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1 Ecosystems monitoring powered by environmental genomics: a
2 review of current strategies with an implementation roadmap.

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36 strategy; ecosystem management

37

38 **Abstract**

39 A decade after environmental scientists integrated high-throughput sequencing technologies in
40 their toolbox, the genomics-based monitoring of anthropogenic impacts on the biodiversity and
41 functioning of ecosystems is yet to be implemented by regulatory frameworks. Despite the
42 broadly acknowledged potential of environmental genomics to this end, technical limitations and
43 conceptual issues still stand in the way of its broad application by end-users. In addition, the
44 multiplicity of potential implementation strategies may contribute to a perception that the routine
45 application of this methodology is premature or “in development”, hence restraining regulators
46 from binding these tools into legal frameworks. Here, we review recent implementations of
47 environmental genomics-based methods, applied to the biomonitoring of ecosystems. By taking
48 a general overview, without narrowing our perspective to particular habitats or groups of
49 organisms, this paper aims to compare, review and discuss the strengths and limitations of four
50 general implementation strategies of environmental genomics for monitoring: (A) Taxonomy-
51 based analyses focused on identification of known bioindicators or described taxa; (B) *De novo*
52 bioindicator analyses; (C) Structural community metrics including inferred ecological networks;
53 and (D) Functional community metrics (metagenomics or metatranscriptomics). We emphasise
54 the utility of the three latter strategies to integrate meiofauna and microorganisms that are not
55 traditionally utilised in biomonitoring because of difficult taxonomic identification. Finally, we
56 propose a roadmap for the implementation of environmental genomics into routine monitoring
57 programs that leverage recent analytical advancements, while pointing out current limitations
58 and future research needs.

59

60

61 The need for broad scale ecosystem monitoring strategies

62 Biodiversity drives the fundamental processes of ecosystems and provides invaluable
63 services on which we depend. Anthropogenic, detrimental impacts on ecosystems, including
64 accelerating climate change, are unprecedented (Waters et al., 2016) and have led to a decline
65 of biodiversity across the globe (Butchart et al., 2010; Cardinale et al., 2012; Hughes et al.
66 2018). Recent reports stress that one out of the 8 million known species are presently at risk of
67 extinction (IPBES report, 2019). This threatens ecosystem function(ing) and services.
68 Therefore, the urgent challenge is now to build a set of efficient tools to enhance our capacity to
69 predict or detect early warnings of critical ecological shifts efficiently, in order to forecast the
70 direction of such shifts and their impacts on ecosystem functions and services (Carpenter et al.,
71 2011; Barnosky et al., 2012; Ratajczak et al., 2018).

72 Because our societies aim to reach a trade-off between socioeconomic development and
73 ecosystems sustainability (UN A/RES/70/1, 2015), regulatory frameworks have been
74 established worldwide for the sustainable development of industries within environmental
75 constraints (Niemeijer 2002; Grizetti et al., 2015). Such regulatory systems have been
76 incorporated into various national and international directives, especially for aquatic ecosystems
77 (e.g. the Water Framework Directive, WFD, Directive 2000/60/EC and Marine Strategy
78 Framework Directive, MSFD, Directive 2008/56/EC in Europe, the Clean Water Act of the US
79 Environmental Protection Agency in the USA, as well as the United Nations Convention on the
80 Law of the Sea, UNCLOS). The backbone of such monitoring programs is the biological
81 component of ecosystems, as a measure of ecosystem 'health' or 'integrity' (Karr, 1999). This
82 biological component is often referred to as the Biological Quality Elements in those regulations
83 (BQEs, Borja et al., 2013; Hering et al., 2018). Most monitoring strategies implemented in
84 regulations rely on the bioindication principle (autecology, Box 1), i.e. significant correlations
85 between the occurrence of specific organisms and a set of environmental variables. Although
86 chemical and hydrological monitoring techniques provide an environmental quality snapshot,
87 biological indicators convey a cumulative time-integrated measure as their occurrence is the
88 product of their local adaptation and their responses to ecosystem variations and/or
89 disturbances across an extended period of time (Carignan & Villard, 2002; Lear et al., 2011; Birk
90 et al., 2012).

91

92

93 The limits of currently implemented ecosystem monitoring strategies

94 Traditionally, morphologically distinguishable invertebrates have been used as
95 bioindicators in both aquatic and terrestrial ecosystems (Reynoldson & Metcalfe-Smith, 1992;
96 Bongers & Ferris, 1999; Hodgkinson & Jackson, 2005; Gerlach et al., 2013). Fishes, amphibians,
97 macrophytes, phytoplankton and diatoms, are also routinely used in aquatic ecosystems (Birk et
98 al., 2012). Various Biotic Indices (BIs) have been formalized, based on the predictable
99 responses of bioindicator species to environmental disturbances (autecological value) in marine
100 (Maurer et al., 1999; Borja et al., 2000; Rygg et al., 2013), freshwater (Kelly et al., 1995; Stark et
101 al., 1998; Prygiel & Coste, 2000) and terrestrial (Urzelai et al., 2000; Marull et al., 2007)
102 ecosystems. Almost half of the monitoring methodologies currently used in Europe rely on such
103 BIs (Birk et al., 2012). However, for environments or geographical regions for which no BI has
104 been calibrated, ecological assessments rely instead on biodiversity measures of “charismatic”
105 groups such as fishes (Pont et al., 2006), amphibians (Welsh et al., 1998) and insects (Basset
106 et al., 2004).

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

121

122

123 The environmental genomics revolution for biodiversity research and ecosystem 124 monitoring

125 Over the last decade, the development of environmental genomics (EG) coupled with
126 high-throughput sequencing (HTS) technologies has led to a marked improvement in our ability
127 to document biodiversity patterns, for both species occurrence (amplicon sequencing, i.e.
128 metabarcoding, reviewed in Bohmann et al., 2014; Valentini et al., 2016; Deiner et al., 2017;
129 Cristescu et al., 2018; Taberlet et al. 2018; Ruppert et al., 2019) and their metabolic functions
130 (metagenomics and metatranscriptomics, reviewed in Ungerer et al., 2008; Vandenkoornhuys
131 et al., 2010; Quince et al., 2017; Singer et al., 2017; Escalas et al., 2019). Multidisciplinary
132 teams and consortiums have initiated large scale projects aiming at collecting biodiversity data
133 using EG throughout the globe, to address fundamental ecological questions. Among these
134 initiatives, the large barcoding projects led by the international Barcode of Life (Ratnasingham &
135 Hebert, 2007), the Earth Microbiome Project (Gilbert et al., 2010) and the TARA Oceans Project
136 (Karsenti et al., 2011) represent three of the most emblematic examples. Those projects have
137 unraveled an unexpected cryptic (Bickford et al., 2007) and novel microbial diversity (the
138 'unseen majority') guiding reconstruction of the eukaryotic tree of life (Adl et al., 2019). Even
139 though this microbial diversity is known to represent a key component of ecosystem functioning
140 (Delgado-Baquerizo et al., 2016; Guidi et al., 2016; Cavicchioli et al., 2019), the ecology of most
141 microorganisms remains largely enigmatic.

142 The potential of EG for surveying biodiversity and monitoring natural ecosystems at a
143 broad spatio-temporal scale was quickly identified and implemented by environmental scientists
144 (Baird & Hajibabaei, 2012; Taberlet et al., 2012; Davies et al., 2012; Kelly et al., 2014). This
145 work has been boosted by the massive drop in sequencing costs, with over four orders of
146 magnitude within the last 15 years (<https://www.genome.gov>). This has enabled numerous
147 clinical and environmental routine applications. Indeed, fueled by the continuous efforts to
148 optimize laboratory protocols and bioinformatic tools, all steps from large-scale collection of
149 samples, generation of HTS data, statistical analysis, and interpretation of results, can now be
150 performed in matter of days or weeks (Juul et al., 2015; Quinn et al., 2016; Deshpande et al.,
151 2019; Reintjes et al., 2019). For aquatic ecosystems especially, the next breakthrough of this
152 revolution is now expected to be the development and deployment of low-cost, automated and
153 miniaturized *in situ* environmental nucleic acids (eDNA/RNA) samplers (Carr et al., 2017; Gan et

154 al., 2017). These may be integrated into autonomous instruments for broad-scale and
155 continuous ecosystem monitoring programs (Brandt et al., 2016; Bohan et al., 2017; Aguzzi et
156 al., 2019; Benway et al., 2019; Levin et al., 2019).

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

174 Multiple pilot and methodological EG studies have highlighted important variation in
175 terms of compliance with current regulatory programs (reviewed in Hering et al., 2018), leading
176 to the proposition of multiple implementation strategies for current and future ecosystem
177 monitoring programs. Here, we compare and review the strengths and limitations of these EG-
178 based strategies for ecosystem monitoring. Our objective is to pinpoint the criteria of existing
179 monitoring programs that could be fulfilled by EG methods as of today, and clarify the work
180 ahead for the monitoring programs that could benefit from EG in the near future, given
181 continued technological and analytical advancements. To this end, we classify these strategies
182 into four broad categories (Figure 1, Table S1): (A) Taxonomy-based analyses that focus on
183 known bio-indicator species, or the identification and enumeration of formally or informally
184 described taxa; (B) *De novo* bioindicator analyses aiming to identify and utilise novel

185 bioindicators, independent of formal taxonomy; (C) Structural community metrics relying on
186 community structure or inferred ecological networks, where taxa are interchangeable; and (D)
187 Functional community metrics or indicators that focus on protein-coding genes or transcripts
188 instead of taxonomic composition. Based on the specificities of each strategy, their level of
189 maturity and their compatibility with existing regulations (Table 1), we propose an
190 implementation roadmap to integrate EG into ecosystems monitoring programs and highlight
191 future research needs to be undertaken.

192

193

194 “Taxonomy-based” strategy: screening known species and bioindicators with 195 environmental genomics

196 This strategy relies on the enumeration of known biodiversity from DNA obtained from an
197 environmental sample (e.g. sediment, soil, biofilm, water) or from bulk material prepared from
198 an environmental sample by e.g. elutriation, trapped individuals or biofilm scratching (Figure
199 1A). This strategy closely fits the conventional, morphology-based monitoring approach,
200 because it primarily aims at reaching a satisfactory level of congruence in terms of both
201 qualitative and quantitative biodiversity inventories. The taxonomy-based strategy is *de facto*
202 limited to the morphologically characterized fraction of biodiversity for which reference
203 sequences are available in public databases. Hence, approaches using it have usually
204 overlooked meiofaunal or microbial taxa, difficult to identify on the basis of morphological traits,
205 and for most of which the autecology is poorly known (but see Pawlowski et al., 2016). The
206 reference databases routinely used by EG studies include for instance the universal but
207 essentially non-curated GenBank nucleotide repository from the National Center for
208 Biotechnology Information (Benson et al., 1999, but see Leray et al., 2019), or the curated
209 databases BOLD for COI barcodes, primarily from animals (Ratnasingham & Hebert, 2007),
210 SILVA for universal ribosomal markers (Quast et al., 2013), PR² for protists (Guillou et al.,
211 2013), Diat.barcode for diatoms (Rimet et al., 2016), and Unite for fungi (Nilsson et al., 2018).

212 Depending on the environment assessed and the taxonomic group considered, the
213 performance of taxonomy-based approaches varies considerably (Hering et al., 2018).
214 Benchmarking studies comparing EG-based and conventional morphology-based taxonomic
215 inventories (Table S1) have shown mixed degrees of congruence. For the non-invasive

216 detection of fish species from DNA traces in filtered marine water, the rate of success from
217 taxonomy-based monitoring is reported near perfect (Thomsen et al., 2012; Bakker et al., 2017;
218 but see DiBattista et al., 2017). For freshwater macroinvertebrate bulk samples, the rate of
219 species detection varied from 67% (Elbrecht et al., 2017) to 73-83% (Hajibabaei et al., 2011;
220 2012). In contrast, for benthic diatoms sampled from biofilms, the congruence of morphological
221 taxonomy and EG-inferred taxonomy, in terms of shared taxa at species level, ranged only from
222 15-18% (Rivera et al., 2017; Vasselon et al., 2017a) to 28% (Visco et al., 2015). The reported
223 congruence for macroinvertebrates sampled from marine sediments ranged from 20%
224 (Lejzerowicz et al., 2015) up to 60% (Aylagas et al., 2016). Noteworthy, those studies also
225 detected numerous species that were unnoticed in morphological inventories (Hajibabaei et al.,
226 2011; 2012; Elbrecht et al., 2017). Despite these discrepancies, the studies inferring BI values
227 from the detected bioindicators species show very promising results, for both freshwater
228 diatoms (Kermarrec et al., 2014; Visco et al., 2015; Vasselon et al., 2017b; Kelly et al., 2018)
229 and macroinvertebrates (Elbrecht et al., 2017) as well as for marine macroinvertebrates
230 (Lejzerowicz et al., 2015; Aylagas et al., 2016). While acknowledging that the congruence for
231 both qualitative and quantitative inventories are not fully satisfactory, these studies have
232 demonstrated that EG tools are still able to detect sufficient bioindicator taxa to infer accurate BI
233 values, even when considering only presence/absence (Aylagas et al., 2016). The EG
234 methodology has therefore been promoted as a promising tool for fast and cost-effective
235 biodiversity screening for ecosystem monitoring, even while the simultaneous collection of
236 classical morphological samples for validation is univocally suggested. Nonetheless, further
237 improvements in molecular protocols as well as BI inter-calibration is a necessity towards
238 harmonization and standardization across Europe (Poikane et al., 2014; Hering et al., 2018) and
239 beyond (Jeunen et al., 2019).

240 Various biological and technical limitations still impede the implementation of the
241 taxonomy-based strategy for routine monitoring applications (Leese et al., 2018). These
242 limitations mainly stem from the fact that the methods sample fundamentally different units of
243 presence (molecules *versus* individuals), resulting in different biases affecting richness,
244 abundance and taxonomic composition. The richness of “molecular species”, i.e. Operational
245 Taxonomic Units (OTUs) or Amplicon Sequence Variants (ASVs, the new operational unit
246 paradigm, Callahan et al., 2017), should not be considered analogous to morpho-species

247 richness even in the theoretical absence of noise resulting from PCR and sequencing biases.
248 This discrepancy is due to cryptic diversity (Stork, 2018), intragenomic or intra-specific marker
249 variation (Bik et al., 2013, Sun et al., 2013), and the presence of DNA from dead and inactive
250 organisms or as extracellular DNA (Collins et al., 2018). Likewise, the abundance of taxa
251 inferred from HTS read counts can typically not be used to infer the number of individuals.
252 Indeed, the number of sampled DNA molecules and sequence reads are a consequence of the
253 number of individuals, but also of the biomass and the variable number of copies of the targeted
254 marker in the genome (Bik et al., 2013, Vetrovský, et al., 2013), in addition to variations in DNA
255 extractability and primer-specific amplification bias (Elbrecht et al., 2015; Piñol et al., 2015;
256 Krehenwinkel et al., 2017). Finally, EG studies suffer from a strong sampling effect because
257 DNA extractions are typically performed from small amounts of material, making large-size
258 organisms less well-represented in eDNA extracts (Lanzén et al., 2017). However, bulk samples
259 (Elbrecht et al., 2017), larger extraction volume (Nascimento et al., 2018) or more aggressive
260 homogenization (Lanzén et al., unpublished data) can partially alleviate this issue.

261 Since the taxonomy-based strategy depends on reference sequences for organism
262 identification, the incompleteness of reference databases can also have a major impact. Hence,
263 completing databases, both by the “vertical” addition of more taxa and by the “horizontal”
264 coverage of wider geographical areas, would certainly contribute to an improvement in
265 identification (Vasselon et al., 2017; McGee et al., 2019). However, despite sustained efforts,
266 reference databases will likely remain skewed towards some taxa, while suffering from
267 important gaps across other taxonomic groups or biogeographical regions (Weigand et al.,
268 2019; McGee et al., 2019). All these issues directly impact both of the key parameters for
269 applying BIs to assess impact, namely the qualitative and quantitative measures of biodiversity
270 (Pawlowski et al., 2018).

271 Nevertheless, multiple studies have shown that there is room for considerable
272 improvements to better bridge the current gaps between taxonomy-dependent molecular and
273 morphology-based approaches. Taxonomic breadth in HTS data could be broadened by
274 carefully designing novel amplification primers (Elbrecht et al., 2019) or using more than one
275 primer pair (Corse et al., 2019). Applying correction factors to read counts, based on
276 established knowledge of the biovolume (Vasselon et al., 2018), the number of copies of the
277 targeted marker (Vetrovský, et al., 2013) or by spiking samples with known internal standard for

278 quantitative determinations (Tkacz et al., 2018; Ji et al., 2019), are all promising methods for
279 resolving these challenges. Finally, the integration of bioinformatic tools for the automated
280 curation of databases from mislabeled sequences will improve their reliability (Ashelford et al.,
281 2005; Kozlov et al., 2016).

282

283 “*De novo*” strategy: discovering new bioindicators and harnessing them for 284 routine monitoring.

285 In contrast to the taxonomy-based strategy, the *de novo* one does not immediately
286 generate an ecological assessment, because it does not employ previous knowledge
287 associated with bioindicators. Instead, the *de novo* strategy aims at establishing new
288 bioindicators using EG-based profiling of communities and independently generated ecological
289 status or known disturbance gradients (Figure 1B). Harnessing EG and HTS technologies to
290 explore a broader range of biological diversity, formally labelled or not (i.e. taxonomically
291 described or identified), represents an opportunity to move towards a more holistic monitoring
292 paradigm (Chariton et al., 2010; Bik et al., 2012). By considering all the OTU (or ASV) profiles
293 along a known impact gradient of typical anthropogenic origin, studies applying this strategy
294 have shown that HTS data represent a virtually unlimited reservoir of new bioindicators.
295 Examples (listed in Table S1) include contamination by pesticides (Thompson et al., 2016;
296 Andújar et al., 2017) or other agricultural stressors (Salis et al., 2017), and gradients of
297 eutrophication and urban contamination in freshwater systems (Apothéloz-Perret-Gentil et al.,
298 2017; Martínez-Santos et al. 2018; Simonin et al., 2019; Tapolczai et al., 2019a, 2019b). In
299 marine environments, the utility of this strategy has been demonstrated after an oil spill (Bik et
300 al., 2012), in the vicinity of offshore drilling platforms (Lanzén et al., 2016; Laroche et al., 2016,
301 2018a) and aquaculture sites (Pawłowski et al. 2014, Pochon et al., 2015; Dowle et al., 2015;
302 Keeley et al., 2018; Stoeck et al. 2018a, 2018b) as well as along eutrophication and urban or
303 industrial contamination gradients in estuaries (Chariton et al., 2010, 2015; Angly et al., 2015;
304 Lallias et al., 2015; Obi et al., 2016). Interestingly, most of the studies sampling marine
305 sediments highlighted that meiofaunal invertebrates, such as nematodes, gastrotrichs and
306 platyhelminths (Chariton et al., 2010; Bik et al., 2012; Lanzén et al., 2016), large groups of
307 protists such as diatoms, oomycetes and ciliates (Lanzén et al., 2016; Stoeck et al., 2018a) or
308 foraminifera (Pawłowski et al., 2014; Laroche et al., 2016; Frontalini et al., 2018) but also fungi

309 (Bik et al., 2012) and bacteria (Angly et al., 2015; Dowle et al., 2015; Martínez-Santos et al.
310 2018; Obi et al., 2016; Aylagas et al., 2017; Stoeck et al., 2018b; Keeley et al. 2018) have great
311 potential as bioindicators of anthropogenic impacts and can readily be captured by EG studies.

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

320 The indicator value approach ascribes autecological values (or discrete “eco-groups”) to
321 OTUs or ASVs based on their occurrence in samples of known disturbance level, in a similar
322 manner as for the establishment of morphology-based bioindicators. Hence, the autecological
323 values of these *de novo* bioindicators are directly calibrated on the HTS data, which alleviates
324 the qualitative and quantitative biases encountered with the taxonomy-based EG strategy. This
325 has proven successful for both freshwater benthic diatoms (Apothéloz-Perret-Gentil et al.,
326 2017; Tapolczai et al., 2019a, 2019b) and for bacterial and eukaryotic communities in streams
327 and estuarine systems (Chariton et al., 2015; Li et al., 2018). An analogous approach is the use
328 of polynomial quantile regression splines (Andersson, 2008). This has shown great promise for
329 the prediction of impacts from organic enrichment in aquaculture sites using eukaryotic and
330 prokaryotic metabarcoding data in parallel (Keeley et al., 2018). For diatoms, the accuracy of
331 the assessment can be largely improved, arguably because the indicator value approach makes
332 use of a larger number of OTUs or ASVs, compared to an approach relying solely on their
333 taxonomic assignments (Apothéloz-Perret-Gentil et al., 2017; Tapolczai et al. 2019a, 2019b).

334 Supervised machine learning (SML) also requires training datasets, i.e. reference
335 disturbance levels (labels) associated with the community profiles of the samples (features).
336 These algorithms are best at classification problems involving multidimensional and noisy
337 datasets (Libbrecht & Noble, 2015), which are common attributes of HTS data. The task is to
338 automatically disentangle the feature signal (OTU or ASV profiles) and their co-occurrence that
339 convey an ecological signal from background noise. This extracted knowledge is self-contained

340 in a trained model that can be used to make predictions of disturbance level on new samples,
341 based on their compositional profiles (Cordier et al., 2019a). Supervised machine learning also
342 alleviates the qualitative and quantitative biases that hamper the taxonomy-based strategy in a
343 more straightforward manner, because the model is trained directly on HTS data. The
344 applicability of SML has been demonstrated in marine environments, for the detection of various
345 pollutants (Smith et al., 2015) and for the prediction of aquaculture impacts on benthic
346 biodiversity (Cordier et al., 2017; 2018). The SML-based inference of BI values has also been
347 shown to outperform the taxonomy-based strategy, relying on the detection of established
348 macroinvertebrates bioindicators DNA (Cordier et al., 2018), and may be more powerful than the
349 IndVal approach (Frühe et al., 2020). Supervised machine learning applications have also
350 succeeded in predicting the origin of container ship ballast waters (Gerhard & Gunsch, 2019).

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

364
365

366 “Structural community metrics” strategy: blending theoretical ecology into routine 367 ecosystem monitoring.

368 This strategy relies on metrics extracted from the community structure or from inferred
369 ecological networks – where taxa are interchangeable – in order to assess the impact of
370 disturbance and its ramifications on ecosystem functioning (Figure 1C). This represents a clear

371 paradigm-shift for ecosystem monitoring programs, because the evaluation of bioindicators,
372 based on the compositional variation of communities, is not the main aim of the strategy.
373 Instead, its focus is to discover and understand the ecological processes shaping biological
374 communities and their response to disturbances, which is indeed one of the core questions of
375 ecological research. It has long driven the exploration of the links between generic, taxonomy
376 and composition-independent biodiversity metrics or species functional traits distribution and
377 ecosystems functioning and resilience, to reach a more general theoretical framework
378 (Cardinale et al., 2000; McCann, 2000; Hooper et al., 2005; Tilman et al., 2006; Ives &
379 Carpenter, 2007; Mouillot et al., 2013; Loreau & de Mazancourt, 2013).

380 Structural community metrics can be computed from compositional data generated by
381 EG studies, including alpha diversity (e.g. OTU or ASV richness, Shannon diversity or Pielou
382 evenness; reviewed in Daly et al., 2018), along with its phylogeny-aware derivatives (reviewed
383 in Tucker et al., 2017; Washburne et al., 2018). Under anthropogenic impact, alpha diversity in
384 marine sediment has been found to decrease for foraminifera (Pawlowski et al., 2014; 2016;
385 Laroche et al. 2018b), ciliates (Stoeck et al., 2018a) and bacterial communities (Stoeck et al.,
386 2018b). Conversely, disturbances in marine sediments can also trigger increases in bacterial
387 diversity and metabolic activity (Galand et al., 2016; Pérez-Valera et al., 2017). This suggests
388 that the variation of alpha diversity alone is insufficient as a widely applicable indicator of
389 disturbance. Phylogeny-aware metrics attempt to account for the evolutionary relationships
390 among taxa composing communities, to provide insights into community assembly processes
391 and by extension their predictable responses to environmental variations (Webb et al., 2002;
392 Cavender-Bares et al., 2009, but see Mayfield & Levine, 2010; Gerhold et al., 2015). This
393 relationship between phylogenetic diversity and ecosystem functioning has received a lot of
394 attention by plant ecologists (Flynn et al., 2011). However, only few studies have employed EG
395 data to this end, targeting mostly microbial groups, which, as for simple alpha-diversity metrics,
396 has resulted in contrasting conclusions (Galand et al., 2015; Pérez-Valera et al., 2017, Liu et al.,
397 2017; but see Venail & Vives, 2013; Keck & Kahlert, 2019 for studies employing sequencing
398 data but not strictly EG).

399 Metrics based upon alpha diversity may be misleading (Santini et al., 2017) because
400 their variation is often non-linear, strongly scale-dependent (Chase et al., 2019) and valuable
401 only in comparing contexts sampled using the same methodology (Shade, 2017). It also

402 implicitly conveys the idea that 'higher diversity is better' which is not necessarily true (Shade,
403 2017). The inference of ecological functioning based on phylogeny-aware metrics relies on the
404 niche conservatism concept, which postulates that closely related taxa share similar functional
405 traits (Webb et al., 2002; Cavender-Bares et al., 2009; Srivastava et al., 2012). Under this
406 assumption, increased phylogenetic diversity may support functionally diverse or multifunctional
407 ecosystems (Hector & Baghi, 2007 but see Manning et al., 2018). By extension, higher
408 phylogenetic diversity may also support ecosystem resilience, provided that the species fulfilling
409 similar functions have differing responses to disturbances (Cadotte et al., 2012; Oliver et al.,
410 2015). However, because not all functional traits necessarily have a phylogenetic signal
411 (Srivastava et al., 2012), including for microbes (Martiny et al., 2013), inferring ecosystem
412 functioning and the level of anthropogenic impact based on phylogeny-aware metrics alone may
413 prove to be misguided. Likewise, conservation strategies based on these metrics may also be
414 suboptimal (Mazel et al., 2018).

415 Another set of structural community metrics can be computed from the topology of
416 inferred ecological or co-occurrence networks, representing potential biotic interactions
417 (reviewed in Faust & Raes, 2012; Vacher et al., 2016; Layeghifard et al., 2017). Based on
418 empirical evidence of the variation in network structure under environmental disturbance
419 (Tylianakis et al., 2007; Zhou et al., 2011; Karimi et al., 2016; Ma et al., 2018), their properties
420 have been suggested as potential indicators of ecosystem functioning and integrity (Gray et al.,
421 2014; Karimi et al., 2017; Bohan et al., 2011, 2017; Lau et al., 2017; Tylianakis et al., 2017;
422 Pellissier et al., 2018; Delmas et al., 2019). In recent years, a growing interest in these
423 approaches has led to a series of studies employing EG to infer ecological networks from
424 microbial community data (Zhou et al., 2011; Lupatini et al., 2014; Zappellini et al., 2015; Pérez-
425 Valera et al., 2017; Pauvert et al., 2019) or from macroinvertebrates (Compson et al., 2019), in
426 order to explore the links between network properties such as connectance, centrality or
427 nestedness, and ecosystem functioning. For instance, it has been shown that bacterial
428 communities in anthropized soil may have fewer potentially interacting taxa, than in natural soil
429 (Lupatini et al., 2014). Likewise, in aquatic ecosystems, anthropogenic impacts are reflected in
430 co-occurrence networks by a lower connectivity (Lawes et al., 2017; Laroche et al., 2018b; Li et
431 al., 2018) and a lower ratio of positive interactions (Laroche et al., 2018b).

432 While promising, exploring the links between the properties of ecological networks
433 inferred from EG data and ecosystem functioning is still in its infancy (Faust et al., 2012; 2015;
434 Lima-Mendez et al., 2015; Lawes et al., 2017; Laroche et al., 2018b; Li et al., 2018; Pauvert et
435 al., 2019). Multiple methodological issues limit the inference of robust networks from EG data
436 based on co-occurrences in space or time. For example, read counts are strictly compositional,
437 representing relative abundance of the marker itself, rather than presence or absolute
438 abundances (but see Friedman & Alm, 2012; Kurtz et al., 2015). Further, it is challenging to
439 control for covariates and confounding environmental parameters (but see Tammadoni-Nezhad
440 et al., 2013; Tackmann et al., 2018; Cougoul et al., 2018; Chiquet et al., 2018; Momal et al.,
441 2019), replicability of inference (Pauvert et al., 2019) and the relative merits of statistical and
442 logical inference (Vacher et al., 2016). Robust networks also require considerably more
443 replicates than are typically collected in EG studies, which increase both time and costs.
444 Nevertheless, as more benchmark datasets containing both EG data and independently
445 confirmed interactions between taxa become available to complement simulated datasets (see
446 Lima-Mendez et al., 2015), making robust network inference to explore the applicability of their
447 metrics for ecosystem monitoring will likely come within reach in the years to come.

448

449 “Functional community metrics” strategy: employing functional environmental 450 genomics for routine monitoring.

451 Another avenue of implementation of EG for ecosystem monitoring is the use of shotgun
452 metagenomics and metatranscriptomics, depicting the metabolic capabilities of the community,
453 and the expressed genes at the moment of sampling, respectively (Figure 1D). However,
454 ecologists have yet to disentangle the relative importance and relationship of taxonomic
455 diversity and functional traits for ecosystems functioning (Flynn et al., 2011; Gagic et al., 2015).
456 This is particularly true in microbial ecology with the “who’s there” *versus* “what they are doing”
457 paradigms that often relate to the employed molecular methodologies, i.e metabarcoding *versus*
458 metagenomics and metatranscriptomics (Xu et al., 2014). Some metagenomic contigs and
459 functional transcripts were indeed found to represent efficient bioindicators of anthropogenic
460 disturbances (Table S1), in terrestrial (de Menezes et al., 2012), groundwater (He et al., 2018),
461 freshwater (Thompson et al., 2016; Cheaib et al., 2018; Falk et al., 2019) and marine
462 environments (Kisand et al., 2012; Galand et al., 2016; Birrer et al., 2019), opening up potential

463 avenues for future routine ecosystem monitoring applications. Functional and taxonomic profiles
464 may respond differently under anthropogenic disturbance (Cheaib et al., 2018), as well as under
465 natural environmental variation (Barberà et al., 2012; Louca et al., 2016a; 2016b; Louca et al.,
466 2018). This taxon-function decoupling paves the way towards a molecular trait-based ecology
467 (Raes et al., 2011; Lajoie & Kembel 2019).

468 In an ecosystem monitoring context, functional profiles present two important features
469 that anticipate these proxies to be more accurate than taxonomic profiles for the detection of a
470 given environmental disturbance. First, because prokaryotes functional redundancy may be
471 widespread (Louca et al., 2018; Pearman et al., 2019; but see Galand et al., 2018 and see
472 Ramond et al. 2019 for protists), any given anthropogenic disturbance might trigger a similar
473 response across multiple taxonomic groups. Under this assumption, ecosystem monitoring
474 based on functional profiles may be less sensitive to biogeographical effects, random
475 demographic drift, and species dispersal limitation than a monitoring strategy based on
476 taxonomic profiles. This functional redundancy would also allow the establishment of a direct
477 and mechanistic link between a measured functional response to a given anthropogenic
478 disturbance. Second, because functional shifts are likely to occur prior to compositional ones, as
479 a response of the taxa present to the disturbance, the variation of functional profiles may
480 constitute useful early warnings for a timelier ecosystem management, especially the ones
481 detected by means of metatranscriptomics. However, RNA molecules are reportedly less stable
482 than genomic DNA, which would add challenging practical constraints that could preclude their
483 implementation in routine ecosystem monitoring programs (but see Fordyce et al., 2013;
484 Pochon et al., 2017; Cristescu, 2019; von Ammon et al. 2019). As a possible cost-effective
485 “shortcut”, bacterial 16S rRNA profiles can be used to predict functional community profiles,
486 based on evolutionary models (Langille et al., 2013; Aßhauer et al., 2015). Thus, 16S data could
487 be also explored for searching potential functional bioindicators by this approach (Mukherjee et
488 al., 2017; Laroche et al., 2018; Cordier 2020).

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492 [A roadmap for the implementation of environmental genomics for ecosystem](#)
493 [monitoring](#)

494 [The emergence of standards for EG methodologies to be applied for monitoring](#)
495 [programs.](#)

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

505 The field of EG for ecosystems monitoring is experiencing similar issues and has yet to
506 overcome some of the barriers to the necessary paradigm-shift in monitoring programs (Hering
507 et al., 2018). Some of the noteworthy steps towards this goal were achieved with the
508 widespread adoption of the MIGS, MIMARKS and MIxS standards in genomics, specifying the
509 minimum information that should accompany any genome, marker gene sequences or any
510 sequence (Field et al., 2008; Yilmaz et al., 2011). Now the most challenging part resides in the
511 adoption of standardized methodologies to produce, store and analyze EG data for a given
512 environmental setting. Given the variety of biological models and environmental matrices,
513 reaching a consensus in the scientific community and formalizing standards appears very
514 challenging, especially for metabarcoding (Pollock et al., 2018; Knight et al., 2018; Wilcox et al.,
515 2018; Zinger et al., 2019) and its application to ecosystem monitoring (Cristescu & Hebert,
516 2018; Hering et al., 2018). Yet, these hurdles are not specific to genomics methodologies, but
517 also exist for the morphology-based ones (Birk et al., 2012). Building robust, shared
518 methodological standards is of course necessary and important efforts are deployed to reach
519 this aim (Leese et al., 2018; Hering et al., 2018; Working Group CEN/TC230/WG28), for the
520 sampling of eDNA (Dickie et al., 2018; Wilcox et al., 2018; CEN 2018a), the molecular protocols
521 (Goldberg et al., 2016; Blackman et al., 2019) as well as for bioinformatics (Roy et al., 2018;

522 Knight et al., 2018), data interoperability (McDonald et al., 2012; Callahan et al., 2017) and
523 reference databases (CEN, 2018b).

524

525 Matching the right implementation strategy to the right monitoring program.

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

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

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

566 Structural and functional community metrics represent alternative implementation
567 strategies that may ultimately lead to a more generic, broadly applicable ecological monitoring
568 framework (Bohan et al., 2017; Karimi et al., 2017; Tylanakis et al., 2017; Quince et al., 2017;
569 Singer et al., 2017; Pellissier et al., 2018; Escalas et al., 2019). These strategies hold the
570 potential to provide a more mechanistic and functional understanding of the response of
571 biological communities to ecosystem variation. Such knowledge could hence be included in
572 predictive models to forecast shifts in biodiversity structure and possibly their consequences on
573 their associated ecosystem services under different disturbance scenarios. However, an
574 operational ecosystem monitoring framework remains to be built upon this theoretical ecological
575 work (Figure 2), that has only partially been experimentally validated (but see Laroche et al.,
576 2018; Ma et al., 2019). In addition, the extraction of structural or functional community metrics
577 remain active fields of ecological research, and the emergence of a molecular trait-based
578 ecology using metagenomics and metatranscriptomics profiles is in its infancy (Lajoie et al.,
579 2019). Hence, it is premature to discuss their operational implementation and regulatory
580 establishment, but their ecological benefit should be anticipated. Nevertheless, the collected
581 labelled datasets including samples for the production of EG data in the course of future
582 ecosystem monitoring campaigns will certainly contribute to move these possibilities forward.

583

584 Collecting reference data and eDNA/eRNA samples in parallel.

585 If EG-based methods are to complement or replace current morphology-based ones, the
586 prerequisite is to establish whether they can provide similar ecological diagnostics, to ensure a
587 smooth implementation and compatibility with existing time series (Leese et al., 2016; Bálint et
588 al., 2018). This inevitably implies extensive parallel sampling of currently implemented and EG
589 methodologies for some time, to build reference datasets on which the applicability can be
590 assessed and the calibration with previous methodology performed (Leese et al., 2016; Keeley
591 et al. 2018). To be reliable, such reference datasets have to cover a broad range of possible
592 environmental conditions for a given ecosystem across multiple spatiotemporal scales, ideally in
593 a balanced manner, to account for biotic interactions, random demographic drift and dispersal
594 limitations that may interact with the anthropogenic pressures in the assembly of communities.

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

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610 Conclusion and further research needs

611 The potential for EG-based methods for ecosystems monitoring is enormous and can
612 presently fulfil most of the requirements of current monitoring programs. Moving towards a
613 routine use of EG is certainly a paradigm-shift, but this technological breakthrough will

614 overcome the limitations of current morpho-taxonomy methodologies and enable the required
615 up-scaling to meet monitoring needs in a changing world. Without doubts, EG-based methods
616 will pave the way for a more cost-effective, faster, reproducible and semi-automatable
617 ecosystem monitoring framework. Regardless of the implementation strategy envisioned, the
618 following key technological, scientific and societal improvements will be beneficial for a
619 smoother transition:

- 620 ● A collaborative and transdisciplinary design of monitoring campaigns, involving both
621 experts, stakeholders and regulators would allow monitoring programs to more easily
622 bridge the science-policy gap.
- 623 ● A collection of reference morphological and molecular data in parallel, at least in a
624 subset of reference points or during a transition period, will assure backward and
625 forward compatibility of time series datasets, regardless of the envisioned
626 implementation strategy to be decided in future monitoring campaigns.
- 627 ● The efforts to complete reference sequence databases need to be sustained, by adding
628 more representatives of the known biodiversity, with a wider geographical coverage.
- 629 ● A reference database framework for *de novo* strategies needs to be established. A key
630 requirement is the ability to reliably compare OTUs or ASVs identified in monitoring
631 programs to formally establish knowledge about their sensitivity to disturbance.
- 632 ● The taxonomic resolution level (haplotype, species, genus, family, order, class) at which
633 HTS reads are most informative as genetic bioindicators for a given situation remains to
634 be identified.
- 635 ● For the identification of novel genetic bioindicators in complex communities, it will be
636 important to distinguish the effect of natural (seasonal) variation from disturbance-
637 induced community changes with rigorous experimental designs.
- 638 ● Basic and replicable research is highly needed to develop a structural and functional
639 community metrics-based implementation strategy. Such effort will likely contribute to
640 the establishment of a more broadly applicable monitoring framework and less
641 constrained by the database and geographical coverage limitations.

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647 **Box 1: Glossary of terms used in this paper**

- 648 ● Implementation strategy: Refers to the way environmental genomics data is produced
649 and analysed in an ecosystem monitoring context. It includes the choice of all the
650 molecular biology steps, i.e. targeted molecules (DNA versus RNA), metabarcoding
651 (amplicon sequencing) versus metagenomics or metatranscriptomics (shotgun
652 sequencing), and the computational biology steps (analytical approach), i.e. focusing on
653 the taxonomically assigned sequences or considering all the sequences, the use of
654 compositional turnovers (beta-diversity), structural metrics (alpha or phylogenetic
655 diversity and ecological network properties) or functional metrics (functional genes or
656 transcripts diversity).
- 657 ● Environmental genomics: Suite of molecular tools to sample, process and analyse
658 nucleic acids from an environmental sample (soil, water, sediment, feces)
- 659 ● Environmental DNA/RNA: Nucleic acids present in an environmental sample. It
660 encompasses the DNA/RNA within living multi or unicellular organisms, dead or
661 decaying as well as extracellular material.
- 662 ● Metabarcoding: A molecular workflow to simultaneously study the diversity of PCR-
663 selected organisms from environmental samples using high-throughput sequencing. This
664 is equivalent to amplicon sequencing of a taxonomic marker.
- 665 ● Metagenomics: Shotgun sequencing of the genomic DNA isolated from an
666 environmental sample. There is no PCR selection of particular taxonomic group and
667 include coding as well as non-coding genomic material.
- 668 ● Metatranscriptomics: Shotgun sequencing of retro-transcribed RNA isolated from an
669 environmental sample. As for metagenomics, there is no PCR selection but includes
670 only transcribed RNA (mRNA, rRNA), supposedly functional.
- 671 ● Bioindicator: A taxon, marker sequence, gene or transcript that is used as an indicator of
672 the ecological status of an environment.
- 673 ● Autecological value: Ecological knowledge about the distribution and abundance of
674 particular species obtained by studying interactions of individual organisms with their
675 environments.
- 676 ● Biotic Indices: Continuous or discrete variables that measure the level of disturbance of
677 an environment based on the composition and relative abundance of bioindicator taxa
678 (or OTUs/ASVs). Around half of the existing monitoring programs rely on biotic indices
679 (BIs). The BIs usually includes several ordered discrete classes, usually from 'poor' to
680 'high' ecological status.

681 ● Ecological network: Representation of statistically inferred biotic interactions through
682 spatial or temporal co-occurrence or co-exclusion. Taxa (nodes) are connected by
683 pairwise links (edges). Network ecology aims to understand how these network
684 properties are linked to the functioning of ecosystems.

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689 **Figures and tables**

690

691 Figure 1: Overview of the current methodology for the monitoring of ecosystems, that relies
692 mostly on the morphological identification of biodiversity and / or bioindicators of anthropogenic
693 impacts. Ecological diagnostics are performed based on reference biodiversity or on reference
694 biotic indices for a given ecosystem. The development of environmental genomics
695 methodologies has led to the proposition of multiple implementation strategies that can
696 intervene at different levels of the monitoring workflow, to produce an ecological diagnostic.
697 Green colors and smileys within boxes indicate reference or “high” ecological status while red
698 colors and smileys represent non-reference biodiversity or “poor” ecological status (i.e.
699 impacted environments). The colors on tags besides organisms or sequences indicate their bio-
700 indication value (red: indicator of impact, yellow: indicator of intermediate status, green:
701 indicator of good status). In this review paper, these strategies have been grouped in four broad
702 categories: (A) Taxonomy-based analyses focused on identification of known bio-indicators or
703 described taxa; (B) *De novo* bioindicator analyses; (C) Structural community metrics including
704 inferred ecological networks; and (D) Functional community metrics (metagenomics or
705 metatranscriptomics).

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708 Figure 2: Strengths and limitations of the currently envisioned implementation strategies of
709 environmental genomics for the monitoring of ecosystems, and their ability to fulfill the criteria of
710 existing monitoring programs.

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

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718 Table S1: List of studies employing environmental genomics for ecosystem monitoring sorted by
719 strategy, ecosystem, targeted taxonomic group and objective.

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