Opinion

Describing functional diversity of communities from environmental DNA

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Comprehensive assessments of functional diversity are needed to understand ecosystem alterations under global changes. The 'Fun-eDNA' approach characterises functional diversity by assigning traits to taxonomic units obtained through environmental DNA (eDNA) sampling. By simultaneously analysing an unprecedented number of taxa over broad spatial scales, the approach provides a whole-ecosystem perspective of functional diversity. Fun-eDNA is increasingly used to tackle multiple questions, but aligning eDNA with traits poses several conceptual and technical challenges. Enhancing trait databases, improving the annotation of eDNA-based taxonomic inventories, interdisciplinary collaboration, and conceptual harmonisation of traits are key steps to achieve a comprehensive assessment of diverse taxa. Overcoming these challenges can unlock the full potential of eDNA in leveraging measures of ecosystem functions from multi-taxa assessments.

eDNA metabarcoding to assess functional diversity

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

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

Recent years have seen a growing number of studies adopting a functional perspective through eDNA, with over 100 articles published between 2012 and 2024, assessing various taxonomic groups of eukaryotes (e.g., protists, plants, animals) and diverse environments (Box 1). These studies characterise the functional diversity of communities by linking traits to taxonomic units identified through eDNA. Traits are gathered from a variety of origins, including scientific literature, open databases, and direct observations or measurements of organisms collected independently from the

Highlights

By combining environmental DNA (eDNA) samples with traits, Fun-eDNA can depict functional diversity at large spatial and temporal scales.

Leveraging ever-increasing trait databases together with eDNA, Fun-eDNA has the potential to give a consistent and unified view of multi-taxa and multi-trophic communities by summarising them along a consistent trait-based perspective.

While promising, combining eDNA-based inventories with traits presents conceptual and technical challenges that need to be addressed, particularly when applied across a broad spectrum of taxa and/or at large spatial scales.

The full implementation of Fun-eDNA will allow a new framework that integrates the efficient biodiversity sampling based on eDNA and the mechanistic understanding of functional ecology. This will help to assess and mitigate the impact of global changes on the functions of ecosystems.

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eDNA samples (Box 1). This approach, hereafter referred to as 'Fun-eDNA' (Figure 1), enables three key types of trait-based applications (see the supplemental information online). First, **functional groups** can be identified at sampling sites based on the presence of traits indicative of specific functions, such as trophic level or growth form [13]. Second, researchers can calculate indices that synthesise the variability of multiple traits, thereby characterising aspects of functional diversity for a given community (e.g., functional richness and redundancy [14]). Finally, trait values can be attributed to taxonomic units to derive mean community values for specific traits (e.g., body size [15]). The resulting functional groups, indices, and community trait values can then be related to environmental parameters.

By assigning traits to eDNA-based inventories, researchers can consider a broader array of functions within the sampled communities compared with assigning traits to traditional inventories, due to the reduced selectivity towards specific traits, taxa or habitats inherent in eDNA sampling [16–18]. Furthermore, the Fun-eDNA approach offers novel insights into difficult-to-sample communities, such as microscopic organisms arduous to identify [19,20], or species-rich ecosystems [21], which often require extensive expertise for taxonomic identification. Despite its potential to advance functional and ecological studies, trait-based assessments from eDNA data face major conceptual and technical challenges. Anticipating an increasing number of studies embracing this functional perspective, we summarise the major achievements of the Fun-eDNA approach, identify recurring challenges, and recommend solutions while acknowledging their limitations. Finally, we propose future research avenues aimed at addressing the challenges posed by this emerging and dynamic approach.

The untapped potential of integrating eDNA with trait-based approaches

eDNA-based inventories provide efficient means for conducting biodiversity analyses across multiple sites within reasonable timeframes. A significant advantage of Fun-eDNA lies in its ability to integrate trait information into large-scale inventories, thereby offering additional insights into ecological questions at broad spatial and temporal resolutions [16]. For instance, Fun-eDNA has identified key drivers of functional diversity at both continental [19] and global scales [15,22], illustrating consistent effects of ecological factors on traits (see Figure 2A for an example). As global changes accelerate, large-scale spatio-temporal patterns derived from Fun-eDNA can enhance rapid assessments of biodiversity alterations. Traits assigned to comprehensive eDNA-based inventories can elucidate the impacts of global stressors at regional and continental scales, yielding valuable implications for management. For instance, Fun-eDNA revealed a functional

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

We reviewed studies assigning traits to taxonomic units obtained from eDNA ('Fun-eDNA approach'). The review was conducted through a Web of Science query (on 12 September 2024). See the supplemental information online for the search string and the selection of the relevant articles. We identified 129 published articles employing the Fun-eDNA approach starting from 2012, with a notable increase in the past 5 years (Figure IA). In these studies, a very diverse range of taxa (Figure IB) was assessed by analysing the eDNA obtained from the water, soil, sediment, or organic material (e.g., faeces, plant organs) (Figure IC). Aims were heterogeneous across studies (Figure ID), with the majority of articles testing the methodological robustness of functional assessments and assessing the natural (e.g., climate, soil properties) and/or anthropogenic drivers (e.g., deforestation) of functional diversity. Feeding characteristics were largely the traits most frequently used to assess functional diversity, followed by habitat and morphology (Figure IE). Traits were assigned to eDNA-based inventories by gathering information from different sources (Figure IF). Functional diversity was characterised using three general trait-based applications. Over 70% of studies inferred functional diversity on the basis of the number of functional groups (e.g., herbivores, decomposers, predators) or from the proportion/number of taxa within defined functional groups. Over 25% of studies calculated functional indices by combining information of several traits for each taxa to summarise different aspects of the functional structure of a given community (e.g., functional richness, redundancy, specialisation). Finally, few studies attributed values for a given trait to the taxonomic unit and derived mean trait values for the entire community (e.g., mean community values) by averaging the values of the taxa detected in the sample.
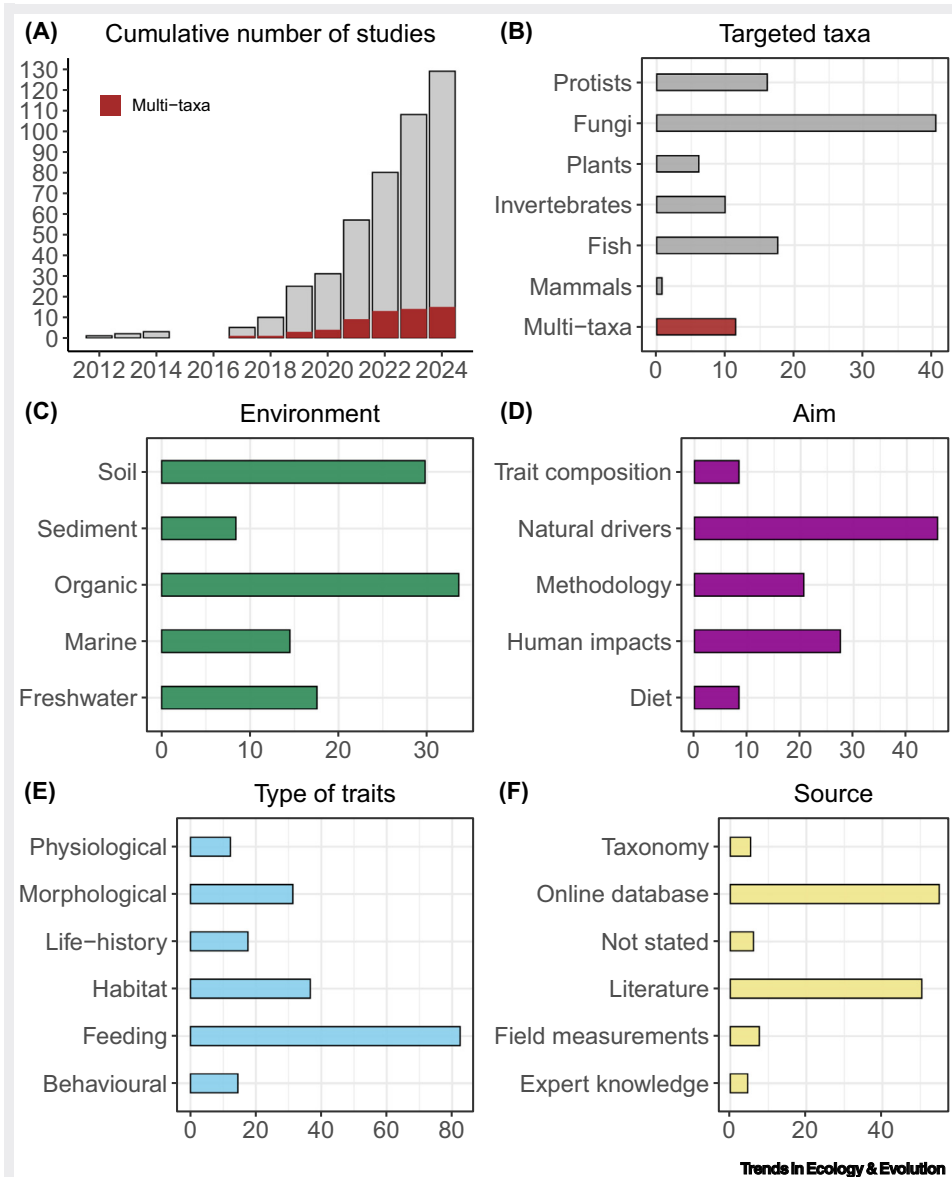
Figure I. Results of the literature review of the articles using the Fun-eDNA approach. (A) Cumulative number of studies using the Fun-eDNA approach for each year from 2012 to 2024. Multi-taxa studies in red correspond to the studies assessing the traits of at least three different taxonomic groups from the same eDNA sample. We then report the percentages of Fun-eDNA studies categorised by the sampled taxa (B), sampled environments (C), research aims (D), trait typologies (E), and the source from which the traits were collected (F). See the supplemental information online for details on the definition of the categories and the list of selected articles.

Glossary

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

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

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

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

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

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

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

homogenization of fish communities in human-impacted rivers in French Guiana and showed that assemblages poorly monitored by the Water Framework Directive exhibited unique traits from the regional pool of species' traits [21]. In Europe, assessing the impacts of land-use changes on the structure and functions of soil communities with Fun-eDNA revealed higher microbial richness in croplands compared with less-disturbed environments, with potential negative impacts on ecosystems due to increased proportion of fungal pathogens [19].
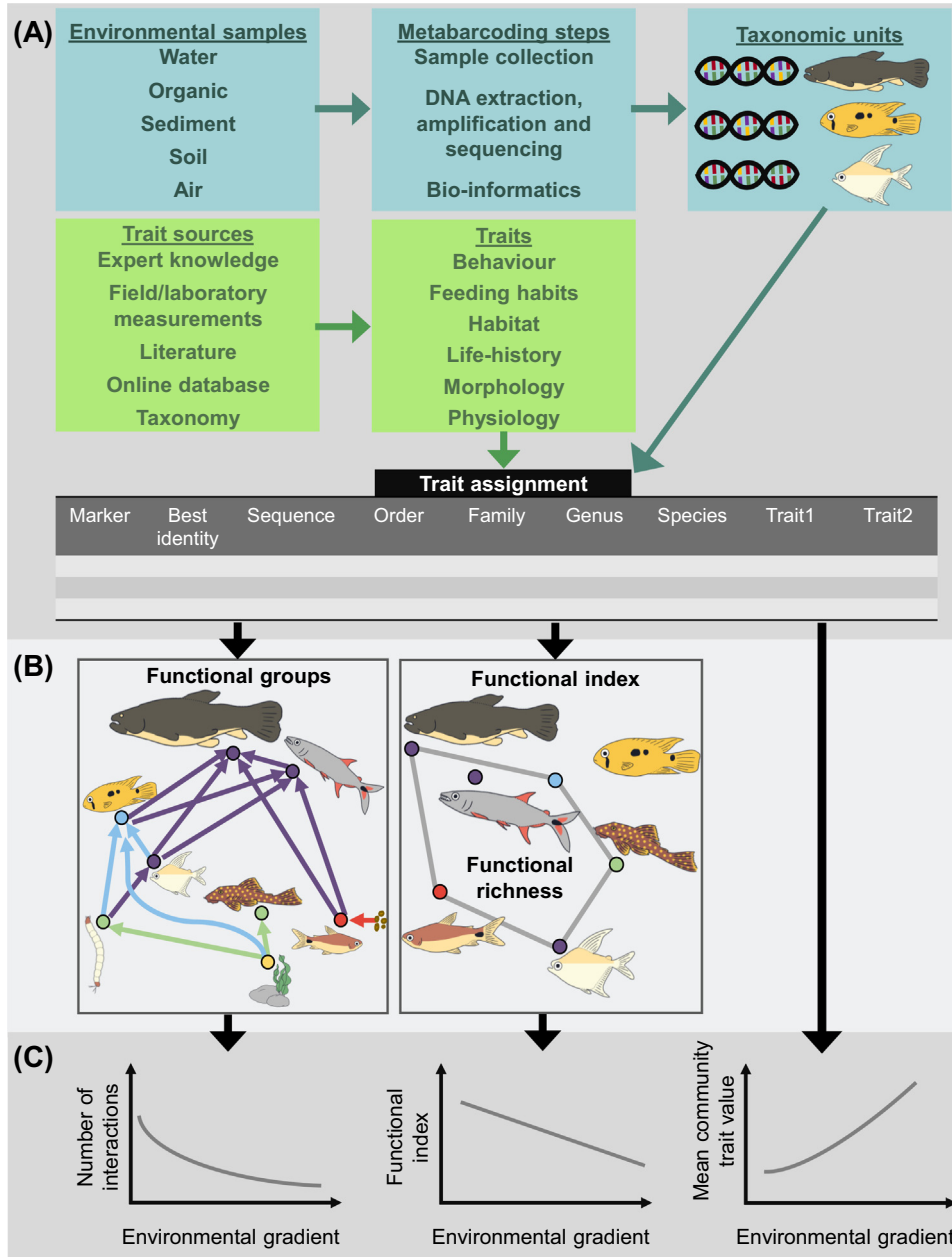
Figure 1. Fun-eDNA procedure. (A) The Fun-eDNA approach links eDNA (blue) and functional ecology (green) methods to assign traits to taxonomic units identified with eDNA. Traits from various sources are assigned to taxonomic units enabling characterisation of the functional diversity of communities. (B) Functional diversity can be characterised through the identification of functional groups (categorised based on specific traits), community indexes, or community mean trait values. Functional groups can be used to reconstruct food-webs, from which different variables can be derived (e.g., number of interactions). Community indexes, synthesising the variability of multiple traits in a given community, can be also quantified. Here, we represented functional richness, as the convex hull volume occupied by the community in a functional space. Values for a given trait can be attributed to taxa to calculate mean community values for the trait. (C) The obtained functional variables can be related to natural or anthropogenic drivers. In (B), arrows represent the flow of energy and matter from one organism to another, indicating the direction of consumption (from the resource to the consumer). Coloured dots represent trophic groups: yellow = primary producer, red = detrivore, green = herbivore, blue = omnivore, purple = secondary consumer.
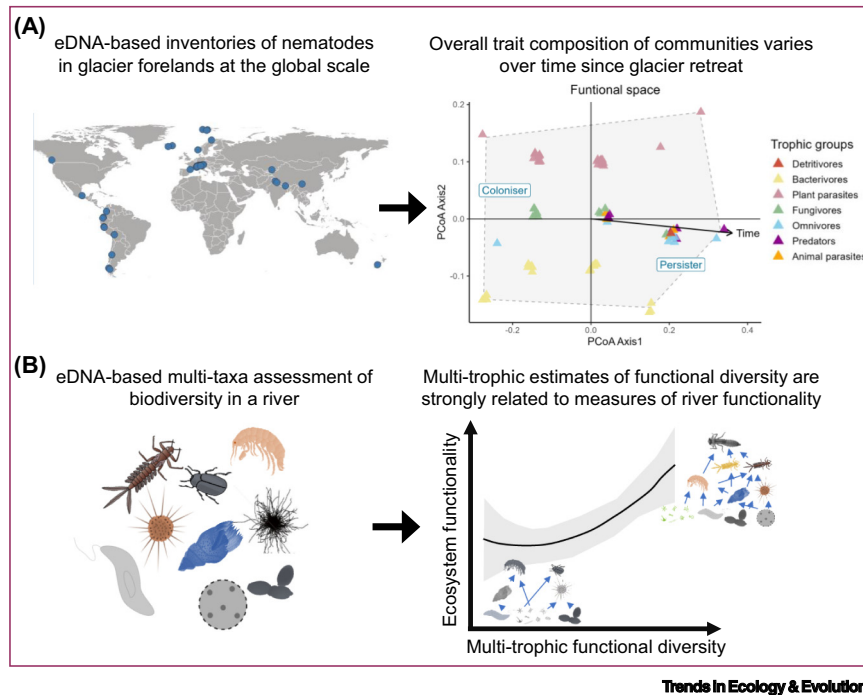
Figure 2. Major advances enabled by the Fun-eDNA approach. Exploiting the full potential of Fun-eDNA allows for the assessment of functional diversity in communities across spatial scales and taxonomic extents that would be challenging with traditional methods. (A) Guerrieri *et al*. [22] used soil eDNA to describe the functional diversity of nematodes in 46 glacier forelands distributed across the world (blue dots). Building a functional space by ordinating nematode taxonomic units (triangles) based on their traits showed a general shift in trait composition over time since glacier retreat. In recently deglaciated sites, 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 feeding habits. If trait information is available, this approach can be extended to other soil taxa communities to assess whole-ecosystem functional diversity. (B) Li *et al*. [23] used eDNA to assess functional diversity across a broad range of taxa (invertebrates, fungi, protists, and bacteria) in a Chinese river and showed that ecosystem functionality increases in sites hosting the communities with highest functional diversity.

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

Since ecosystem functions are outcomes of ecological processes directly or indirectly influenced by organisms and their interactions [28,29], multi-taxa measures of functional diversity can serve as proxies for ecosystem multifunctionality [26,30]. Such measures can reflect key ecosystem

functions, such as decomposition and enzyme activities ([23]; Figure 2B). Specifically, trophic interactions enable the evaluation of how biodiversity affects essential ecosystem functions, including carbon sequestration, nutrient cycling, and pest regulation, as well as overall ecosystem resilience and stability [31,32]. **Food-webs** integrate interactions and energy fluxes occurring at different trophic levels in a given community and alterations in their structure may reflect impacts on ecosystem functions [30]. However, comprehensive reconstructions of actual trophic interactions are complex, requiring the integration of extensive, heterogeneous information [33], as mere co-occurrences of taxa do not imply trophic relationships [34]. Nevertheless, large-scale, multi-taxa inventories obtained through eDNA can be integrated with available trait information, such as feeding habits, size, and protective strategies, to infer the likelihood of trophic interactions [30,31,33]. Employing machine learning or Molecular Ecological Network Analysis [35] can further increase the accuracy of these reconstructions, using observations of real interactions [33,36]. So far, only 11% of the studies using Fun-eDNA considered at least three taxonomic groups (Box 1), often at small spatial scales, and even fewer have attempted to reconstruct food-webs or other interaction networks. Nonetheless, some studies have successfully used Fun-eDNA to identify trophic cascades and trophic niche overlaps [26,35], yielding promising results. We anticipate that food-web reconstructions based on eDNA will gain popularity in coming years.

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

Challenges inherent to eDNA methods
Taxonomic uncertainty. eDNA inventories frequently contain assignments at taxonomic levels coarser than species (e.g., genus or family). This is typically due to low taxonomic resolution of the markers used – common in generalist markers targeting all eukaryotes – and incomplete local reference databases of sequences. Incomplete sequence databases pose significant challenges for broad-scale studies and/or assessments in species-rich ecosystems [37]. Nevertheless, if correctly addressed, these limitations need not preclude robust functional estimates. Recent analyses suggested that taxonomic uncertainty, stemming from incomplete sequence databases, has minimal impact on functional diversity estimates (maximum underestimation compared with actual values of <30%) [38].

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

- Assigning traits only to taxa identified at the species level (e.g., [17]), which is feasible when using comprehensive reference databases and specific markers, because most assignments are at species level.
- Attributing trait values or categories based on a randomly chosen species expected to inhabit the study area within the identified genus or family [18,38].
- Condensing information into higher taxonomic ranks: calculating mean values of all available species within the genus/family for continuous traits (e.g., [22]) or assigning general traits conserved at the genus/family level for categorical traits (e.g., [22]). Traits may also be inferred from the phylogeny [39,40]. These solutions cluster the taxonomic units obtained from eDNA into broader functional groups often closely aligned with taxonomy.

Although these strategies facilitate a functional perspective on communities, the last two points presuppose a phylogenetic signal among species within a given genus or family (i.e., closely

related taxa share traits). This assumption does not always hold, as some traits may have evolved independently and different traits can exist within the same clade. Consequently, these strategies may underestimate functional diversity by reducing functional heterogeneity within clades. To enhance Fun-eDNA accuracy, comprehensive sequence databases should be developed [41,42]. Future research should prioritise sequencing efforts for under-represented taxa and regions, as well as taxa within functionally diverse genera/families or bearing extreme traits [38].

eDNA mostly provides presence/absence information. eDNA primarily provides presence/absence data, complicating the ability to ascertain whether an organism is alive, inactive, or dead [43]. eDNA can also detect the stochastic occurrence of windblown spores of micro-organisms, even though these can have little influence on actual ecosystem dynamics. These limitations pose challenges for testing ecological hypotheses regarding the contribution of species and their traits to ecosystem functioning, particularly when mass ratio effects are expected – that is, when ecosystem functions are primarily determined by the traits of taxa with the highest biomass or abundance [6,44]. Nonetheless, taxa with the highest relative abundance or occurrence in eDNA datasets often are the ones with the highest actual abundance [43,45–48]. Emerging technologies, such as environmental RNA (eRNA, which degrades more quickly in the environment than eDNA) or shotgun sequencing, may help address these limitations. However, further methodological advances are needed for their wider application [24,43,49,50].

Challenges inherent to trait-based approaches

Available trait information is dispersed and not exhaustive. Over the past decades, trait-based research has generated extensive but unconnected and heterogeneous datasets across various contexts, resulting in trait information being scattered across disparate repositories. This makes trait assignment time-consuming and cumbersome. Researchers using the Fun-eDNA approach often pool trait data from various sources (Box 1) [51–53] and have to deal with significant database heterogeneity. In addition, available data tend to be biased toward a limited number of taxonomic groups (e.g., plants, vertebrates), regions (e.g., Europe), as well as toward specific trait types, (e.g., feeding habits) [54], which limits the research questions that can be addressed. Finally, for some groups (e.g., tardigrades, rotifers), knowledge about the biology of the species is scarce. To enhance accessibility, integration, and reuse of trait information, we recommend that future trait measurements adhere to established standards for dataset description and structuring [55–59], while expanding these frameworks to encompass additional taxa, traits, and ecosystems.

Variability in terminology and concepts. The considerable variability in terminology across studies and databases creates semantic inconsistencies, complicating the effective integration of traits [60]. For example, in the reviewed articles (Box 1), terms related to feeding exhibited considerable variation ('nutrition', 'diet', 'food acquisition', 'trophy', 'consumption'). Combining traits from very different organisms exacerbates semantic inconsistencies, as preferred terminology differs across taxonomic groups, and can pose conceptual challenges. Indeed, the same trait can exert different functional effects on the ecosystem, depending on the taxon, its specific characteristics, scales of action, and interactions within its respective ecosystems. For instance, for microbes, the term 'predation' can refer to phagotrophic protists grazing on bacteria, thereby regulating bacterial populations and local nutrient cycling with rapid and localised effects. The ecological effects of 'predation' performed by large vertebrates are not fully comparable with that of microbes. Vertebrate predation involves complex behavioural mechanisms and promotes cascading effects that can persist for long periods, as these animals occur over broad spatial scales and often show long generation times [61].

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

Challenges to integrate eDNA with trait-based approaches

Assigning traits to eDNA-based inventories is not straightforward, as traits are collected independently from eDNA data and are described at the species or even individual level. Consequently, trait assignment to inventories is arduous. Trait databases coupling sequencing and detailed taxonomic information with specific guidelines have been recently developed to facilitate and accelerate the functional annotation of eDNA-based inventories [55,64]. Researchers should consistently upload the assembled trait databases in a freely accessible way with detailed taxonomic and sequencing information (Figure 1). Additionally, assigned trait values can derive from individuals inhabiting other habitats and thus likely subjected to very different environmental conditions than those studied. This can overlook local adaptation and intraspecific variation across sites, seasons, and/or life stages [6,43], which are crucial aspects shaping species interactions and the ecosystem's ability to cope with environmental changes [65,66]. A growing number of studies are exploring eDNA applications to describe within-species genetic variation from short markers [67,68], potentially enabling teasing apart lineages with functional differences [69]. However, eDNA samples often contain degraded DNA in low quantities, which can lead to erroneous sequence variants, complicating population genetic analyses [70]. Moreover, not all markers are suitable for population analyses or may not reflect variability in phenotypic traits. Combining eDNA sampling across several sites with a stratified *in situ* collection of specimens at a strategically chosen subset of the sampling sites (covering key environmental conditions) is an optimal strategy to efficiently measure fine-scale trait variation and better represent local trait measures [54].

Concluding remarks

The expansion of eDNA analyses is revolutionising biodiversity inventories, offering unparalleled opportunities for the comprehensive exploration of whole-ecosystem biodiversity across wide spatio-temporal scales. By integrating traits with eDNA-based inventories of a broad spectrum of taxa, we can link community ecology with functional ecology. This opens up accessible avenues to deepen our understanding of the relationships between biodiversity and ecosystem functions by providing proxies for trophic interactions and multifunctionality. To achieve a consistent and unified view of multiple taxonomic groups, methodological developments including sequencing prioritisation, standards for describing and structuring trait datasets, *in situ* specimen collection, along with conceptual integration, and interdisciplinary collaborations are essential (see Outstanding questions). Furthermore, to achieve a mechanistic understanding of the links between biodiversity and ecosystem function, the functional measures of communities obtained with Fun-eDNA (e.g., multi-taxa measures, food-webs, synchronous responses) need to be related with explicit assessments of ecosystem functions (e.g., decomposition, productivity) [23,27]. Future work integrating the competencies of taxonomists, field biologists, ecosystem ecologists, and molecular biologists is expected to improve the effectiveness of eDNA-based analyses of functional diversity, providing more complete information that will help addressing the challenges of global change.

Outstanding questions

The use of available trait databases (online and/or from the literature) can greatly speed up analyses of traits and functions across broad spatial scales, but overlooks intraspecific variation of traits and local adaptation. To what extent does this determine loss of information?

How do we best tailor trait selection to obtain pertinent traits for the Fun-eDNA approach? Pertinent traits are those that can be assigned to eDNA-based inventories, and their selection must align with the specific purpose of the research. For instance, if the research question involves the response to changing environmental drivers, the pertinent traits would be response traits linked to those drivers. Alternatively, if the focus is on the links between biodiversity and ecosystem functions, effect traits should be selected. For the latter, the choice of traits will depend on the specific function of interest (e.g., if the aim is to assess trophic interactions or carbon fluxes).

Can we detect the interdependence of traits between interacting groups to provide a more mechanistic understanding of synergies between traits involved in a given interaction? For instance, coupling data on effect traits of predators and response traits of their prey might allow identifying functional linkages of their interactions.

How can the coordination of disciplines and experts be achieved to reach a harmonised terminology for the traits of all the taxa inhabiting the same environment? This is pivotal to develop trait-based frameworks to synthesise the whole-ecosystem functional variation across diverse taxa.

Declaration of interests

The authors declare no competing interests.

Supplemental information

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