



**HAL**  
open science

## **Describing functional diversity of communities from environmental DNA**

Isabel Cantera, Simone Giachello, Tamara Münkemüller, Marco Caccianiga, Mauro Gobbi, Gianalberto Losapio, Silvio Marta, Barbara Valle, Krzysztof Zawierucha, Wilfried Thuiller, et al.

► **To cite this version:**

Isabel Cantera, Simone Giachello, Tamara Münkemüller, Marco Caccianiga, Mauro Gobbi, et al..  
Describing functional diversity of communities from environmental DNA. 2024. hal-04769072

**HAL Id: hal-04769072**

**<https://hal.science/hal-04769072v1>**

Preprint submitted on 7 Nov 2024

**HAL** is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

1 **Describing functional diversity of communities from environmental DNA**

2

3 **Cantera Isabel**\*<sup>1</sup>, **Giachello Simone**<sup>1,2</sup>, **Münkemüller Tamara**<sup>3</sup>, **Caccianiga Marco**<sup>4</sup>, **Gobbi Mauro**<sup>5</sup>,  
4 **Losapio Gianalberto**<sup>4,6</sup>, **Marta Silvio**<sup>7</sup>, **Valle Barbara**<sup>8,9</sup>, **Zawierucha Krzysztof**<sup>10</sup>, **Thuiller Wilfried**<sup>3</sup>  
5 and **Ficetola Francesco**<sup>1</sup>

6

7 <sup>1</sup>Department of Environmental Science and Policy, Università degli Studi di Milano, Milano, Italy.

8 <sup>2</sup>Department of Sciences, Technologies and Society, University School for Advanced Studies IUSS  
9 Pavia, Pavia, Italy.

10 <sup>3</sup>Université Grenoble Alpes, Université Savoie Mont Blanc, CNRS, LECA, Grenoble, France.

11 <sup>4</sup>Department of Biosciences, Università degli Studi di Milano, Milan, Italy.

12 <sup>5</sup>Research and Museum Collections Office, Climate and Ecology Unit, MUSE-Science Museum of  
13 Trento, Italy.

14 <sup>6</sup>Institute of Earth Surface Dynamics, University of Lausanne, Lausanne, Switzerland.

15 <sup>7</sup>Institute of Geosciences and Earth Resources, CNR, Via Moruzzi 1, 56124, Pisa, Italy.

16 <sup>8</sup>Università degli Studi di Siena, Siena, Italy.

17 <sup>9</sup>NBFC- Nature Biodiversity Future Center, Palermo, Italy.

18 <sup>10</sup>Department of Animal Taxonomy and Ecology, Adam Mickiewicz University, Poznań, Poland.

19 \*Corresponding author : isa\_cantera@hotmail.com

20

21 **Abstract**

22

23 Comprehensive assessments of functional diversity are needed to understand ecosystem alterations  
24 under global changes. The Fun-eDNA approach characterises functional diversity on the basis of  
25 eDNA-based inventories by assigning functional traits to taxonomic units. Fun-eDNA allows the joint  
26 analysis of an unprecedented number of taxa over broad spatial scales, providing a whole-ecosystem  
27 perspective of functional diversity. Fun-eDNA is increasingly used to tackle multiple questions, but  
28 aligning eDNA with traits poses several conceptual and technical challenges. Enhancing trait and  
29 eDNA databases, improving the annotation of eDNA inventories, interdisciplinary collaboration and  
30 conceptual harmonisation of traits are key steps for a comprehensive assessment of diverse taxa.  
31 Overcoming these challenges can unlock the full potential of eDNA in leveraging measures of  
32 ecosystem functioning from multi-taxa assessments.

33 **Keywords:** biodiversity, traits, metabarcoding, biotic interactions

34

35 **Highlights**

36

- 37 - By combining environmental DNA (eDNA) samples from water, soil, air or canopy with traits, Fun-  
38 eDNA can depict functional diversity at large spatial and temporal scales.
- 39 - Leveraging ever-increasing trait databases together with eDNA, Fun-eDNA has the potential to give  
40 a consistent and unified view of multi-taxa and multi-trophic communities by summarising them  
41 along a consistent trait-based perspective.
- 42 - While promising, combining eDNA with traits presents conceptual and technical challenges that  
43 need to be addressed to exploit its full potential.
- 44 - The full implementation of Fun-eDNA will allow a new framework that integrates the efficient  
45 biodiversity sampling based on eDNA and the mechanistic understanding of functional ecology. This  
46 will help to assess and mitigate the impact of global changes on the functions of ecosystems.

47

48

49 **Glossary**

50 **Ecosystem function:** physical, chemical, and biological processes that contribute to the self-  
51 maintenance of ecosystems, including energy flow, nutrient cycling, buffering of contaminants,  
52 regulation of natural populations and climate. These functions are essential for sustaining life and  
53 maintaining the services and resilience of ecosystems.

54  
55 **Environmental DNA (eDNA):** genetic material shed by an organism in the surrounding environment  
56 (e.g., water, soil, air). By capturing and analysing the DNA from the sampled environment, the  
57 presence of an organism can be potentially inferred without directly observing or capturing it.

58  
59 **Food-web:** network describing the trophic interactions and flow of energy among different trophic  
60 levels within ecological communities, ranging from primary producers, such as plants and microbes,  
61 to apex predator animals. It illustrates the interdependence of taxa and the transfer of energy and  
62 matter through various trophic levels throughout the community.

63  
64 **Functional diversity:** the variety of traits present within a given community. This diversity facet can  
65 be described by the dissimilarity, mean and/or distribution of traits, as well as their values and/or  
66 categories.

67  
68 **Functional groups:** taxa that express similar combinations of traits. Functional groups can be defined  
69 according to specific purposes and functions. Taxa within these groups are considered functionally  
70 redundant, as they perform similar ecological functions.

71  
72 **Functional trait:** biological characteristic that influences the organismal performance within its  
73 environment and its contribution to ecosystem functioning. Functional traits can encompass habitat,  
74 trophic, morphological, behavioural, optical, physiological, or life-history characteristics.

75  
76 **Taxonomic diversity:** variety of different taxa (or molecular taxonomic units) within a given  
77 community.

78

79 **Main** body

80

81 *eDNA metabarcoding to assess functional diversity*

82 **Environmental DNA** (eDNA) (see **glossary**) metabarcoding has emerged as a powerful tool for  
83 rapidly compiling biodiversity inventories across diverse taxonomic groups, habitats and ecosystems  
84 [1,2]. This method facilitates extensive biodiversity assessments across numerous sites, achieving  
85 goals that would be unattainable with traditional sampling techniques. eDNA-based inventories  
86 effectively capture **taxonomic diversity** [3], thereby revolutionising the acquisition of standardised,  
87 large-scale biodiversity data.

88 While conventional biodiversity metrics predominantly focus on taxonomic diversity, there is  
89 a growing recognition of **functional diversity** as a more proximate measure of **ecosystem functions**  
90 [4–6]. **Functional traits**, such as body size, feeding behaviour, photosynthetic capacity, reproductive  
91 strategies, and dispersal ability, are intricately linked to community structure and dynamics [7].  
92 Analysing these traits reveals how they influence ecosystem functions (effect traits) and how they  
93 respond to environmental drivers (response traits) [8]. When thoughtfully selected, traits (see [9]) can  
94 enhance our understanding of ecosystem functions [10], enabling the quantification and prediction of  
95 biodiversity’s impacts on ecosystem performance [11,12].

96 Recent years have seen a growing number of studies adopting a functional perspective through  
97 eDNA, with over 100 articles published between 2012 and 2024, assessing various taxonomic groups  
98 of eukaryotes (e.g., protists, plants, animals) and diverse environments (Box 1). These studies  
99 characterise the functional diversity of communities by linking traits to taxonomic units identified  
100 through eDNA. Traits are gathered from a variety of origins, including scientific literature, open  
101 databases, and direct observations or measurements of organisms collected independently from the  
102 eDNA samples (Box 1). This approach, hereafter referred to as “Fun-eDNA” (see Figure 1), enables  
103 three key types of trait-based applications (Suppl. Info. Table S1). First, it allows for the identification  
104 of **functional groups** at sampling sites based on the presence of key traits indicative of specific  
105 functions, such as trophic levels or growth form - an approach used in 71% of the studies [13]. Second,  
106 researchers can calculate indices that synthesise the variability of multiple traits, thereby  
107 characterising aspects of functional diversity, such as functional richness and redundancy [14].  
108 Finally, trait values can be attributed to taxonomic units to derive mean community values for specific  
109 traits (e.g., body size [15]). The resulting functional groups, indices, and mean trait values can then  
110 be related to environmental parameters.

111 By assigning traits to eDNA-based inventories, researchers can consider a broader array of  
112 functions within the sampled communities compared to assigning traits to traditional inventories, due  
113 to the reduced selectivity towards specific traits, taxa or habitats inherent in eDNA sampling [16–18].  
114 Furthermore, the Fun-eDNA approach offers novel insights into difficult-to-sample communities,

115 such as microscopic organisms arduous to identify [19,20], or species-rich ecosystems [21], which  
116 often require extensive expertise for identification. Despite its potential to advance functional and  
117 ecological studies, trait-based assessments from eDNA data face major conceptual and technical  
118 challenges. Anticipating an increasing number of studies embracing this functional perspective, we  
119 summarise the major achievements of the Fun-eDNA approach, identify recurring challenges and  
120 recommend solutions while acknowledging their limitations. Finally, we propose future research  
121 avenues aimed at addressing the challenges posed by this emerging and dynamic approach.

122

### 123 **The untapped potential of integrating eDNA with trait-based approaches**

124 eDNA-based inventories provide efficient means for conducting biodiversity analyses across multiple  
125 sites within reasonable timeframes. A significant advantage of Fun-eDNA lies in its ability to  
126 integrate trait information into large-scale inventories, thereby offering additional insights into  
127 ecological questions at broad spatial and temporal resolutions [16]. For instance, Fun-eDNA has  
128 identified key drivers of functional diversity at both continental [19] and global scales [15,22],  
129 illustrating consistent effects of ecological factors on traits (see Figure 2A for an example). As global  
130 changes accelerate, large-scale spatio-temporal patterns derived from Fun-eDNA can enhance rapid  
131 assessments of biodiversity alterations. Traits assigned to comprehensive eDNA-based inventories  
132 have elucidated the impacts of global stressors at regional and continental scales, yielding valuable  
133 implications for management. For instance, Fun-eDNA revealed a functional homogenization of fish  
134 communities in human-impacted rivers in French Guiana, showing that assemblages poorly  
135 monitored by the Water Framework Directive exhibited unique traits of the regional pool of species'  
136 traits [21]. In Europe, Fun-eDNA has allowed understanding the impacts of land-use changes on the  
137 structure and functions of soil communities [19], revealing higher microbial richness in croplands  
138 compared to less-disturbed environments, with potential negative impacts on ecosystems due to  
139 increasing pathogen prevalence.

140 Another advantage of Fun-eDNA is its applicability to multi-taxa inventories. Characterising  
141 functional diversity across many taxonomic and trophic groups is inherently challenging, requiring  
142 diverse expertise and multiple sampling techniques. Fun-eDNA allows for the simultaneous coverage  
143 of functional groups from various trophic levels and domains of life from a single sample, using  
144 multiple markers or a generalist one [23,24]. Analysing traits across multiple taxa is crucial for  
145 understanding the intricate relationships between biodiversity losses and ecosystem functions [6], and  
146 for identifying whole-ecosystem responses to environmental drivers [25]. Fun-eDNA can reveal  
147 whether functional groups respond synchronously to particular drivers and whether these coordinated  
148 responses arise from direct effects (shared responses of individual functional groups) or through  
149 trophic cascades [26,27]. For instance, in grassland ecosystems, multiple groups can show consistent

150 and synchronous responses to land-use intensification because of both direct effects on trophic groups  
151 and cascading effects across trophic levels [27].

152 Since ecosystem functions are outcomes of ecological processes directly or indirectly  
153 influenced by organisms and their interactions [28,29], multi-taxa measures of functional diversity  
154 can serve as proxies for ecosystem multifunctionality [26,30]. Such measures relate to key ecosystem  
155 functions, such as decomposition and enzyme activities ([23]; Figure 2B). Specifically, trophic  
156 interactions enable evaluation of how biodiversity affects essential ecosystem processes, including  
157 carbon sequestration, nutrient cycling, and pest regulation, as well as overall resilience and stability  
158 [31,32]. Food-webs integrate interactions and energy fluxes occurring at different trophic levels in a  
159 given community, and alterations in their structure may reflect impacts on ecosystem functions [30].  
160 However, comprehensive reconstructions of actual trophic interactions are complex, requiring the  
161 integration of extensive, heterogeneous information [33], as mere co-occurrence of taxa does not  
162 imply trophic relationships [34]. Nevertheless, large-scale, multi-taxa inventories obtained through  
163 eDNA can be integrated with trait information, such as food preferences, size and protective  
164 strategies, to infer the likelihood of trophic interactions [30,31,33]. Employing machine learning or  
165 Molecular Ecological Network Analysis [35] can further increase the accuracy of these  
166 reconstructions, using observations of real interactions [33,36]. So far, only 10% of the studies using  
167 Fun-eDNA considered at least three taxonomic groups (Box 1), often at small spatial scales, and even  
168 fewer have attempted to reconstruct food-webs or other interaction networks. Nonetheless, some  
169 studies have successfully used Fun-eDNA to identify trophic cascades and trophic niche overlaps  
170 [26,35], yielding promising results. We anticipate that eDNA-based food web reconstructions will  
171 gain popularity in coming years.

172

173

## 174 **Challenges and solutions to promote Fun-eDNA use in functional ecology**

175 Despite its successes, the full exploitation of Fun-eDNA is challenged by limitations inherent to both  
176 eDNA and trait-based approaches, as well as their integration, particularly when applied across a  
177 broad spectrum of taxa and/or at large spatial scales.

178

### 179 ***Challenges inherent to eDNA methods***

#### 180 *Taxonomic uncertainty*

181 eDNA inventories frequently contain assignments at taxonomic levels coarser than species (*e.g.*,  
182 genus or family). This is typically due to low taxonomic resolution of the markers used - common in  
183 generalist markers targeting all eukaryotes - and incomplete local reference databases of sequences.  
184 Incomplete reference databases pose significant challenges for broad-scale studies and assessments

185 in species-rich ecosystems [37]. Nevertheless, if correctly addressed, these limitations need not  
186 preclude robust functional estimates. Recent analyses suggested that taxonomic uncertainty,  
187 stemming from incomplete sequence databases, has minimal impact on functional diversity estimates  
188 (maximum underestimation compared to actual values of <30%)[38].

189

190 Several methodological strategies can mitigate taxonomic uncertainty when assigning traits to eDNA-  
191 based inventories:

- 192 ● Assigning traits only to taxa identified at the species level [e.g., 17], which is feasible  
193 when using comprehensive reference databases and specific markers, because most  
194 assignments are at species level.
- 195 ● Attributing trait values or categories based on a randomly chosen species expected to  
196 inhabit the study area within the identified genus or family [18,38].
- 197 ● Condensing information into higher taxonomic ranks: calculating mean values of all  
198 available species within the genus / family for continuous traits [e.g., 22] or assigning  
199 general traits conserved at the genus / family level for categorical traits [e.g., 40]. Traits  
200 may also be inferred from the phylogeny [40,41]. These solutions cluster the taxonomic  
201 units obtained from eDNA into broader functional groups often closely aligned with  
202 taxonomy.

203

204 Although these solutions facilitate a functional perspective on communities, they presuppose a  
205 phylogenetic signal among species within a given genus or family (i.e., closely related taxa share  
206 traits). This assumption does not always hold, as some traits may have evolved independently and  
207 different traits can exist within the same clade. Consequently, this approach may underestimate  
208 functional diversity by reducing functional heterogeneity within clades. To enhance Fun-eDNA  
209 accuracy, comprehensive sequence databases should be developed, particularly for understudied  
210 regions and taxonomic groups [42,43]. Future research should prioritise sequencing efforts for  
211 underrepresented taxa and regions, as well as taxa within functionally diverse genera/families or  
212 bearing extreme traits [38].

213

214 *eDNA mostly provides presence/absence information*

215 eDNA primarily provides presence/absence data, complicating the ability to ascertain whether an  
216 organism is alive, inactive, or dead [44]. eDNA can also detect the stochastic occurrence of  
217 windblown spores of micro-organisms, even though these can have little influence on actual  
218 ecosystem dynamics. These limitations pose challenges for testing ecological hypotheses regarding  
219 the contribution of species and their traits to ecosystem functioning, particularly when mass ratio  
220 effects are expected - that is, when ecosystem processes are primarily determined by the traits of taxa



221 with the highest biomass or abundance [6,45]. Nevertheless, taxa with the highest relative abundance  
222 or occurrence in eDNA datasets often are the ones with the highest actual abundance, suggesting a  
223 coherence between approaches [44,46–49]. Emerging technologies, such as environmental RNA  
224 (eRNA, which degrades more quickly in the environment than eDNA) or shotgun sequencing, may  
225 help address these limitations. However, further methodological advances are needed for their wider  
226 application [24,44,50,51].

227

### 228 ***Challenges inherent to trait-based approaches***

229

230 *Available trait information is dispersed and not exhaustive.*

231 Over the last decades, trait-based research has generated extensive but unconnected and  
232 heterogeneous datasets across various contexts, resulting in trait information being scattered across  
233 disparate repositories. This makes trait assignment time-consuming and cumbersome. Researchers  
234 using the Fun-eDNA approach often pool trait data from various sources (Box 1) [52–54], and have  
235 to deal with significant database heterogeneity. In addition, available data tend to be biased toward a  
236 limited number of taxonomic groups (e.g., plants, vertebrates), regions (e.g., Europe), as well as  
237 toward specific trait types, (e.g., feeding habits) [55], which limits the research questions that can be  
238 addressed. Finally, for some groups (e.g., tardigrades, rotifers), information about the biology of the  
239 species is scarce. To enhance accessibility, integration and reuse of trait information, we recommend  
240 that future trait measurements adhere to established standards for dataset description and structuring  
241 [39,56–59], while expanding these frameworks to encompass additional taxa, traits and ecosystems.

242

243

244 *Variability in terminology and concepts.*

245 The considerable variability in terminology across studies and databases creates semantic  
246 inconsistencies, complicating the effective integration of traits [60]. For example, in the reviewed  
247 papers (Box 1), terms related to feeding exhibited considerable variation (‘nutrition’, ‘diet’, ‘food  
248 acquisition’, ‘trophy’, ‘consumption’, ‘feeding habits’). Combining traits from very different  
249 organisms exacerbates semantic inconsistencies, as preferred terminology differs across taxonomic  
250 groups, and can pose conceptual challenges. Indeed, the ecological effects of traits can vary across  
251 taxa, given that the same trait can exert different functional effects depending on the taxon, driven by  
252 specific characteristics, scales of action and interactions within their respective ecosystems. For  
253 instance, for microbes, the term "predation" can refer to phagotrophic protists grazing on bacteria,  
254 thereby regulating bacterial populations and local nutrient cycling with rapid and localised effects.  
255 The ecological effects of "predation" performed by large vertebrates is not fully comparable to that  
256 of microbes. Vertebrate predation involves complex behavioural mechanisms and promotes

257 cascading effects that can persist for long periods, as these animals occur over broad spatial scales  
258 and often show long generation times [61].

259 Collaborative initiatives have been developed to promote standardised terminology for various traits  
260 through consensus among researchers within specific scientific fields and ontology-based  
261 applications [60,62]. Such initiatives might be combined with efforts to homogenise terminology  
262 across diverse taxa and to use traits as a common currency that respond similarly to a given  
263 environmental driver [27,63]. This will facilitate the identification of key traits or strategies to  
264 correctly assess ecosystem-level effects to global changes, such as cascading effects on food-webs  
265 [26] or synchronised *versus* divergent responses across taxa [27].

### 267 ***Challenges to integrate eDNA with trait-based approaches***

268 Assigning traits to eDNA-based inventories is not straightforward, as traits are collected  
269 independently from eDNA data and are described at the species or even individual level.  
270 Consequently, trait assignment to eDNA data is arduous. Trait databases coupling sequencing and  
271 detailed taxonomic information with specific guidelines have been recently developed to facilitate  
272 and accelerate the functional annotation of eDNA-based inventories [39,64]. Researchers should  
273 consistently upload the assembled trait databases in a freely accessible way with detailed taxonomic  
274 and sequencing information (Figure 1). Furthermore, as assigned trait values are not measured at the  
275 individual level, they can derive from organisms inhabiting other habitats and thus likely subjected  
276 to different environmental conditions than those studied. This can overlook local adaptation and  
277 intraspecific variation across sites, seasons and/or life stages [6,44], which are crucial aspects shaping  
278 species interactions and the ecosystem's ability to cope with environmental changes [65,66].

279  
280 A growing number of studies are exploring eDNA applications to describe within-species genetic  
281 variation from short markers [67,68], potentially enabling teasing apart lineages with functional  
282 differences [69]. However, eDNA samples often contain degraded DNA in low quantities, which can  
283 lead to erroneous sequence variants, complicating population genetic analyses [70]. Moreover, not  
284 all markers are suitable for population analyses or may not reflect variability in phenotypic traits.  
285 Combining comprehensive eDNA sampling with a stratified *in situ* collection of specimens (collected  
286 at strategically chosen points that cover key environmental conditions) can allow efficient  
287 measurement of fine-scale variation of traits [55].

### 288 **Concluding Remarks**

289 The expansion of eDNA analyses is revolutionising biodiversity inventories, offering unparalleled  
290 opportunities for the comprehensive exploration of whole-ecosystem biodiversity across wide spatio-  
291 temporal scales. By integrating traits with eDNA-based inventories, we can link community ecology

292 with functional ecology. This opens up accessible avenues to deepen our understanding of the  
293 relationships between biodiversity and ecosystem functions by providing proxies for trophic  
294 interactions and multifunctionality. To fully realise the potential of Fun-eDNA, methodological  
295 developments including sequencing prioritisation, standards for describing and structuring trait  
296 datasets, *in situ* specimen collection, along with conceptual integration, and interdisciplinary  
297 collaborations are essential (see Outstanding questions). Furthermore, to achieve a mechanistic  
298 understanding of the links between biodiversity and ecosystem function, the functional measures  
299 obtained with Fun-eDNA (e.g., multi-taxa measures, food-webs, synchronous responses) need to be  
300 related with explicit assessments of ecosystem functions (e. g., decomposition, productivity) [23,27].  
301 Future work integrating the competencies of taxonomists, field biologists, ecologists, and molecular  
302 biologists is expected to improve the effectiveness of eDNA-based analyses of functional diversity,  
303 providing more complete information that will help addressing the challenges of global change.

304

### 305 **Acknowledgments**

306 This study is funded by the European Research Council under the European Community's Horizon  
307 2020 Programme, Grant Agreement no. 772284 (IceCommunities) and Biodiversa+, the European  
308 Biodiversity Partnership under the 2021–2022 BiodivProtect joint call for research proposals, co-  
309 funded by the European Commission (grant agreement no. 101052342 “PrioritIce- Vanishing  
310 habitats: conservation priorities for glacier-related biodiversity threatened by climate change”). The  
311 study was also funded by the Swiss National Science Foundation (PZ00P3\_202127 to GL) and the  
312 National Recovery and Resilience Plan (NRRP), Mission 4 Component 2 Investment 1.4 - Call for  
313 tender No. 3138 of 16 December 2021, rectified by Decree n.3175 of 18 December 2021 of the Italian  
314 Ministry of University and Research funded by the European Union – NextGenerationEU (Project  
315 code CN\_00000033, Concession Decree No. 1034 of 17 June 2022 adopted by the Italian Ministry  
316 of University and Research, CUP B63C22000650007, Project title “National Biodiversity Future  
317 Center - NBFC”). We are grateful to Daniel Gomez Vega for the illustrations of the organisms on  
318 figure 1.

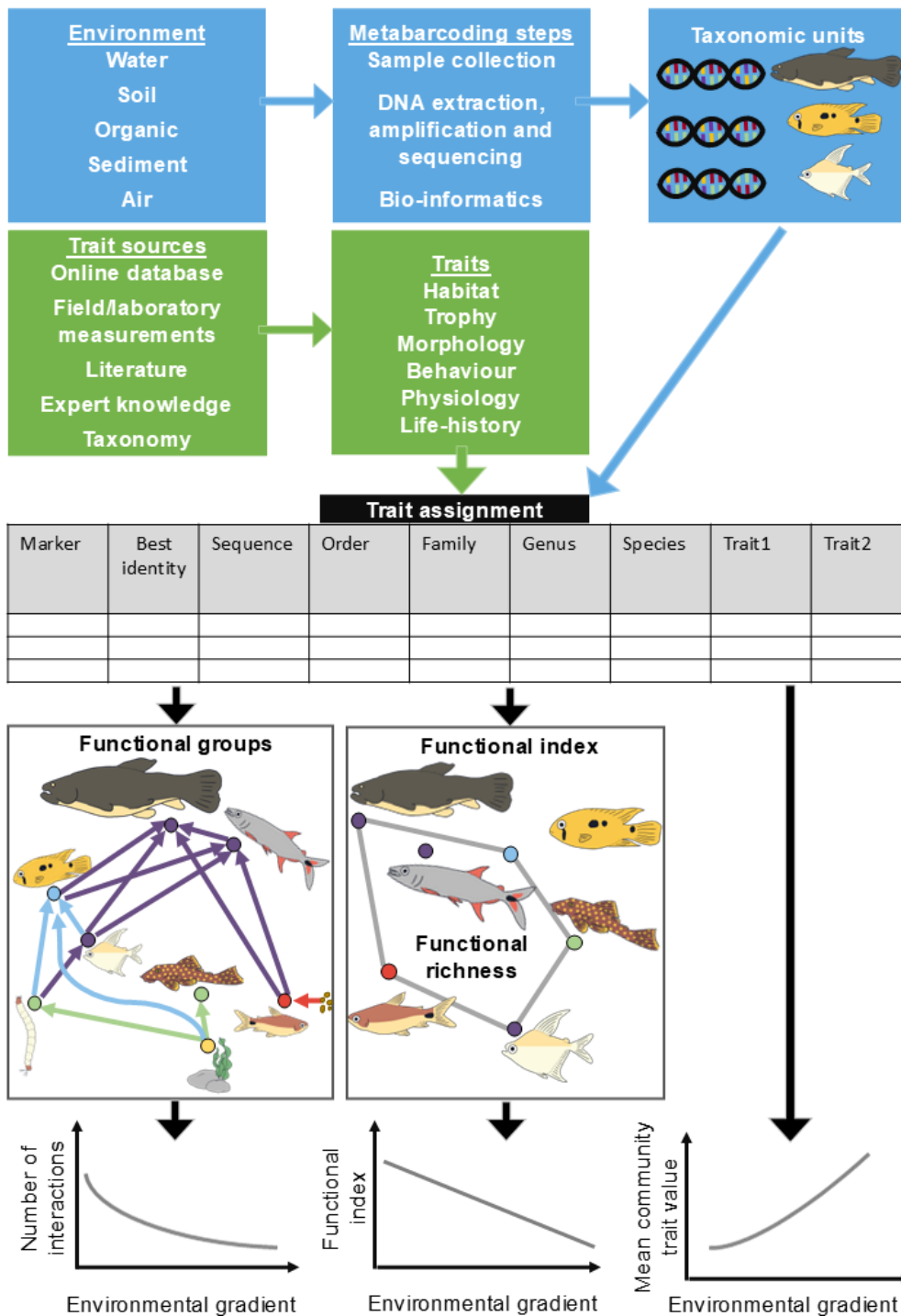
319

### 320 **Conflict of interest statement**

321 The authors declare no competing interests.

322 **Outstanding Questions**

- 323 - The use of available trait databases (online and/or from the literature) can greatly speed up  
324 broad spatial scale analyses, but overlooks intraspecific variation of traits. To what extent  
325 does this determine loss of information?
- 326 - How do we best tailor trait selection to obtain pertinent traits for the Fun-eDNA approach?  
327 Pertinent traits are those that can be assigned to eDNA-based inventories, and their selection  
328 must align with the specific purpose of the research. For instance, if the research question  
329 involves the response to changing environmental drivers, the pertinent traits would be  
330 response traits linked to those. Alternatively, if the focus is on the links between biodiversity  
331 and ecosystem functions, effect traits should be selected. For the latter, the choice of traits  
332 will depend on the specific function of interest (e.g., if the aim is to assess trophic interactions  
333 or carbon fluxes).
- 334 - Can we detect the interdependence of traits between interacting groups to provide a more  
335 mechanistic understanding of synergies between traits involved in a given interaction? For  
336 instance, coupling data on effect traits of predators and response traits of their prey might  
337 allow identifying functional linkages of their interactions.
- 338 - How can the coordination of disciplines and experts be achieved to reach a harmonised  
339 terminology for the traits of all the taxa inhabiting the same environment? This is pivotal to  
340 develop trait-based frameworks to synthesise the whole-ecosystem functional variation across  
341 diverse taxa.
- 342
- 343



344

345

346

347

348

349

350

351

352

353

354

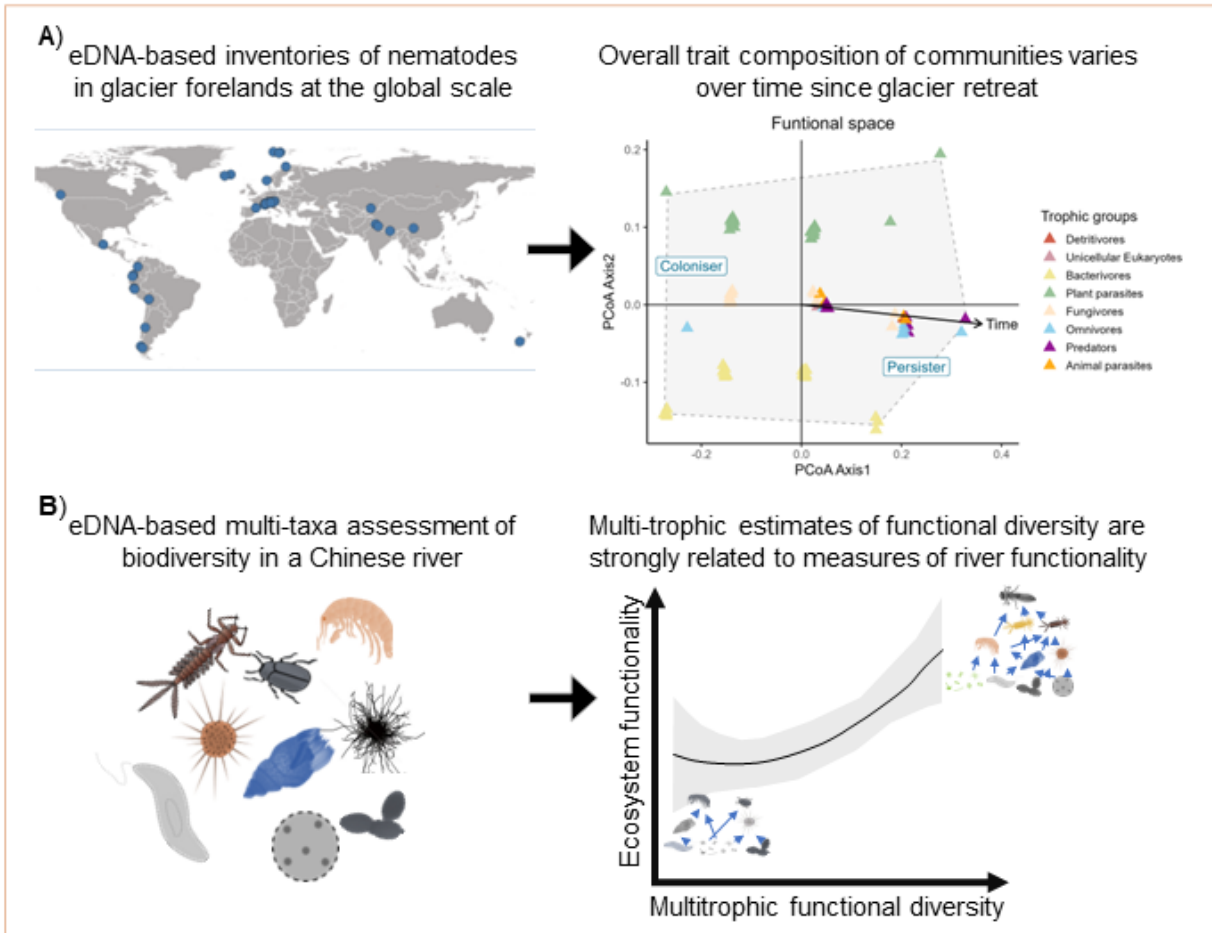
355

356

357

358

**Figure 1: Fun-eDNA procedure.** A) The Fun-eDNA approach links eDNA (blue) and functional ecology (green) methods to assign traits to taxonomic units identified with eDNA. Traits from various sources are assigned to these units, enabling the characterization of the functional diversity of communities. B) Functional diversity can be characterised through the identification of functional groups, community indexes or mean trait values. Functional groups can be counted to quantify functional richness (number of functional groups) or categorised based on specific purposes, processes and functions, and can also help to reconstruct food-webs, from which different variables (e.g. number of interactions) can be derived. Community indexes, like functional richness (represented here by the convex hull occupied by the community in a multidimensional functional space), can be also quantified. Values for a given trait can be attributed to calculate mean community values for a specific trait. C) The obtained functional variables can be related to natural or anthropogenic drivers. In the B panels, arrows represent the flow of energy and matter from one organism to another, indicating the direction of consumption (from prey to predator or from a producer to a consumer). Coloured dots represent trophic groups: yellow=primary producers, red=detrivore, green=herbivores, blue=omnivores, purple=secondary consumers.



359

360

361

362

363

364

365

366

367

368

369

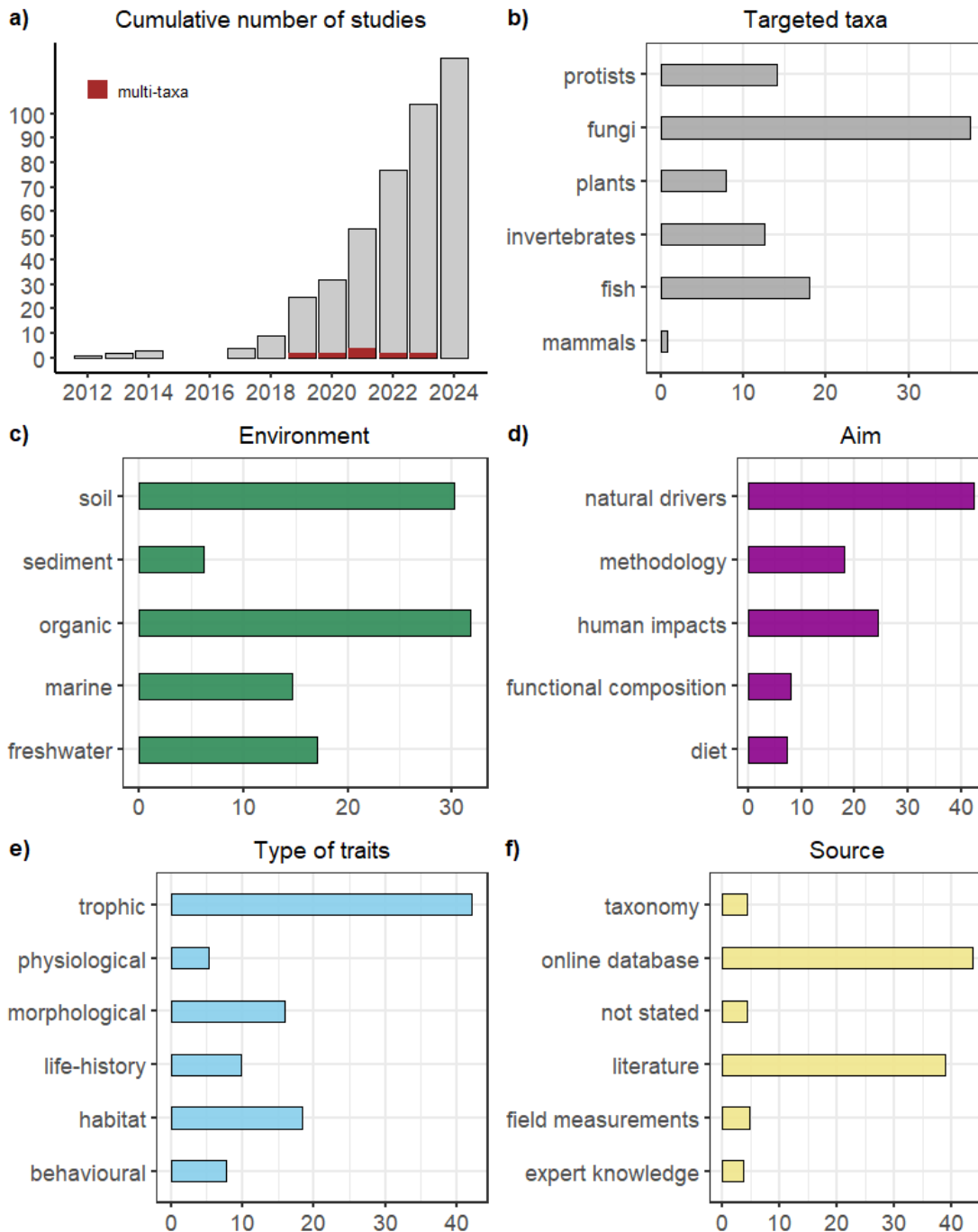
370

371

**Figure 2: Major advances enabled by the Fun-eDNA approach.** Exploiting the full potential of Fun-eDNA allows for the assessment of functional diversity in communities across spatial scales and taxonomic extents that would be challenging with traditional methods. **A)** Guerrieri et al.[22] used soil eDNA to describe the functional diversity of nematodes in 46 glacier forelands distributed across the world, and showed a general functional shift over time since glacier retreat. In recently deglaciated terrains, coloniser nematodes with an r-strategy and mostly feeding on bacteria and fungi prevailed, while in later stages, communities hosted more persistent nematodes with a K-strategy and representing more diverse trophic ranges. If trait information is available, this approach can be extended to other soil taxa communities to assess whole-ecosystem biodiversity. **B)** Li et al.[23] used eDNA to assess functional diversity across a broad range of taxa (invertebrates, fungi, protists, algae and bacteria), and showed that the ecosystem functionality of a river increases in sites hosting the communities with highest functional diversity.

372 **Box 1. Literature review of articles using the Fun-eDNA approach**

373 We reviewed studies assigning traits to taxonomic units obtained from eDNA (“Fun-eDNA approach”). The  
374 review was conducted through a Web of Science query (on September 12th, 2024). See Suppl. Info. for the  
375 search string and the selection of the relevant articles. We identified 123 published articles employing the Fun-  
376 eDNA approach starting from 2012, with a notable increase in the last five years (Figure 1a). In these studies,  
377 a very diverse range of taxa (Figure 1b) was assessed by analysing the eDNA obtained from the water, soil,  
378 sediment or organic material (e.g., faeces, plant organs) (Figure 1c). Aims were heterogeneous across studies  
379 (Figure 1d), with some papers testing the methodological robustness of functional assessments, and others  
380 assessing the natural (e.g., climate, soil properties) and/or anthropogenic drivers (e.g., deforestation) of  
381 functional diversity. Traits were assigned to eDNA-based inventories by gathering information from the  
382 literature, online databases, previous field measurements, expert knowledge and/or inferred from taxonomy  
383 (Figure 1e). Trophic characteristics were the traits most frequently used to assess functional diversity, followed  
384 by habitat (Figure 1f). Functional diversity was characterised using three general trait-based applications. 71%  
385 of studies inferred functional diversity on the basis of the number of functional groups (e.g., herbivores,  
386 decomposers, predators) or from the proportion / number of taxa within defined functional groups. 25% of  
387 studies calculated functional indices from several traits to summarise different aspects of the functional  
388 structure of a given community (e.g., functional richness, redundancy, specialisation). 4% of studies attributed  
389 values for a given trait to the taxonomic unit and derived mean trait values for the entire community (e.g.,  
390 mean community values).  
391



392  
393  
394  
395  
396  
397  
398  
399  
400

Figure I. Results of the literature review of the articles using the Fun-eDNA approach. (a) Cumulative number of studies using the Fun-eDNA approach for each year from 2012 to 2024. Multi-taxa studies in red correspond to the studies assessing the traits of at least three different taxonomic groups from the same eDNA sample. We then report the percentages of Fun-eDNA studies targeting a) different taxa, b) different environments, c) research aims, d) trait typologies e) and the source from which the traits were collected (f). See Suppl. Info. for details on the definition of the categories.

401  
402  
403  
404  
405  
406  
407

## References

1. Beng, K.C. and Corlett, R.T. (2020) Applications of environmental DNA (eDNA) in ecology and conservation: opportunities, challenges and prospects. *Biodivers. Conserv.* 29, 2089–2121
2. Taberlet, P. *et al.* (2018) *Environmental DNA for biodiversity research and monitoring*, Oxford University Press
3. Fediajevaite, J. *et al.* (2021) Meta-analysis shows that environmental DNA outperforms traditional surveys, but warrants better reporting standards. *Ecol. Evol.* 11, 4803–4815



- 408 4. Mammola, S. *et al.* (2021) Concepts and applications in functional diversity. *Funct. Ecol.* 35,  
409 1869–1885
- 410 5. Díaz, S. *et al.* (2006) Biodiversity Loss Threatens Human Well-Being. *PLOS Biol.* 4, e277
- 411 6. Reiss, J. *et al.* (2009) Emerging horizons in biodiversity and ecosystem functioning research.  
412 *Trends Ecol. Evol.* 24, 505–514
- 413 7. Nolte, D. *et al.* (2019) Habitat specialization, distribution range size and body size drive  
414 extinction risk in carabid beetles. *Biodivers. Conserv.* 28, 1267–1283
- 415 8. Lavorel, S. *et al.* (2013) A novel framework for linking functional diversity of plants with other  
416 trophic levels for the quantification of ecosystem services. *J. Veg. Sci.* 24, 942–948
- 417 9. Streit, R.P. and Bellwood, D.R. (2023) To harness traits for ecology, let's abandon  
418 'functionality.' *Trends Ecol. Evol.* 38, 402–411
- 419 10. Lavorel, S. *et al.* (2011) Using plant functional traits to understand the landscape distribution of  
420 multiple ecosystem services. *J. Ecol.* 99, 135–147
- 421 11. Mouillot, D. *et al.* (2013) A functional approach reveals community responses to disturbances.  
422 *Trends Ecol. Evol.* 28, 167–177
- 423 12. Gobbi, M. and Fontaneto, D. (2008) Biodiversity of ground beetles (Coleoptera: Carabidae) in  
424 different habitats of the Italian Po lowland. *Agric. Ecosyst. Environ.* 127, 273–276
- 425 13. Jaus, R.-T. *et al.* (2021) A Parasite's Paradise: Biotrophic Species Prevail Oomycete  
426 Community Composition in Tree Canopies. *Front. For. Glob. CHANGE* 4
- 427 14. Cantera, I. *et al.* (2023) Functional responses to deforestation in fish communities inhabiting  
428 neotropical streams and rivers. *Ecol. Process.* 12, 52
- 429 15. Aslani, F. *et al.* (2022) Towards revealing the global diversity and community assembly of soil  
430 eukaryotes. *Ecol. Lett.* 25, 65–76
- 431 16. Seymour, M. *et al.* (2021) Environmental DNA provides higher resolution assessment of  
432 riverine biodiversity and ecosystem function via spatio-temporal nestedness and turnover  
433 partitioning. *Commun. Biol.* 4, 512
- 434 17. Aglieri, G. *et al.* (2021) Environmental DNA effectively captures functional diversity of coastal  
435 fish communities. *Mol. Ecol.* 30, 3127–3139
- 436 18. Marques, V. *et al.* (2021) Use of environmental DNA in assessment of fish functional and  
437 phylogenetic diversity. *Conserv. Biol.* 35, 1944–1956
- 438 19. Labouyrie, M. *et al.* (2023) Patterns in soil microbial diversity across Europe. *Nat. Commun.*  
439 14, 3311
- 440 20. Singer, D. *et al.* (2021) Protist taxonomic and functional diversity in soil, freshwater and marine  
441 ecosystems. *Environ. Int.* 146, 106262
- 442 21. Coutant, O. *et al.* (2023) Environmental DNA reveals a mismatch between diversity facets of  
443 Amazonian fishes in response to contrasting geographical, environmental and anthropogenic  
444 effects. *Glob. Change Biol.* 29, 1741–1758
- 445 22. Guerrieri, A. *et al.* (2024) Local climate modulates the development of soil nematode  
446 communities after glacier retreat. *Glob. Change Biol.* 30, e17057
- 447 23. Li, F. *et al.* (2020) Human activities' fingerprint on multitrophic biodiversity and ecosystem  
448 functions across a major river catchment in China. *Glob. Change Biol.* 26, 6867–6879
- 449 24. Ficetola, G.F. and Taberlet, P. (2023) Towards exhaustive community ecology via DNA  
450 metabarcoding. *Mol. Ecol.* 32, 6320–6329
- 451 25. Montagna, M. *et al.* (2018) Differential biodiversity responses between kingdoms (plants, fungi,  
452 bacteria and metazoa) along an Alpine succession gradient. *Mol. Ecol.* 27, 3671–3685
- 453 26. Calderón-Sanou, I. *et al.* (2021) Cascading effects of moth outbreaks on subarctic soil food  
454 webs. *Sci. Rep.* 11, 15054
- 455 27. Neyret, M. *et al.* (2024) A slow-fast trait continuum at the whole community level in relation to  
456 land-use intensification. *Nat. Commun.* 15, 1–23
- 457 28. Cardinale, B.J. *et al.* (2002) Species diversity enhances ecosystem functioning through  
458 interspecific facilitation. *Nature* 415, 426–429
- 459 29. Cardinale, B.J. (2011) Biodiversity improves water quality through niche partitioning. *Nature*  
460 472, 86–89

- 461 30. Potapov, A.M. (2022) Multifunctionality of belowground food webs: resource, size and spatial  
462 energy channels. *Biol. Rev.* 97, 1691–1711
- 463 31. Potapov, A.M. *et al.* (2024) Rainforest transformation reallocates energy from green to brown  
464 food webs. *Nature* 627, 116–122
- 465 32. Soliveres, S. *et al.* (2016) Biodiversity at multiple trophic levels is needed for ecosystem  
466 multifunctionality. *Nature* 536, 456–459
- 467 33. Fricke, E.C. *et al.* (2022) Collapse of terrestrial mammal food webs since the Late Pleistocene.  
468 *Science* 377, 1008–1011
- 469 34. Thuiller, W. *et al.* (2024) Navigating the integration of biotic interactions in biogeography. *J.*  
470 *Biogeogr.* 51, 550–559
- 471 35. Meyer, J.M. *et al.* (2020) Molecular Ecological Network Analyses: An Effective Conservation  
472 Tool for the Assessment of Biodiversity, Trophic Interactions, and Community Structure.  
473 *Front. Ecol. Evol.* 8
- 474 36. Pichler, M. *et al.* (2020) Machine learning algorithms to infer trait-matching and predict species  
475 interactions in ecological networks. *Methods Ecol. Evol.* 11, 281–293
- 476 37. Marques, V. *et al.* (2021) GAPeDNA: Assessing and mapping global species gaps in genetic  
477 databases for eDNA metabarcoding. *Divers. Distrib.* 27, 1880–1892
- 478 38. Condachou, C. *et al.* (2023) Inferring functional diversity from environmental DNA  
479 metabarcoding. *Environ. DNA* 5, 934–944
- 480 39. Giachello, S. *et al.* (2023) Toward a common set of functional traits for soil protists. *Soil Biol.*  
481 *Biochem.* 187, 109207
- 482 40. Kim, S.W. *et al.* (2018) Transcending data gaps: a framework to reduce inferential errors in  
483 ecological analyses. *Ecol. Lett.* 21, 1200–1210
- 484 41. Johnson, T.F. *et al.* (2021) Handling missing values in trait data. *Glob. Ecol. Biogeogr.* 30, 51–  
485 62
- 486 42. Ruppert, K. *et al.* (2019) Past, present, and future perspectives of environmental DNA (eDNA)  
487 metabarcoding: A systematic review in methods, monitoring, and applications of global eDNA.  
488 *Glob. Ecol. Conserv.* 17, e00547
- 489 43. Weigand, H. *et al.* (2019) DNA barcode reference libraries for the monitoring of aquatic biota  
490 in Europe: Gap-analysis and recommendations for future work. *Sci. Total Environ.* 678, 499–  
491 524
- 492 44. Yao, M. *et al.* (2022) Fishing for fish environmental DNA: Ecological applications,  
493 methodological considerations, surveying designs, and ways forward. *Mol. Ecol.* 31, 5132–5164
- 494 45. Smith, M.D. *et al.* (2020) Mass ratio effects underlie ecosystem responses to environmental  
495 change. *J. Ecol.* 108, 855–864
- 496 46. Calderón-Sanou, I. *et al.* (2019) From environmental DNA sequences to ecological conclusions:  
497 How strong is the influence of methodological choices? *J. Biogeogr.* 47, 193–206
- 498 47. Wu, L. *et al.* (2024) Monitoring of multiple fish species by quantitative environmental DNA  
499 metabarcoding surveys over two summer seasons. *Mol. Ecol. Resour.* 24, e13875
- 500 48. Pansu, J. *et al.* (2015) Long-lasting modification of soil fungal diversity associated with the  
501 introduction of rabbits to a remote sub-Antarctic archipelago. *Biol. Lett.* 11, 20150408
- 502 49. Ariza, M. *et al.* (2023) Plant biodiversity assessment through soil eDNA reflects temporal and  
503 local diversity. *Methods Ecol. Evol.* 14, 415–430
- 504 50. Stat, M. *et al.* (2017) Ecosystem biomonitoring with eDNA: metabarcoding across the tree of  
505 life in a tropical marine environment. *Sci. Rep.* 7, 12240
- 506 51. Peel, N. *et al.* (2019) Semi-quantitative characterisation of mixed pollen samples using MinION  
507 sequencing and Reverse Metagenomics (RevMet). *Methods Ecol. Evol.* 10, 1690–1701
- 508 52. Cantera, I. *et al.* (2022) Low level of anthropization linked to harsh vertebrate biodiversity  
509 declines in Amazonia. *Nat. Commun.* 13, 3290
- 510 53. Veron, P. *et al.* (2023) Environmental DNA complements scientific trawling in surveys of  
511 marine fish biodiversity. *ICES J. Mar. Sci.* 80, 2150–2165
- 512 54. Calderon-Sanou, I. *et al.* (2022) Energy and physiological tolerance explain multi-trophic soil  
513 diversity in temperate mountains. *Divers. Distrib.* 28, 2549–2564

- 514 55. Pereira, C.L. *et al.* (2023) Future-proofing environmental DNA and trait-based predictions of  
515 food webs. *BioScience* 73, 862–878
- 516 56. Dawson, S.K. *et al.* (2019) Handbook for the measurement of macrofungal functional traits: A  
517 start with basidiomycete wood fungi. *Funct. Ecol.* 33, 372–387
- 518 57. Klimešová, J. *et al.* (2019) Handbook of standardized protocols for collecting plant modularity  
519 traits. *Perspect. Plant Ecol. Evol. Syst.* 40, 125485
- 520 58. Pérez-Harguindeguy, N. *et al.* (2013) New handbook for standardised measurement of plant  
521 functional traits worldwide. *Aust. J. Bot.* 61, 167–234
- 522 59. Moretti, M. *et al.* (2017) Handbook of protocols for standardized measurement of terrestrial  
523 invertebrate functional traits. *Funct. Ecol.* 31, 558–567
- 524 60. Pey, B. *et al.* (2014) A Thesaurus for Soil Invertebrate Trait-Based Approaches. *PLOS ONE* 9,  
525 e108985
- 526 61. Ripple, W.J. *et al.* (2014) Status and Ecological Effects of the World’s Largest Carnivores.  
527 *Science* 343, 1241484
- 528 62. Le Guillarme, N. *et al.* (2023) The Soil Food Web Ontology: Aligning trophic groups,  
529 processes, resources, and dietary traits to support food-web research. *Ecol. Inform.* 78, 102360
- 530 63. Zhang, C. *et al.* (2024) Linking nematodes and ecosystem function: a trait-based framework.  
531 *Trends Ecol. Evol.* DOI: <https://doi.org/10.1016/j.tree.2024.02.002>
- 532 64. Nguyen, N.H. *et al.* (2016) FUNGuild: An open annotation tool for parsing fungal community  
533 datasets by ecological guild. *Fungal Ecol.* 20, 241–248
- 534 65. Violle, C. *et al.* (2012) The return of the variance: intraspecific variability in community  
535 ecology. *Trends Ecol. Evol.* 27, 244–252
- 536 66. Bolnick, D.I. *et al.* (2011) Why intraspecific trait variation matters in community ecology.  
537 *Trends Ecol. Evol.* 26, 183–192
- 538 67. Sigsgaard, E.E. *et al.* (2020) Population-level inferences from environmental DNA—Current  
539 status and future perspectives. *Evol. Appl.* 13, 245–262
- 540 68. Yatsuyanagi, T. *et al.* (2024) Environmental DNA unveils deep phylogeographic structure of a  
541 freshwater fish. *Mol. Ecol.* 33, e17337
- 542 69. Steudel, B. *et al.* (2016) Contrasting biodiversity–ecosystem functioning relationships in  
543 phylogenetic and functional diversity. *New Phytol.* 212, 409–420
- 544 70. Andres, K.J. *et al.* (2023) Detecting and analysing intraspecific genetic variation with eDNA :  
545 From population genetics to species abundance. *Mol. Ecol.* 32, 4118–4132
- 546