments. Taken together, the need for faster, more objective, robust and cost-effective tools and strategies to deliver a more efficient ecosystem monitoring has never been more pressing.

3 | ENVIRONMENTAL GENOMICS REVOLUTION FOR BIODIVERSITY RESEARCH AND ECOSYSTEM MONITORING

Over the last decade, the development of environmental genomics (EG) coupled with high-throughput sequencing (HTS) technologies has led to a marked improvement in our ability to document biodiversity patterns, for both species occurrence (amplicon sequencing, i.e., metabarcoding, reviewed in Bohmann et al., 2014; Cristescu & Hebert, 2018; Deiner et al., 2017; Ruppert, Kline, & Rahman, 2019; Taberlet, Bonin, Zinger, & Coissac, 2018; Valentini et al., 2016) and their metabolic functions (metagenomics and metatranscriptomics, reviewed in Ungerer, Johnson, & Herman, 2008; Escalas et al., 2019; Quince, Walker, Simpson, Loman, & Segata, 2017; Singer, Wagner, & Woyke, 2017; Vandenkoornhuyse et al., 2010). Multidisciplinary teams and consortiums have initiated large-scale projects aiming at collecting biodiversity data using EG throughout the globe, to address fundamental ecological questions. Among these initiatives, the large barcoding projects led by the international Barcode of Life (Ratnasingham & Hebert, 2007), the Earth Microbiome Project (Gilbert et al., 2010) and the TARA Oceans Project (Karsenti et al., 2011) represent three of the most emblematic examples. Those projects have unravelled an unexpected cryptic (Bickford et al., 2007) and novel microbial diversity (the "unseen majority") guiding reconstruction of the eukaryotic tree of life (Adl et al., 2019). Even though this microbial diversity is known to represent a key component of ecosystem functioning (Cavicchioli et al., 2019;

Delgado-Baquerizo et al., 2016; Guidi et al., 2016), the ecology of most microorganisms remains largely enigmatic.

The potential of EG for surveying biodiversity and monitoring natural ecosystems at a broad spatiotemporal scale was quickly identified and implemented by environmental scientists (Baird & Hajibabaei, 2012; Davies et al., 2012; Kelly et al., 2014; Taberlet, Coissac, Pompanon, Brochmann, & Willerslev, 2012). This work has been boosted by the massive drop in sequencing costs, with over four orders of magnitude within the last 15 years (<https://www.genome.gov>). This has enabled numerous clinical and environmental routine applications. Indeed, fueled by the continuous efforts to optimize laboratory protocols and bioinformatic tools, all steps from large-scale collection of samples, generation of HTS data, statistical analysis, and interpretation of results, can now be performed in matter of days or weeks (Deshpande et al., 2019; Juul et al., 2015; Quinn et al., 2016; Reintjes et al., 2019). For aquatic ecosystems especially, the next breakthrough of this revolution is now expected to be the development and deployment of low-cost, automated and miniaturized *in situ* environmental nucleic acids (eDNA/RNA) samplers (Carr et al., 2017; Gan et al., 2017). These may be integrated into autonomous instruments for broadscale and continuous ecosystem monitoring programmes (Aguzzi et al., 2019; Benway et al., 2019; Bohan et al., 2017; Brandt et al., 2016; Levin et al., 2019).

These advances in genomics-based research have led to a series of pilot studies assessing the applicability of EG for the monitoring of ecosystem changes by collecting biodiversity data from various taxonomic groups (e.g., fishes, macroinvertebrates, protists, bacteria) and environments (e.g., water, biofilms, soil or sediment). Several such pilot studies have targeted multicellular organisms as a replacement for arduous morphological identification of the same taxa (Hajibabaei, Shokralla, Zhou, Singer, & Bair, 2011; Hajibabaei, Spall, Shokralla, & van Konynenburg, 2012; Lejzerowicz et al., 2015; Thomsen et al., 2012; Zhou et al., 2013). However, the potential of EG to leverage the general eukaryotic and prokaryotic diversity for ecological monitoring, has also been explored (Bik Halanych, Sharma, & Thomas, 2012; Chariton, Court, Hartley, Colloff, & Hardy, 2010; Dowle, Pochon, Keeley, & Wood, 2015; Lallias et al., 2015), and indeed advocated (Bouchez et al., 2016; Chariton et al., 2016; Creer et al., 2010; Graham et al., 2016; Payne, 2013). Encouraged by the immense opportunities for ecosystem monitoring, over 45 countries recently decided to join their efforts within the European COST Action DNAqua-Net, to anticipate upcoming paradigm shifts and develop genomic tools tailored for the monitoring of aquatic ecosystems (<http://dnaqua.net>, Leese et al., 2016). Similarly, other large-scale collaborative projects were recently launched, including STREAM in Canada (<https://stream-dna.com/>), Lakes380 in New Zealand (<https://lakes380.com/>) and NGB in France (<http://next-genbiomonitoring.org/>), aiming at the unbridling of EG for ecosystem monitoring.

Multiple pilot and methodological EG studies have highlighted important variation in terms of compliance with current regulatory programmes (reviewed in Hering et al., 2018), leading to the proposition of multiple implementation strategies for current and future ecosystem monitoring programmes. Here, we compare and review the

strengths and limitations of these EG-based strategies for ecosystem monitoring. Our objective is to pinpoint the criteria of existing monitoring programmes that could be fulfilled by EG methods as of today, and clarify the work ahead for the monitoring programmes that could benefit from EG in the near future, given continued technological and analytical advancements. To this end, we classify these strategies into four broad categories (Figure 1, Table S1): (a) Taxonomy-based analyses that focus on known bioindicator species, or the identification and enumeration of formally or informally described taxa; (b) De novo bioindicator analyses aiming to identify and utilise novel bioindicators, independent of formal taxonomy; (c) Structural community metrics relying on community structure or inferred ecological networks, where taxa are interchangeable; and (d) Functional community metrics or indicators that focus on protein-coding genes or transcripts instead of taxonomic composition. Based on the specificities of each strategy, their level of maturity and their compatibility with existing regulations (Table 1), we propose an implementation roadmap to integrate EG into ecosystems monitoring programmes and highlight future research needs to be undertaken.

4 | “TAXONOMY-BASED” STRATEGY: SCREENING KNOWN SPECIES AND BIOINDICATORS WITH ENVIRONMENTAL GENOMICS

This strategy relies on the enumeration of known biodiversity from DNA obtained from an environmental sample (e.g., sediment, soil, biofilm, water) or from bulk material prepared from an environmental sample by e.g., elutriation, trapped individuals or biofilm scratching (Figure 1a). This strategy closely fits the conventional, morphology-based monitoring approach, because it primarily aims at reaching a satisfactory level of congruence in terms of both qualitative and quantitative biodiversity inventories. The taxonomy-based strategy is *de facto* limited to the morphologically characterized fraction of biodiversity for which reference sequences are available in public databases. Hence, approaches using it have usually overlooked meiofaunal or microbial taxa, difficult to identify on the basis of morphological traits, and for most of which the autecology is poorly known (but see Pawlowski, Esling, et al., 2016). The reference databases routinely used by EG studies include for instance the universal but essentially noncurated GenBank nucleotide repository from the National Center for Biotechnology Information (Benson et al., 1999, but see Leray, Knowlton, Ho, Nguyen, & Machida, 2019), or the curated databases BOLD for COI barcodes, primarily from animals (Ratnasingham & Hebert, 2007), SILVA for universal ribosomal markers (Quast et al., 2013), PR² for protists (Guillou et al., 2013), Diat.barcode for diatoms (Rimet et al., 2016), and Unite for fungi (Nilsson, Sharma, Bhatnagar, Bertilsson, & Terenius, 2019).

Depending on the environment assessed and the taxonomic group considered, the performance of taxonomy-based approaches varies considerably (Hering et al., 2018). Benchmarking studies comparing EG-based and conventional morphology-based taxonomic

TABLE 1 Comparison of the four implementation strategies in terms of compatibility with current standards, backward and forward compatibility, performance, biodiversity coverage, generalization potential and ease of standardization

Implementation strategy	Subcategory	Compatibility with current standards	Backward and forward compatibility	Performance for monitoring programme	Potential biodiversity coverage ^a	Potential spatiotemporal generalization	Ease of standardization
(A) Taxonomy-based	Screening species	+++	++	+++	++	+	+
(A) Taxonomy-based	Screening bioindicators (for BIs)	+++	++	++	+	+	+
(B) De novo	Bioindicators discovery	–	–	–	+++	++	+
(B) De novo	Supervised learning and predictions	+++	+++	+++	+++	++	+
(C) Structural community metrics	Alpha and phylogeny-aware metrics	+ (alpha diversity)	–	+	+++	+	+
(C) Structural community metrics	Co-occurrence networks	–	–	–	+++	+++	+
(D) Functional community metrics	Functional genes/transcripts	–	–	–	+++	+++	++

^aDepends on molecular methodology, i.e., amplicon versus shotgun. If amplicon, it will depend on PCR primers targets, e.g., universal eukaryotes and prokaryotes versus group specific primers.

inventories (Table S1) have shown mixed degrees of congruence. For the noninvasive detection of fish species from DNA traces in filtered marine water, the rate of success from taxonomy-based monitoring is reported near perfect (Thomsen et al., 2012; Bakker et al., 2017; but see DiBattista et al., 2017). For freshwater macroinvertebrate bulk samples, the rate of species detection varied from 67% (Elbrecht, Vamos, Meissner, Aroviita, & Leese, 2017) to 73%–83% (Hajibabaei et al., 2011, 2012). In contrast, for benthic diatoms sampled from biofilms, the congruence of morphological taxonomy and EG-inferred taxonomy, in terms of shared taxa at species level, ranged only from 15%–18% (Rivera et al., 2018; Vasselon, Domaizon, et al., 2017) to 28% (Visco et al., 2015). The reported congruence for macroinvertebrates sampled from marine sediments ranged from 20% (Lejzerowicz et al., 2015) up to 60% (Aylagas, Borja, Irigoien, & Rodríguez-Ezpeleta, 2016). Noteworthy, those studies also detected numerous species that were unnoticed in morphological inventories (Elbrecht et al., 2017; Hajibabaei et al., 2011, 2012). Despite these discrepancies, the studies inferring BI values from the detected bioindicators species show very promising results, for both freshwater diatoms (Kelly et al., 2018; Kermarrec et al., 2014; Vasselon, Rimet, et al., 2017; Visco et al., 2015) and macroinvertebrates (Elbrecht et al., 2017) as well as for marine macroinvertebrates (Aylagas et al., 2016; Lejzerowicz et al., 2015). While acknowledging that the congruence for both qualitative and quantitative inventories are not fully satisfactory, these studies have demonstrated that EG tools are still able to detect sufficient bioindicator taxa to infer accurate BI values, even when considering only presence/absence (Aylagas et al., 2016). The EG methodology has therefore been promoted as a promising tool for fast and cost-effective biodiversity screening for ecosystem monitoring, even while the simultaneous collection of classical morphological samples for validation is univocally suggested. Nonetheless, further improvements in molecular protocols as well as BI intercalibration is a necessity towards harmonization and standardization across Europe (Hering et al., 2018; Poikane et al., 2014) and beyond (Jeunen et al., 2019).

Various biological and technical limitations still impede the implementation of the taxonomy-based strategy for routine monitoring applications (Leese et al., 2018). These limitations mainly stem from the fact that the methods sample fundamentally different units of presence (molecules vs. individuals), resulting in different biases affecting richness, abundance and taxonomic composition. The richness of “molecular species”, i.e., operational taxonomic units (OTUs) or Amplicon Sequence Variants (ASVs, the new operational unit paradigm, Callahan, McMurdie, & Holmes, 2017), should not be considered analogous to morphospecies richness even in the theoretical absence of noise resulting from PCR and sequencing biases. This discrepancy is due to cryptic diversity (Stork, 2018), intragenomic or intraspecific marker variation (Bik, Fournier, Sung, Bergeron, & Thomas, 2013; Sun, Jiang, Wu, & Zhou, 2013), and the presence of DNA from dead and inactive organisms or as extracellular DNA (Collins et al., 2018). Likewise, the abundance of taxa inferred from HTS read counts can typically not be used to infer the number of individuals. Indeed, the number of sampled DNA molecules and

sequence reads are a consequence of the number of individuals, but also of the biomass and the variable number of copies of the targeted marker in the genome (Bik et al., 2013; Větrovský & Baldrian 2013), in addition to variations in DNA extractability and primer-specific amplification bias (Elbrecht & Leese, 2015; Krehenwinkel et al., 2017; Piñol, Mir, Gomez-Polo, & Agustí, 2015). Finally, EG studies suffer from a strong sampling effect because DNA extractions are typically performed from small amounts of material, making large-size organisms less well-represented in eDNA extracts (Lanzén, Lekang, Jonassen, Thompson, & Troedsson, 2017). However, bulk samples (Elbrecht et al., 2017), larger extraction volume (Nascimento, Lallias, Bik, & Creer, 2018) or more aggressive homogenization (Lanzén et al., unpublished data) can partially alleviate this issue.

Since the taxonomy-based strategy depends on reference sequences for organism identification, the incompleteness of reference databases can also have a major impact. Hence, completing databases, both by the “vertical” addition of more taxa and by the “horizontal” coverage of wider geographical areas, would certainly contribute to an improvement in identification (McGee, Robinson, & Hajibabaei, 2019; Vasselon, Domaizon, et al., 2017). However, despite sustained efforts, reference databases will probably remain skewed towards some taxa, while suffering from important gaps across other taxonomic groups or biogeographical regions (McGee et al., 2019; Weigand et al., 2019). All these issues directly impact both of the key parameters for applying BIs to assess impact, namely the qualitative and quantitative measures of biodiversity (Pawłowski et al., 2018).

Nevertheless, multiple studies have shown that there is room for considerable improvements to better bridge the current gaps between taxonomy-dependent molecular and morphology-based approaches. Taxonomic breadth in HTS data could be broadened by carefully designing novel amplification primers (Elbrecht et al., 2019) or using more than one primer pair (Corse et al., 2019). Applying correction factors to read counts, based on established knowledge of the biovolume (Vasselon et al., 2018), the number of copies of the targeted marker (Větrovský & Baldrian 2013) or by spiking samples with known internal standard for quantitative determinations (Ji et al., 2020; Tkacz, Hortala, & Poole, 2018), are all promising methods for resolving these challenges. Finally, the integration of bioinformatic tools for the automated curation of databases from mislabeled sequences will improve their reliability (Ashelford, Chuzhanova, Fry, Jones, & Weightman, 2005; Kozlov, Zhang, Yilmaz, Glöckner, & Stamatakis, 2016).

5 | “DE NOVO” STRATEGY: DISCOVERING NEW BIOINDICATORS AND HARNESSING THEM FOR ROUTINE MONITORING

In contrast to the taxonomy-based strategy, the *de novo* one does not immediately generate an ecological assessment, because it does not employ previous knowledge associated with bioindicators. Instead, the *de novo* strategy aims at establishing new bioindicators using EG-based profiling of communities and independently

generated ecological status or known disturbance gradients (Figure 1b). Harnessing EG and HTS technologies to explore a broader range of biological diversity, formally labelled or not (i.e., taxonomically described or identified), represents an opportunity to move towards a more holistic monitoring paradigm (Bik et al., 2012; Chariton et al., 2010). By considering all the OTU (or ASV) profiles along a known impact gradient of typical anthropogenic origin, studies applying this strategy have shown that HTS data represent a virtually unlimited reservoir of new bioindicators. Examples (listed in Table S1) include contamination by pesticides (Thompson et al., 2016; Andújar et al., 2017) or other agricultural stressors (Salis, Bruder, Piggott, Summerfield, & Matthaei, 2017), and gradients of eutrophication and urban contamination in freshwater systems (Apothéloz-Perret-Gentil et al., 2017; Martínez-Santos et al., 2018; Simonin et al., 2019; Tapolczai, Vasselon, et al., 2019; Tapolczai, Vasselon, et al., 2019). In marine environments, the utility of this strategy has been demonstrated after an oil spill (Bik et al., 2012), in the vicinity of offshore drilling platforms (Cordier, Frontalini et al., 2019; Lanzén, Lekang, Jonassen, Thompson, & Troedsson, 2016; Laroche, Pochon, et al., 2018; Laroche et al., 2016) and aquaculture sites (Pawłowski, Esling et al., 2014, Pochon et al., 2015; Dowle et al., 2015; Keeley, Wood, & Pochon, 2018; Stoeck, Frühe, et al., 2018; Stoeck, Frühe, et al., 2018 as well as along eutrophication and urban or industrial contamination gradients in estuaries (Chariton et al., 2010; Chariton et al., 2015; Angly et al., 2016; Lallias et al., 2015; Obi et al., 2016). Interestingly, most of the studies sampling marine sediments highlighted that meiofaunal invertebrates, such as nematodes, gastrotrichs and platyhelminths (Bik et al., 2012; Chariton et al., 2010; Lanzén et al., 2016), large groups of protists such as diatoms, oomycetes and ciliates (Lanzén et al., 2016; Stoeck, Frühe, et al., 2018) or foraminifera (Pawłowski, Esling, et al., 2014; Laroche et al., 2016; Frontalini et al., 2018) but also fungi (Bik et al., 2012) and bacteria (Angly et al., 2016; Aylagas et al., 2017; Dowle et al., 2015; Keeley et al. 2018; Martínez-Santos et al., 2018; Obi et al., 2016; Stoeck, Frühe, et al., 2018) have great potential as bioindicators of anthropogenic impacts and can readily be captured by EG studies.

Unfortunately, most proof-of-concept studies employing the *de novo* strategy have not yet validated their results by performing ecological assessments based on newly identified bioindicators as a reference in a new environmental context. For this information to be useful on new samples, the data obtained from known disturbance gradients (i.e., reference or training data set) must be operational in different spatiotemporal contexts. To this end, two main approaches have been proposed and tested, namely indicator value (e.g., the IndVal approach, Dufrêne & Legendre, 1997) and supervised machine learning (SML, Crisci, Ghattas, & Perera, 2012; Libbrecht & Noble, 2015).

The indicator value approach ascribes autecological values (or discrete “eco-groups”) to OTUs or ASVs based on their occurrence in samples of known disturbance level, in a similar manner as for the establishment of morphology-based bioindicators. Hence, the autecological values of these *de novo* bioindicators are directly calibrated on the HTS data, which alleviates the qualitative

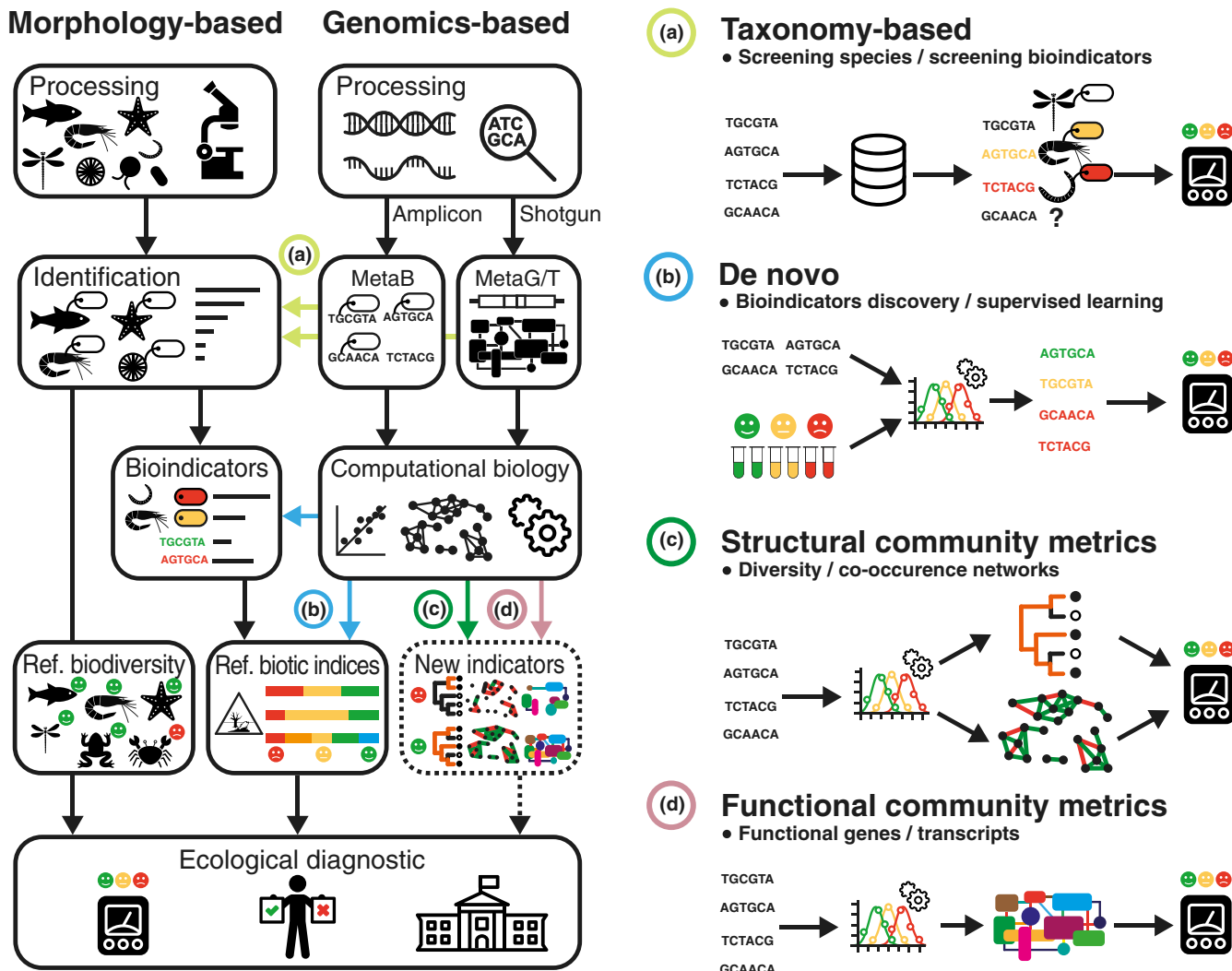


FIGURE 1 Overview of the current methodology for the monitoring of ecosystems, that relies mostly on the morphological identification of biodiversity and/or bioindicators of anthropogenic impacts. Ecological diagnostics are performed based on reference biodiversity or on reference biotic indices for a given ecosystem. The development of environmental genomics methodologies has led to the proposition of multiple implementation strategies that can intervene at different levels of the monitoring workflow, to produce an ecological diagnostic. Green colours and smileys within boxes indicate reference biodiversity and "good" or "high" ecological status while red colours and smileys represent nonreference biodiversity and "poor" ecological status (i.e., impacted environments) Green colours and smileys within boxes indicate reference biodiversity and "good" or "high" ecological status while red colours and smileys represent nonreference biodiversity and "poor" ecological status (i.e., impacted environments). The colours on tags besides organisms or sequences indicate their bioindication value (red: indicator of impact, yellow: indicator of intermediate status, green: indicator of good status). In this review paper, these strategies have been grouped into four broad categories: (a) Taxonomy-based analyses focused on identification of known bioindicators or described taxa; (b) De novo bioindicator analyses; (c) Structural community metrics including inferred ecological networks; and (d) Functional community metrics (metagenomics or metatranscriptomics)

and quantitative biases encountered with the taxonomy-based EG strategy. This has proven successful for both freshwater benthic diatoms (Apothéloz-Perret-Gentil et al., 2017; Tapolczai, Vasselon, et al., 2019; Tapolczai, Vasselon, et al., 2019) and for bacterial and eukaryotic communities in streams and estuarine systems (Chariton et al., 2015; Li et al., 2018). An analogous approach is the use of polynomial quantile regression splines (Anderson 2008). This has shown great promise for the prediction of impacts from organic enrichment in aquaculture sites using eukaryotic and prokaryotic metabarcoding data in parallel (Keeley et al., 2018). For diatoms, the accuracy

of the assessment can be largely improved, arguably because the indicator value approach makes use of a larger number of OTUs or ASVs, compared to an approach relying solely on their taxonomic assignments (Apothéloz-Perret-Gentil et al., 2017; Tapolczai, Vasselon, et al., 2019; Tapolczai, Vasselon, et al., 2019).

Supervised machine learning (SML) also requires training data sets, i.e., reference disturbance levels (labels) associated with the community profiles of the samples (features). These algorithms are best at classification problems involving multidimensional and noisy data sets (Libbrecht & Noble, 2015), which are common attributes

of HTS data. The task is to automatically disentangle the feature signal (OTU or ASV profiles) and their co-occurrence that convey an ecological signal from background noise. This extracted knowledge is self-contained in a trained model that can be used to make predictions of disturbance level on new samples, based on their compositional profiles (Cordier, Lanzén, et al., 2019). Supervised machine learning also alleviates the qualitative and quantitative biases that hamper the taxonomy-based strategy in a more straightforward manner, because the model is trained directly on HTS data. The applicability of SML has been demonstrated in marine environments, for the detection of various pollutants (Smith et al., 2015) and for the prediction of aquaculture impacts on benthic biodiversity (Cordier et al., 2017, 2018). The SML-based inference of BI values has also been shown to outperform the taxonomy-based strategy, relying on the detection of established macroinvertebrates bioindicators DNA (Cordier et al., 2018), and may be more powerful than the IndVal approach (Frühe et al., 2020). Supervised machine learning applications have also succeeded in predicting the origin of container ship ballast waters (Gerhard & Gunsch, 2019).

The *de novo* strategy provides numerous advantages over the taxonomy-based one. First, it can reduce or bypass the dependence on reference sequence databases for taxonomic assignments of HTS reads to known bioindicators. Instead, new ecological knowledge is hypothesised *de novo* during the calibration of OTUs or ASVs autecological values (IndVal) or during the supervised training of a model (SML). Second, it can leverage powerful but previously inaccessible groups of bioindicators among prokaryotes, protists, meiofauna and mesozooplankton, that are widespread and may react both faster and stronger to environmental disturbances (Bouchez et al., 2016; Creer et al., 2010; Pawlowski, Esling, et al., 2016; Payne, 2013). Finally, when applied for the inference of BIs that are currently employed in routine monitoring programmes, a *de novo* strategy is directly compatible with current regulations, because the assessment categories remain the same and the BI values are simply inferred indirectly. Hence, this strategy assures a full backward and forward compatibility with current monitoring programmes, facilitating continuity of important time series data sets (Bálint et al., 2018).

6 | “STRUCTURAL COMMUNITY METRICS” STRATEGY: BLENDING THEORETICAL ECOLOGY INTO ROUTINE ECOSYSTEM MONITORING

This strategy relies on metrics extracted from the community structure or from inferred ecological networks – where taxa are interchangeable – in order to assess the impact of disturbance and its ramifications on ecosystem functioning (Figure 1c). This represents a clear paradigm shift for ecosystem monitoring programmes, because the evaluation of bioindicators, based on the compositional variation of communities, is not the main aim of the strategy. Instead, its focus is to discover and understand the ecological processes shaping biological communities and their response to disturbances, which is

indeed one of the core questions of ecological research. It has long driven the exploration of the links between generic, taxonomy and composition-independent biodiversity metrics or species functional traits distribution and ecosystems functioning and resilience, to reach a more general theoretical framework (Cardinale, Nelson, & Palmer, 2000; Hooper et al., 2005; Ives & Carpenter, 2007; Loreau & de Mazancourt, 2013; McCann, 2000; Mouillot, Graham, Villéger, Mason, & Bellwood, 2013; Tilman, Reich, & Knops, 2006).

Structural community metrics can be computed from compositional data generated by EG studies, including alpha diversity (e.g., OTU or ASV richness, Shannon diversity or Pielou evenness; reviewed in Daly, Baetens, & De Baets, 2018), along with its phylogeny-aware derivatives (reviewed in Tucker et al., 2017; Washburne et al., 2018). Under anthropogenic impact, alpha diversity in marine sediment has been found to decrease for foraminifera (Laroche, Wood, et al., 2018; Pawlowski, Esling, et al., 2014, 2016), ciliates (Stoeck, Kochems et al., 2018) and bacterial communities (Stoeck, Frühe, et al., 2018). Conversely, disturbances in marine sediments can also trigger increases in bacterial diversity and metabolic activity (Galand et al., 2016; Pérez-Valera et al., 2017). This suggests that the variation of alpha diversity alone is insufficient as a widely applicable indicator of disturbance. Phylogeny-aware metrics attempt to account for the evolutionary relationships among taxa composing communities, to provide insights into community assembly processes and by extension their predictable responses to environmental variations (Webb, Ackerly, McPeck, & Donoghue, 2002; Cavender-Bares, Kozak, Fine, & Kembel, 2009, but see Mayfield & Levine, 2010; Gerhold, Cahill, Winter, Bartish, & Prinzing, 2015). This relationship between phylogenetic diversity and ecosystem functioning has received a lot of attention by plant ecologists (Flynn, Mirotnick, Jain, Palmer, & Naeem, 2011). However, only few studies have employed EG data to this end, targeting mostly microbial groups, which, as for simple alpha-diversity metrics, has resulted in contrasting conclusions (Galand, Salter, & Kalenitchenko, 2015; Pérez-Valera et al., 2017, Liu et al., 2017; but see Venail & Vives, 2013; Keck & Kahlert, 2019 for studies employing sequencing data but not strictly EG).

Metrics based upon alpha diversity may be misleading (Santini et al., 2017) because their variation is often nonlinear, strongly scale-dependent (Chase et al., 2019) and valuable only in comparing contexts sampled using the same methodology (Shade, 2017). It also implicitly conveys the idea that “higher diversity is better” which is not necessarily true (Shade, 2017). The inference of ecological functioning based on phylogeny-aware metrics relies on the niche conservatism concept, which postulates that closely related taxa share similar functional traits (Cavender-Bares et al., 2009; Srivastava, Cadotte, Macdonald, Marushia, & Mirotnick, 2012; Webb et al., 2002). Under this assumption, increased phylogenetic diversity may support functionally diverse or multifunctional ecosystems (Hector & Baghi, 2007 but see Manning et al., 2018). By extension, higher phylogenetic diversity may also support ecosystem resilience, provided that the species fulfilling similar functions have differing responses to disturbances (Cadotte, Dinnage, & Tilman, 2012; Oliver et al., 2015). However, because not all functional traits

necessarily have a phylogenetic signal (Srivastava et al., 2012), including for microbes (Martiny, Treseder, & Pusch, 2013), inferring ecosystem functioning and the level of anthropogenic impact based on phylogeny-aware metrics alone may prove to be misguided. Likewise, conservation strategies based on these metrics may also be suboptimal (Mazel et al., 2018).

Another set of structural community metrics can be computed from the topology of inferred ecological or co-occurrence networks, representing potential biotic interactions (reviewed in Faust & Raes, 2012; Layeghifard et al., 2017; Vacher et al., 2016). Based on empirical evidence of the variation in network structure under environmental disturbance (Karimi, Meyer, Gilbert, & Bernard, 2016; Ma et al., 2019; Tylisanakis, Tschantke, & Lewis, 2007; Zhou et al., 2011), their properties have been suggested as potential indicators of ecosystem functioning and integrity (Bohan, Caron-Lormier, Muggleton, Raybould, & Tamaddoni-Nezhad, 2011; Bohan et al., 2017; Delmas et al., 2019; Gray et al., 2014; Karimi et al., 2017; Lau, Borrett, Baiser, Gotelli, & Ellison, 2017; Pellissier et al., 2018; Tylisanakis & Morris, 2017). In recent years, a growing interest in these approaches has led to a series of studies employing EG to infer ecological networks from microbial community data (Lupatini et al., 2014; Pauvert, Vallance, Delière, Buée, & Vacher, 2019; Pérez-Valera et al., 2017; Zappelini et al., 2015; Zhou et al., 2011) or from macroinvertebrates (Compson et al., 2019), in order to explore the links between network properties such as connectance, centrality or nestedness, and ecosystem functioning. For instance, it has been shown that bacterial communities in anthropized soil may have fewer potentially interacting taxa, than in natural soil (Lupatini et al., 2014). Likewise, in aquatic ecosystems, anthropogenic impacts are reflected in co-occurrence networks by a lower connectivity (Laroche, Wood, et al., 2018; Lawes, Dafforn, Clark, Brown, & Johnson, 2017; Li et al., 2018) and a lower ratio of positive interactions (Laroche, Wood, et al., 2018).

While promising, exploring the links between the properties of ecological networks inferred from EG data and ecosystem functioning is still in its infancy (Faust et al., 2012, 2015; Laroche, Wood, et al., 2018; Lawes et al., 2017; Li et al., 2018; Lima-Mendez et al., 2015; Pauvert et al., 2019). Multiple methodological issues limit the inference of robust networks from EG data based on co-occurrences in space or time. For example, read counts are strictly compositional, representing relative abundance of the marker itself, rather than presence or absolute abundances (but see Friedman & Alm, 2012; Kurtz et al., 2015). Further, it is challenging to control for covariates and confounding environmental parameters (but see Chiquet, Mariadassou, & Robin, 2018; Cougoul, Bailly, & Wit, 2019; Momal, Robin, & Ambroise, 2019; Tackmann, Matias Rodrigues, & von Mering, 2019; Tamaddoni-Nezhad et al., 2013), replicability of inference (Pauvert et al., 2019) and the relative merits of statistical and logical inference (Vacher et al., 2016). Robust networks also require considerably more replicates than are typically collected in EG studies, which increase both time and costs. Nevertheless, as more benchmark data sets containing both EG data and independently confirmed interactions between taxa become available

to complement simulated data sets (see Lima-Mendez et al., 2015), making robust network inference to explore the applicability of their metrics for ecosystem monitoring will probably come within reach in the years to come.

7 | “FUNCTIONAL COMMUNITY METRICS” STRATEGY: EMPLOYING FUNCTIONAL ENVIRONMENTAL GENOMICS FOR ROUTINE MONITORING

Another avenue of implementation of EG for ecosystem monitoring is the use of shotgun metagenomics and metatranscriptomics, depicting the metabolic capabilities of the community, and the expressed genes at the moment of sampling, respectively (Figure 1d). However, ecologists have yet to disentangle the relative importance and relationship of taxonomic diversity and functional traits for ecosystems functioning (Flynn et al., 2011; Gagic et al., 2015). This is particularly true in microbial ecology with the “who's there” versus “what they are doing” paradigms that often relate to the employed molecular methodologies, i.e., metabarcoding versus metagenomics and metatranscriptomics (Xu, Malmer, Langille, Way, & Knight, 2014). Some metagenomic contigs and functional transcripts were indeed found to represent efficient bioindicators of anthropogenic disturbances (Table S1), in terrestrial (de Menezes, Clipson, & Doyle, 2012), groundwater (He et al., 2018), freshwater (Cheaib, Boulch, Le Mercier, & Derome, 2018; Falk et al., 2019; Thompson et al., 2016) and marine environments (Birrer et al., 2019; Galand et al., 2016; Kisand, Valente, Lahm, Tanet, & Lettieri, 2012), opening up potential avenues for future routine ecosystem monitoring applications. Functional and taxonomic profiles may respond differently under anthropogenic disturbance (Cheaib et al., 2018), as well as under natural environmental variation (Barberán, Fernández-Guerra, Bohannan, & Casamayor, 2012; Louca, Jacques, et al., 2016; Louca et al., 2018). This taxon-function decoupling paves the way towards a molecular trait-based ecology (Lajoie & Kembel, 2019; Raes, Letunic, Yamada, Jensen, & Bork, 2011).

In an ecosystem monitoring context, functional profiles present two important features that anticipate these proxies to be more accurate than taxonomic profiles for the detection of a given environmental disturbance. First, because prokaryotes functional redundancy may be widespread (Louca et al., 2018; Pearman et al., 2019; but see Galand, Pereira, Hochart, Auguet, & Debroas, 2018 and Ramond et al. 2019 for protists), any given anthropogenic disturbance might trigger a similar response across multiple taxonomic groups. Under this assumption, ecosystem monitoring based on functional profiles may be less sensitive to biogeographical effects, random demographic drift, and species dispersal limitation than a monitoring strategy based on taxonomic profiles. This functional redundancy would also allow the establishment of a direct and mechanistic link between a measured functional response to a given anthropogenic disturbance. Second, because functional shifts are likely to occur prior to compositional ones, as a response of the taxa present to

the disturbance, the variation of functional profiles may constitute useful early warnings for a timelier ecosystem management, especially the ones detected by means of metatranscriptomics. However, RNA molecules are reportedly less stable than genomic DNA, which would add challenging practical constraints that could preclude their implementation in routine ecosystem monitoring programmes (but see von Ammon et al., 2019; Cristescu, 2019; Fordyce et al., 2013; Pochon, Zaiko, Fletcher, Laroche, & Wood, 2017). As a possible cost-effective “shortcut”, bacterial 16S rRNA profiles can be used to predict functional community profiles, based on evolutionary models (Aßhauer, Borja, Irigoien, & Rodríguez-Ezpeleta, 2015; Langille et al., 2013). Thus, 16S data could be also explored for searching potential functional bioindicators by this approach (Cordier, 2020; Laroche, Wood, et al., 2018; Mukherjee et al., 2017).

8 | A ROADMAP FOR THE IMPLEMENTATION OF ENVIRONMENTAL GENOMICS FOR ECOSYSTEM MONITORING

8.1 | The emergence of standards for EG methodologies to be applied for monitoring programmes

The time lag between technological breakthroughs, the uptake by scientists and the implementation of research results into real management applications can be notoriously long. Even for clinical applications where the contributions of genomics have long been anticipated (Dulbecco, 1986; Manolio et al., 2013) and for which economic perspectives are obvious, its implementation for routine healthcare applications is considered to have started five years ago (Stark et al., 2019). This is three times faster than the average 17 years for any healthcare research (Morris, Wooding, & Grant, 2011). The emergence of consensual standards for methodological protocols and data formats for interoperable exchanges, represent the most challenging issue for the routine adoption (Stark et al., 2019).

The field of EG for ecosystems monitoring is experiencing similar issues and has yet to overcome some of the barriers to the necessary paradigm-shift in monitoring programmes (Hering et al., 2018). Some of the noteworthy steps towards this goal were achieved with the widespread adoption of the MIGS, MIMARKS and MixS standards in genomics, specifying the minimum information that should accompany any genome, marker gene sequences or any sequence (Field et al., 2008; Yilmaz et al., 2011). Now the most challenging part resides in the adoption of standardized methodologies to produce, store and analyse EG data for a given environmental setting. Given the variety of biological models and environmental matrices, reaching a consensus in the scientific community and formalizing standards appears very challenging, especially for metabarcoding (Knight et al., 2018; Pollock, Glendinning, Wisedchanwet, & Watson, 2018; Wilcox et al., 2018; Zinger et al., 2019) and its application to ecosystem monitoring (Cristescu & Hebert, 2018; Hering et al., 2018). Yet, these hurdles are not specific to genomics methodologies, but

also exist for the morphology-based ones (Birk et al., 2012). Building robust, shared methodological standards is of course necessary and important efforts are deployed to reach this aim (Hering et al., 2018; Leese et al., 2018; Working Group CEN/TC230/WG28), for the sampling of eDNA (CEN, 2018a; Dickie et al., 2018; Wilcox et al., 2018), the molecular protocols (Blackman et al., 2019; Goldberg et al., 2016) as well as for bioinformatics (Knight et al., 2018; Roy et al., 2018), data interoperability (Callahan et al., 2017; McDonald et al., 2012) and reference databases (CEN, 2018b).

8.2 | Matching the right implementation strategy to the right monitoring programme

Several monitoring programmes may benefit quickly and reliably from an EG implementation, while others may require further optimization of molecular protocols or adjustments of their assessment criteria (Table 1). For instance, monitoring programmes relying primarily on taxonomic inventories are still hindered by the lack of congruence between the recovered species list and their relative abundances, even though the biological and technical biases might be partially alleviated in the future. Furthermore, despite the sustained effort, reference sequence databases for barcoding remain skewed toward some groups and geographical locations (McGee et al., 2019; Weigand et al., 2019), limiting congruence between EG and morphotaxonomic inventories. Hence, the taxonomy-based implementation strategy for these monitoring programmes will require improvements of molecular protocols and reference databases, to generate EG data that better fit the current standards, or an adaptation of the currently implemented assessment criteria to fit the specificities of EG data (Hering et al., 2018).

Monitoring programmes relying on the screening of established bioindicators for the computation of BI values are proposed as being compatible with an implementation of EG (Hering et al., 2018; Pawlowski et al., 2018). Indeed, this compatibility is greatly facilitated by the fact that the assessment criteria, i.e., BIs, are not meant to strictly rely on taxonomic inventories but rather on the autecology of bioindicators. Hence, for the taxonomy-based strategy, the BI formulations can compensate the impact of taxonomic mismatches between morphology and EG and databases incompleteness to some extent, because multiple taxa are ascribed identical autecological values, conveying similar ecological signal (Keck, Vasselon, Rimet, Bouchez, & Kahlert, 2018). The applicability of this approach has been demonstrated in freshwater (Elbrecht et al., 2017; Kelly et al., 2018; Mortagua et al., 2019; Rivera, Vasselon, Bouchez, & Rimet, 2020; Vasselon, Domaizon, et al., 2017) and in marine environments (Aylagas et al., 2016; Lejzerowicz et al., 2015). However, those studies have also shown that a large amount of sequences are not taxonomically assigned and currently omitted for ecological assessment, opening the door to new approaches that could extract ecological information from those unlabelled sequences.

The de novo strategy uses the occurrence of previously scrutinized sequences in samples of known BI values or other impact

measures to ascribe autecological values to sequences directly, or generate a predictive model (Apothéloz-Perret-Gentil et al., 2017; Cordier et al., 2017; Tapolczai, Vasselon, et al., 2019). Hence, these approaches are less sensitive to the biological and technical issues mentioned above, because the ecological signal (autecology) is calibrated directly on the specificities of EG data. From an implementation perspective, this *de novo* strategy thus may represent the most direct path towards implementation of EG into monitoring programmes relying on BIs (Figure 2). Though somewhat unintuitive, this is because inferred BI values with a *de novo* strategy convey the same ecological meaning as they do with current methodologies, which is not the case when BIs values are inferred from bioindicators composition profiles depicted by EG data, as their autecological values were calibrated only on morphology-based data. Thus, the *de novo* strategy assures a better continuity with previous BIs data and time series and expand the range of possible bioindicators to virtually any taxa or sequence.

Structural and functional community metrics represent alternative implementation strategies that may ultimately lead to a more generic, broadly applicable ecological monitoring framework (Bohan et al., 2017; Escalas et al., 2019; Karimi et al., 2017; Pellissier et al., 2018; Quince et al., 2017; Singer et al., 2017; Tylianakis & Morris, 2017). These strategies hold the potential to provide a more mechanistic and functional understanding of the response of biological communities to ecosystem variation. Such knowledge could hence be included in predictive models to forecast shifts in biodiversity structure and possibly their consequences on their associated ecosystem services under different disturbance scenarios. However, an operational ecosystem monitoring framework remains to be built upon this theoretical ecological work (Figure 2), that has only partially been experimentally validated (but see Laroche, Wood, et al., 2018; Ma et al., 2019). In addition, the extraction of structural or functional community metrics remain active fields of ecological research, and the emergence of a molecular trait-based ecology using metagenomics and metatranscriptomics profiles is in its infancy (Lajoie & Kembel, 2019). Hence, it is premature to discuss their operational implementation and regulatory establishment, but their ecological benefit should be anticipated. Nevertheless, the collected labelled data sets including samples for the production of EG data in the course of future ecosystem monitoring campaigns will certainly contribute to move these possibilities forward.

8.3 | Collecting reference data and eDNA/eRNA samples in parallel

If EG-based methods are to complement or replace current morphology-based ones, the prerequisite is to establish whether they can provide similar ecological diagnostics, to ensure a smooth implementation and compatibility with existing time series (Bálint et al., 2018; Leese et al., 2016). This inevitably implies extensive parallel sampling of currently implemented and EG methodologies for some time, to build reference data sets on which the applicability

can be assessed and the calibration with previous methodology performed (Keeley et al. 2018; Leese et al., 2016). To be reliable, such reference data sets have to cover a broad range of possible environmental conditions for a given ecosystem across multiple spatiotemporal scales, ideally in a balanced manner, to account for biotic interactions, random demographic drift and dispersal limitations that may interact with the anthropogenic pressures in the assembly of communities.

The collection of reference data raises concerns regarding the substantial financial investment necessary for monitoring programmes adopting one or a combination of EG strategies, versus the “risk” of technological novelty and/or paradigm shift. However, the collected reference data sets would still be extremely valuable in such case, because the extracted DNA/RNA alongside the accompanying reference metadata can be safely stored and reanalysed later on, assuring a forward compatibility to the limit of availability of stored DNA/RNA material (Hering et al., 2018; Jarman, Berry, & Bunce, 2018). Indeed, molecular costs are usually far less prohibitive than those related to field sampling and metadata collection. Hence, such fully labelled data sets will constitute the ideal benchmarks against which to assess the validity of any new implementation strategy based on novel technology or new paradigm.

9 | CONCLUSION AND FURTHER RESEARCH NEEDS

The potential for EG-based methods for ecosystems monitoring is enormous and can presently fulfil most of the requirements of current monitoring programmes. Moving towards a routine use of EG is certainly a paradigm shift, but this technological breakthrough will overcome the limitations of current morphotaxonomy methodologies and enable the required upscaling to meet monitoring needs in a changing world. Without doubts, EG-based methods will pave the way for a more cost-effective, faster, reproducible and semi-automatable ecosystem monitoring framework. Regardless of the implementation strategy envisioned, the following key technological, scientific and societal improvements will be beneficial for a smoother transition:

- A collaborative and transdisciplinary design of monitoring campaigns, involving both experts, stakeholders and regulators would allow monitoring programmes to more easily bridge the science-policy gap.
- A collection of reference morphological and molecular data in parallel, at least in a subset of reference points or during a transition period, will assure backward and forward compatibility of time series data sets, regardless of the envisioned implementation strategy to be decided in future monitoring campaigns.
- The efforts to complete reference sequence databases need to be sustained, by adding more representatives of the known biodiversity, with a wider geographical coverage.
- A reference database framework for *de novo* strategies needs to be established. A key requirement is the ability to reliably compare

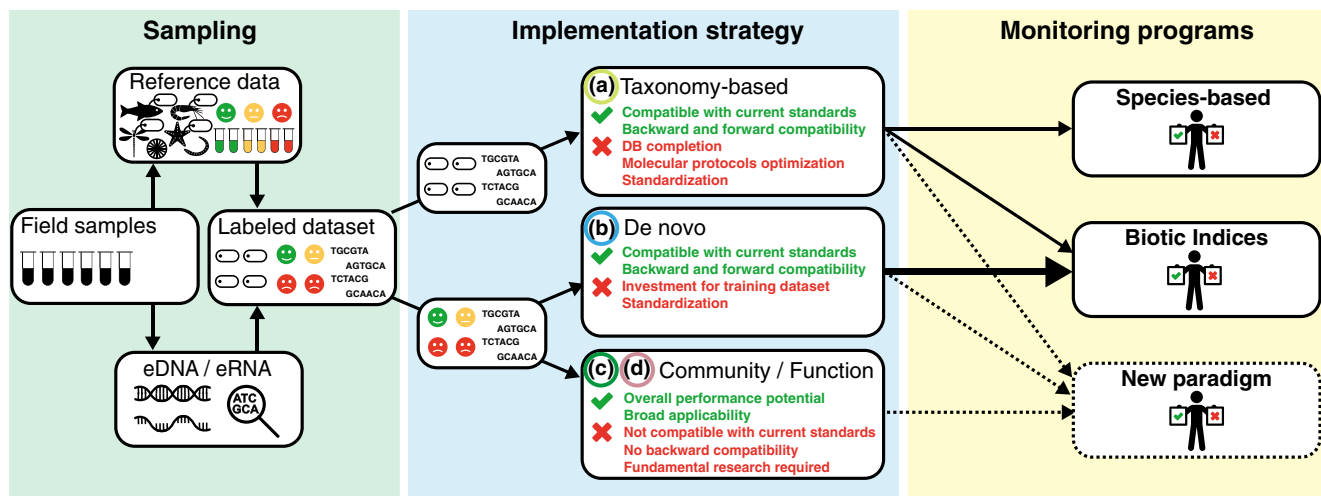


FIGURE 2 Strengths and limitations of the currently envisioned implementation strategies of environmental genomics for the monitoring of ecosystems, and their ability to fulfill the criteria of existing monitoring programmes. The tag above organisms indicate their taxonomic affiliation and smileys indicate the ecological status of the sample (from "poor" to "good")

OTUs or ASVs identified in monitoring programmes to formally establish knowledge about their sensitivity to disturbance.

- The taxonomic resolution level (haplotype, species, genus, family, order, class) at which HTS reads are most informative as genetic bioindicators for a given situation remains to be identified.
- For the identification of novel genetic bioindicators in complex communities, it will be important to distinguish the effect of natural (seasonal) variation from disturbance-induced community changes with rigorous experimental designs.
- Basic and replicable research is highly needed to develop a structural and functional community metrics-based implementation strategy. Such effort will probably contribute to the establishment of a more broadly applicable monitoring framework and less constrained by the database and geographical coverage limitations.

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ORCID

Tristan Cordier [ID https://orcid.org/0000-0001-7398-4790](https://orcid.org/0000-0001-7398-4790)

Laure Apothéloz-Perret-Gentil [ID https://orcid.org/0000-0002-8592-3079](https://orcid.org/0000-0002-8592-3079)

Eva Aylagas [ID https://orcid.org/0000-0001-9792-8451](https://orcid.org/0000-0001-9792-8451)

Anthony Chariton [ID https://orcid.org/0000-0002-5809-3372](https://orcid.org/0000-0002-5809-3372)

François Keck [ID https://orcid.org/0000-0002-3323-4167](https://orcid.org/0000-0002-3323-4167)

Florian Leese [ID https://orcid.org/0000-0002-5465-913X](https://orcid.org/0000-0002-5465-913X)

Thorsten Stoeck [ID https://orcid.org/0000-0001-5180-5659](https://orcid.org/0000-0001-5180-5659)

Anders Lanzén [ID https://orcid.org/0000-0002-7138-6364](https://orcid.org/0000-0002-7138-6364)

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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