Towards exhaustive community ecology via DNA metabarcoding

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Abstract

Exhaustive biodiversity data, covering all the taxa in an environment, would be fundamental to understand how global changes influence organisms living at different trophic levels, and to evaluate impacts on interspecific interactions. Molecular approaches such as DNA metabarcoding are boosting our ability to perform biodiversity inventories. Nevertheless, even though a few studies have recently attempted exhaustive reconstructions of communities, holistic assessments remain rare. The majority of metabarcoding studies published in the last years used just one or two markers and analyzed a limited number of taxonomic groups. Here we provide an overview of emerging approaches that can allow all-taxa biological inventories. Exhaustive biodiversity assessments can be attempted by combining a large number of specific primers, by exploiting the power of universal primers, or by combining specific and universal primers to obtain good information on key taxa while limiting the overlooked biodiversity. Multiplexes of primers, shotgun sequencing and capture enrichment may provide a better coverage of biodiversity compared to standard metabarcoding, but still require major methodological advances. We identify the strengths and limitations of different approaches, and suggest new development lines that might improve broad scale biodiversity analyses in the near future. More holistic reconstructions of ecological communities can greatly increase the value of metabarcoding studies, improving understanding of the consequences of ongoing environmental changes on the multiple components of biodiversity.

Key words:

Environmental DNA; shotgun sequencing; multi-trophic analyses; primer cocktails

1. INTRODUCTION

An exhaustive assessment of biodiversity has always been a major challenge for community ecologists. In principle, all the organisms can have key roles in the ecosystems where they live and can interact with each other: some insects and mammals feed on plants, plants interact with soil fungi, protists can feed on bacteria or parasitize other eukaryotes, and of course many other interactions occur. Ideally, ecologists should assess the occurrence (and perhaps the abundance) of all the organisms, if they want to unravel the multifaceted impact of environmental changes on biodiversity, eventually taking into account the potential biotic interactions (Urban et al., 2016). Unfortunately, this is only rarely possible. By using traditional approaches (e.g. morphological identification of species), thousands systematists would need to work together for months to produce an "all-taxa biological inventory" of just a hectare of tropical forest (Lawton et al., 1998). Molecular approaches (starting with DNA barcoding) have revolutionized biodiversity inventories, as they allow a much faster and cheaper assessment of species occurrence, and are particularly efficient for taxonomic groups including many difficult to identify, cryptic or undescribed taxa (Floyd, Abebe, Papert, & Blaxter, 2002; Hebert, Cywinska, Ball, & DeWaard, 2003; Hebert, Penton, Burns, Janzen, & Hallwachs, 2004). DNA metabarcoding now allows the contemporary assessment of a huge number of species, starting from both environmental DNA (eDNA) and bulk samples (also named whole organism community DNA; Pawlowski, Apothéloz-Perret-Gentil, & Altermatt, 2020). Does this mean that ecologists are finally able to assess the whole community, targeting the different trophic levels?

In recent years, some studies that have successfully applied and integrated multiple markers to broadly assess biodiversity, highlight that exhaustive reconstructions of communities can be possible (Table 1). Nevertheless, holistic ecosystem assessments are not as widespread as they could be. The scarcity of studies targeting the whole community might be related to technical limitations, to the lack of conceptual frameworks, or might arise because the usefulness of such approaches is not fully appreciated by molecular ecologists. In this contribution, we first perform a quantitative assessment of the recent studies that applied metabarcoding for biodiversity assessments. This allowed us to *i*) evaluate how frequently researchers attempted the joint analysis of multiple taxonomic groups for an exhaustive assessment of biodiversity, and *ii*) to identify the used approaches. Subsequently, in order to operationalize and scale up these approaches, *iii*) we describe some new avenues that may be adopted to obtain detailed information over the broadest spectrum of taxa, and to attempt a nearly-complete reconstruction of communities on the basis of the metabarcoding of both eDNA and bulk samples. By discussing the strengths and limitations of some of these approaches, *iv*) we also propose new development lines that might improve the taxonomic breadth of biodiversity analyses, and we hope to encourage a growth of studies targeting holistic reconstructions of biodiversity.

2. HOW FREQUENT IS THE HOLISTIC ANALYSIS OF COMMUNITIES USING METABARCODING? AN ANALYSIS OF THE LITERATURE

2.1 Methods

In order to assess the number and typology of markers used in recent DNA metabarcoding studies, we performed a search on the ISI web of science the 1st of September 2022, using the search terms "DNA metabarcoding", limiting search to research articles published in 2021-2022. The search returned 978 papers. We restricted our search to nine representative journals. We considered: the three journals publishing the largest number of non-methodological papers on the topic (*Scientific Reports*, *Molecular Ecology*, *Ecology and Evolution*); four high-impact factor journals (*Nature Communications*, *Science Advances*, *Nature Ecology and Evolution* and *Proceedings of the National Academy of Sciences USA*) and two of the most popular open-access journals (*PLoS One* and *PeerJ*) The journal *Environmental DNA* was not considered because, in September 2022, it was not indexed in the Web of Science. Overall, we obtained 212 papers (Supplementary Table S1a). We screened the abstracts and retained papers analyzing biodiversity variation in different areas, scales and organisms. We excluded strictly methodological papers (e.g. testing the performance of primers), reviews and meta-analyses, and papers focusing on intra-specific evolutionary patterns. After a detailed screening, we also excluded diet studies, and papers on symbionts or parasites (overall, 72 studies evaluated; see Table S1a) because none of them attempted exhaustive reconstruction of communities, and they used primers focusing on the taxa assumed to be the diet or the symbionts of target organisms (but see also Weber et al., 2023).

For all the papers focusing on biodiversity assessment, we recorded: 1) the number and identity of taxonomic groups analyzed; 2) the number and identity of primers used for DNA amplification; 3) whether the study used universal or specific primers. For the sake of simplicity, universal primers were defined as the ones amplifying an entire domain of life, a kingdom, or multiple distantly related phyla. Specific primers were the ones amplifying a superphylum (e.g. Spermatophyta), a phylum, or finer taxonomic groups. We then used generalized linear mixed models with truncated Poisson error distribution (glmmTMB R package; Brooks et al., 2017) to test whether the number of analyzed taxa was related to the impact factor of journals; journal identity was included as a random factor. The complete list of screened papers, and the features of papers assessing biodiversity, are available in Supplementary Tables S1a-b.

2.2 Frequency of holistic community reconstructions in recent literature: results

Overall, we retained 85 papers using different DNA metabarcoding approaches for biodiversity reconstructions across nine journals during the last two years. The majority of studies (89%) used

just one or two primer pairs and focused on just one (e.g. arthropods, fish, fungi, plants) or two taxa (e.g. plants + mammals; bacteria + micro-eukaryotes; Supplementary Table S1b; Fig. 1a-b). Several studies had a broad taxonomic scope and used universal primers (particularly focusing on COI and 18S) to amplify very broad groups (e.g. all the eukaryotes, all the animals). Conversely, very few studies attempted an exhaustive biodiversity analysis combining multiple primer pairs each of which targets a different taxon (Fig. 1b). Papers published in journals with higher impact factor tended to analyze a larger number of taxa (mixed model: $Z = 3.619$, $P = 0.0003$; Fig. 1c).

3. POTENTIAL STRATEGIES FOR EXAUSTIVE BIODIVERSITY ANALYSES USING MOLECULAR APPROACHES

Although attempts of holistic community reconstruction remain rare, several approaches are already available to address this challenge. Each has its strengths and limitations (Table 1), but ongoing technical and/or conceptual developments may promote their application in the near future.

3.1 Using many markers in the same study

A very large number of primers has been developed and tested for metabarcoding studies. For instance, Taberlet, Bonin, Zinger, and Coissac (2018) proposed 62 distinct primer pairs for DNA metabarcoding, some of which were extremely versatile and amplified very broad taxa (e.g. all the bacteria and archaea; all the eukaryotes) and others being much more specific, focusing on well-defined taxa (e.g. turtles or the plant family Asteraceae). In principle, we can amplify the DNA extracted from one single environmental or bulk sample using multiple primers, and then combine the results to attempt an overall reconstruction of biodiversity (Jurburg, Keil, Singh, & Chase, 2021). For example, we might study the majority of soil biodiversity by analyzing markers specific for bacteria, fungi, earthworms, insects and springtails, while a large portion of freshwater diversity can be assessed by combining primers that amplify bacteria, protists, insects, fishes and amphibians (Bloor, Si-Moussi, Taberlet, Carrère, & Hedde, 2021; Guerrieri et al., 2022; F. Li, Qin, Wang, Zhang, & Yang, 2023).

Combining multiple markers allows a good resolution for the selected focal taxa, particularly if each marker has a well-defined and limited taxonomic scope. The integration of results of different primers can allow assessing the response of multiple taxa to environmental gradients, and even attempting the reconstruction of interaction networks (F. Li et al., 2023).

Unfortunately, using many markers considerably increases the cost and labor associated with the laboratory and sequencing. Furthermore, even if unlimited resources were available (which is

rarely the case), the amount of DNA available for amplification remains limited. Let us assume that 100 μL of eDNA have been extracted from water, each PCR reaction requires 2 μL of template DNA, and the experimental plan requires running eight replicated PCRs per sample to detect rare species with a limited rate of false negatives (Ficetola et al., 2015). In this case, the template DNA is only enough for a maximum of six primers, thus some key taxon will always be missed. For instance, if freshwater biodiversity is analyzed using primers amplifying bacteria, diatoms, mollusks, insects, fishes and amphibians, key taxa such as crustaceans and most micro-eukaryotes will remain undetected. Furthermore, integrating the results of multiple markers to obtain a coherent, homogeneous species lists can be challenging (Bonin, Guerrieri, & Ficetola, 2023; Jurburg et al., 2021; see section 3.3)

3.2 Using universal or degenerate primers

In principle, researchers might choose a few universal primers, such as the ones targeting all the eukaryotes or most of the animals (e.g. 18S rDNA or COI-based primers). Several studies have adopted this approach with both environmental and bulk DNA (Fig. 1b); its advantages include relatively easy implementation and cheap cost (see Jurburg et al., 2021 for additional discussions on limitations and recommendations). In principle, with two pairs of primers (e.g. one eukaryote and one prokaryote marker) we might try amplifying the whole tree of life (e.g. Holman et al., 2021; Martinez-Almoyna et al., 2019). Unfortunately, the search for perfect, truly universal primers has been compared to the search for the Holy Grail (Rubinoff, Cameron, & Will, 2006). On the one hand, some universal primers have limited taxonomic resolution, or have heterogeneous resolution across the three of life. For instance, some primer pairs focusing on 18S (e.g. the Euka02 primer pair, Guardiola et al., 2015) amplify most eukaryotes and have a reasonable resolution for some taxonomic groups (e.g. nematodes), but their resolution is poor for other taxa (e.g. plants), with complex consequences for data analyses (Jurburg et al., 2021). On the other hand, universal primers such as those amplifying COI have heterogeneous amplification rate among the target species. The taxa with less mismatches or with more C/G will be amplified preferentially, and this can reduce the success over other taxa. Highly degenerate primers show additional issues such as frequent amplification of non-target regions and / or non-target taxa (e.g. bacterial DNA amplified with COI primers) (Hintikka, Carlsson, & Carlsson, 2022).

Recently, long-read metabarcoding has been proposed to overcome the limited resolution of many generalist primers (Jamy et al., 2022; Krehenwinkel et al., 2019). With this approach, a very long (e.g. 4500 bp) DNA fragment is amplified with universal primers and then processed through technologies that allow the sequencing of long reads (Jamy et al., 2022). Long-read metabarcoding shows great promise for the recovery of multiple SNPs, and can thus provide unprecedented taxonomic resolution compared to traditional short-read metabarcoding. However, it is still subject to major technical issues (e.g. chimera formation, limited predictability of amplification) and is

more expensive than short-read metabarcoding, even though recent advances (e.g. nanopore technology) are dramatically reducing the associated costs (Krehenwinkel et al., 2019). Furthermore, long metabarcodes pose major methodological challenges, do not always improve resolution, and several aspects of this approach will deserve future adjustments, including the actual universality of primers (Leese et al., 2021; Yeo, Srivathsan, & Meier, 2020).

3.3 Combining universal and more specific primers

In order to overcome the limitations of strategies 3.1 and 3.2, it is possible to analyze the same DNA using both specific primers targeting taxa with particular ecological role (e.g. taxa with taxonomic diversity or with a major functional role), and universal primers. For instance, for the analysis of soil biodiversity it is possible to complement primers amplifying insects, springtails, earthworms and fungi, with a primer that amplifies all the eukaryotes and can give an idea of the diversity of groups not amplified with the previous ones (e.g. micro-eukaryotes, nematodes, rotifers) (Bloor et al., 2021; Calderón-Sanou et al., 2022; Guerrieri et al., 2022). This approach has the advantage of providing a reasonable representation of biodiversity, with good information on selected key taxa and few completely missing, and might thus allow exploring complex relationships between multiple taxonomic groups (Bloor et al., 2021; Calderón-Sanou et al., 2022). Nevertheless, similarly to approach 3.1, it remains costly and labor-intensive.

Furthermore, with this approach the resolution of markers can be highly heterogeneous among taxa amplified by specific and universal primers. For instance, the above-cited combination of primers would provide an excellent taxonomic resolution for earthworms and springtails, but a very coarse one for other taxa (e.g. rotifers). Combining taxonomic tables with very different resolution in ecological analyses can be extremely complex, and comparing the biodiversity (e.g. taxonomic richness) of groups amplified with different markers is certainly problematic. When multiple primers amplify the same taxonomic group (e.g. a universal and a specific marker, but also two universal markers), possible approaches include retaining the information from the marker producing the largest number of taxonomic units (S. Arnaud-Haond, personal communication), or of all the taxonomic units identified by at least one marker. Even if some analytical strategies can help fine-tuning bioinformatics treatments and combining information from disparate groups (Bonin et al., 2023; Jurburg et al., 2021), understanding the potential drawbacks of such integrated datasets remains a major methodological challenge.

3.4 Multiplex of primers

An alternative approach is combining multiple metabarcoding primers in the same PCR mix, to simultaneously amplify and sequence multiple taxonomic groups. So far, primer cocktails have

been rarely used, but might provide extremely comprehensive information on biodiversity (Govender, Singh, Groeneveld, Pillay, & Willows-Munro, 2022; Kennedy et al., 2022). For instance, Govender et al. (2022) used six primer cocktails, each amplifying a different fragment of the COI-5P gene region, to explore the diversity of marine zooplankton. By combining primers optimized for different phyla, they were able to characterize at high resolution the diversity of the major taxonomic groups, including fish, crustaceans, echinoderms, mollusks, cnidarians and more. Govender et al. (2022) included up to four different reverse primers within the same PCR reaction, all targeting the same DNA fragment. Nevertheless, in principle an even larger number of primers could be combined, to maximize the number of taxa that are amplified at high resolution, and the multiplex might include primers targeting different genomic regions, if they have comparable performance (see below). Such multiplexes including a large number of markers might boost the number of taxa amplified at high resolution, efficiently exploiting the available template DNA while limiting costs.

Nevertheless, this approach remains poorly explored and needs major methodological developments. Primers often show strong differences in amplification efficiency, and DNA concentration can be extremely different across taxa. In standard PCRs, this is taken into account by tuning key parameters (e.g. number of cycles), but in a multiplex all the primers undergo the same number of cycles, therefore the mix should ideally include primers with comparable amplification performance, and targeting taxa with similar DNA concentration. Preliminary analyses can assess the similarity of primers, for instance checking via qPCR if they show analogous amplification patterns under the same conditions. Alternatively, multiplexes including markers with different efficiency and / or abundance of template DNA can be optimized by increasing the concentration of the primers with lower performance. Furthermore, designing a multiplex requires the identification of primers with similar annealing temperatures, but amplifying complementary groups. Specific bioinformatics tools have boosted our ability to identify the most appropriate metabarcoding primers (Riaz et al., 2011), but designing a multiplex will certainly need further developments for both bioinformatics and wet lab. Finally, current popular bioinformatics pipelines are optimized to process one marker at a time, and specific developments can be required to retrieve information from multiple metabarcodes from the same study (Porter & Hajibabaei, 2022).

3.5 Shotgun sequencing and capture enrichment

Shotgun sequencing and other metagenomics approaches can extract large amounts of information from eDNA, and potentially allow the reconstruction of the whole community, without targeting a specific group (Gusareva et al., 2019; Parducci et al., 2019; Pedersen et al., 2016; Wang et al., 2021). In principle, the shotgun sequencing approach should bypass the DNA barcode amplification bias, might allow the use of the whole DNA available in the environment, providing

information on all the trophic layers, and can help to estimate the relative abundance of taxa (Garrido-Sanz, Senar, & Piñol, 2022; Gusareva et al., 2019; Parducci et al., 2017), thus overcoming many of the limitations associated to DNA metabarcoding. Nevertheless, several issues continue to limit the broad-scale application of this approach compared to the more standard metabarcoding. First, shotgun sequencing is much more expensive than PCR-based metabarcoding, and the associated bioinformatics pipelines remain complex. Furthermore, to maximize the utility, taxonomic identification should use data across the genome. Unfortunately, so far genomic information outside the barcode regions is mostly limited to vertebrates, some plants (Alsos et al., 2020; Garcés-Pastor et al., 2022), and commercially important species. As a consequence, evidences of the advantage of shotgun sequencing over PCR-based metabarcoding for broad-scale community analyses remain mixed, so far (Bell et al., 2021; Murchie et al., 2020; Parducci et al., 2019; Paula et al., 2022). Despite these issues, the continuing advances of sequencing and bioinformatics technologies suggest that shotgun metagenomics will play an increasingly important role for whole-community analyses, particularly for topical study systems such as ancient eDNA (Pedersen et al., 2016; Wang et al., 2021).

Capture enrichment from next generation sequencing libraries followed by shotgun sequencing has already been implemented to improve the efficiency of direct shotgun sequencing, boosting the retrieved taxonomic information (Murchie et al., 2020). Capture enrichment represents an alternative to PCR-based metabarcoding, and has proven to be very effective for the study of ancient eDNA, for instance to understand temporal changes of the distribution of extinct hominids and large mammals (Slon et al., 2017; Zavala et al., 2021), and allowed reconstructing plant and mammal communities in the Arctic with better performance than both traditional metabarcoding and shotgun sequencing (Murchie et al., 2020). Experiments have also been conducted on modern DNA to identify, for example, fish communities in a tropical river or plants (Mariac et al., 2014; Mariac et al., 2018). So far we are not aware of studies using capture enrichment to address the whole community present in an environment. Nevertheless, one could imagine enrichment in taxonomically informative DNA molecules by designing probes based on highly conserved regions of ribosomal RNAs, and analyzing the more variable flanking regions to retrieve taxonomic information. Of course, as with the shotgun sequencing approach, reference databases constructed from genome skimming are required for both the definition of probes and for identification (Coissac, Hollingsworth, Lavergne, & Taberlet, 2016; Garcés-Pastor et al., 2022).

3.6 Additional issues of using DNA metabarcoding for complete reconstructions of communities

Despite multiple approaches becoming available, all of them share additional issues that must be taken into account for robust assessments of communities. A detailed review of the many technical

aspects of metabarcoding-based assessment of biodiversity is beyond the aim of this work (see e.g. Chen & Ficetola, 2020; Graham, Gillespie, & Krehenwinkel, 2021; Jurburg et al., 2021; Piper et al., 2019; Rodríguez-Ezpeleta et al., 2021; Taberlet et al., 2018; Tedersoo et al., 2022; Zinger et al., 2019 for reviews and discussions), but some of them deserve special attention when the aim is holistic reconstruction of communities.

Abundance data are essential for understanding community dynamics and functioning, but obtaining abundance information from metabarcoding data remains challenging. Within a given group (e.g. fish, J. Li et al., 2019; plants, Pansu et al., 2015), relative abundance can sometimes be estimated from relative abundance of reads, but even within a taxon several factors affect estimates of relative abundance, such as differences in the number of gene copy per cell, or in primer matching (Jurburg et al., 2021; Zinger et al., 2019). These issues are expected to be exacerbated when very different taxa are analyzed in the same study, thus *a priori* calibration (e.g. using mock communities or internal standard DNAs, Garrido-Sanz et al., 2022; Ushio et al., 2018) and the application of analytical frameworks enabling the correction of biases are extremely important (McLaren, Willis, & Callahan, 2019).

Reproducibility is an additional issue. Rare taxa often show limited reproducibility, thus multiple technical and / or biological replicates are needed to assess their occurrence (Stauffer et al., 2021; Zinger et al., 2019). This is particularly problematic when the aim is an exhaustive community assessment, as strong variation in abundance, amplification success and detectability across taxa can lead some taxonomic groups to be inconsistently detected. Appropriate replication levels, and analytical tools taking into account the issues of imperfect detection and MOTU inflation (e.g. pseudogenes, chimera removal), are pivotal to limit the impacts of such biases on ecological conclusions (Alberdi, Aizpurua, Gilbert, & Bohmann, 2018; Graham et al., 2021; Zinger et al., 2019).

So far, strong efforts have been devoted to the development of databases for standard barcodes, but just one or a few barcodes are unlikely to be enough to enable the characterization of the whole community. New, more complete reference databases can be generated using high-throughput sequencing approaches (e.g. genome skimming; Coissac et al., 2016). Genome skimming would allow covering broad sections of the genome (i.e. organelle(s) and nuclear ribosomal DNA), can be useful for all the above-described approaches, and might even serve as starting point for the identification of new markers (Coissac et al., 2016; Garcés-Pastor et al., 2022). An additional issue is related to the completeness of databases, which is highly variable across taxa and geographic areas (Weigand et al., 2019). Despite ongoing efforts, filling the gaps in reference libraries for the diverse components of the tree of life remains a key challenge for the next years.

4. CONCLUSION: OPPORTUNITIES FOR AN EXHAUSTIVE COMMUNITY ECOLOGY USING METABARCODING

One decade of advances on DNA metabarcoding has fostered our ability to obtain biodiversity data, filling long-standing gaps on many components of both terrestrial and aquatic environments. However, so far just a few studies have taken the challenge of covering a broad range of taxonomic groups, or even trying to identify the complex multi-trophic interactions between them (but see Bloor et al., 2021; Calderón-Sanou et al., 2021; Calderón-Sanou et al., 2022; Martinez-Almoyna et al., 2019). We believe that a broader application of holistic community studies will greatly improve our understanding of patterns and processes underlying biodiversity variation. Studies attempting exhaustive reconstructions are more frequent in high-profile journals, suggesting that the research community already recognizes their value to answer long-standing ecological questions. Meeting the challenge of holistic community ecology can greatly increase the value of metabarcoding studies without excessive increase of costs and laboratory burden, as costs and labor are not expected to grow quickly with the number of analyzed taxa (Bálint et al., 2018). Approaches such as the combination of universal and specific primers, or the multiplexes of primers are particularly promising. Nevertheless, both technical and conceptual developments will be required for a more widespread application of the exhaustive community ecology, and some challenges are shared by most approaches.

It is now clear that ongoing global changes determine very intricate effects on organisms and communities. Disparate taxonomic groups can show contrasting responses to climate change and other stressors, and the decline of one taxonomic group can determine dramatic modifications to the whole network of biotic interactions (Fricke et al., 2022). Predicting a species response while ignoring interactions with its predators, food sources or pathogens can thus lead to highly biased results (Sirén, Sutherland, Karmalkar, Duveneck, & Morelli, 2022; Urban et al., 2016). As a consequence, we increasingly need well-resolved information covering the different trophic levels in a community and their manifold interactions (Gilman, Urban, Tewksbury, Gilchrist, & Holt, 2010; Urban et al., 2016). Nonetheless, just obtaining the list of taxa living in a specific environment provides little insights on how they interact, and analyses of biotic interactions involving a large number of taxa remain extremely challenging. Besides species occurrences, metabarcoding studies can provide direct information on species interactions, for instance through the analysis of diet, parasites and the host-associated microbiota (Alberdi et al., 2019; Bass, Stentiford, Littlewood, & Hartikainen, 2015; Ravindran, 2019; Roslin & Majaneva, 2016; Taberlet et al., 2018; Weber et al., 2023), but direct observations of interaction can only focus on a few taxa, and are not enough to reconstruct what happens across all the trophic levels. In the last years, novel frameworks have been proposed for the multi-trophic and multi-taxa analysis of communities in absence of direct observation of interactions, on the basis of species traits, phylogenetic information and machine learning algorithms (Fricke et al., 2022; Gravel et al., 2019), even though a lot of work remains to be done to assess their power, strengths and limitations

(Burian et al., 2021; D'Amen, Mod, Gotelli, & Guisan, 2018; Fricke et al., 2022; Gravel et al., 2019).

Better assessing the impact of global changes on biodiversity requires increasingly complete data covering the multiple components of ecosystems (Urban et al., 2016). DNA metabarcoding can greatly contribute to such endeavors, and we hope that methodological and conceptual advances, allowing a more holistic approach to community ecology, will remain an active research area in the near future.

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DATA ACCESSIBILITY

All the relevant data are provided as supplementary material (Table S1).

AUTHOR CONTRIBUTIONS

The two authors jointly designed the study. GFF wrote the first draft of the manuscript, with substantial contribution from PT.

SUPPORTING INFORMATION

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

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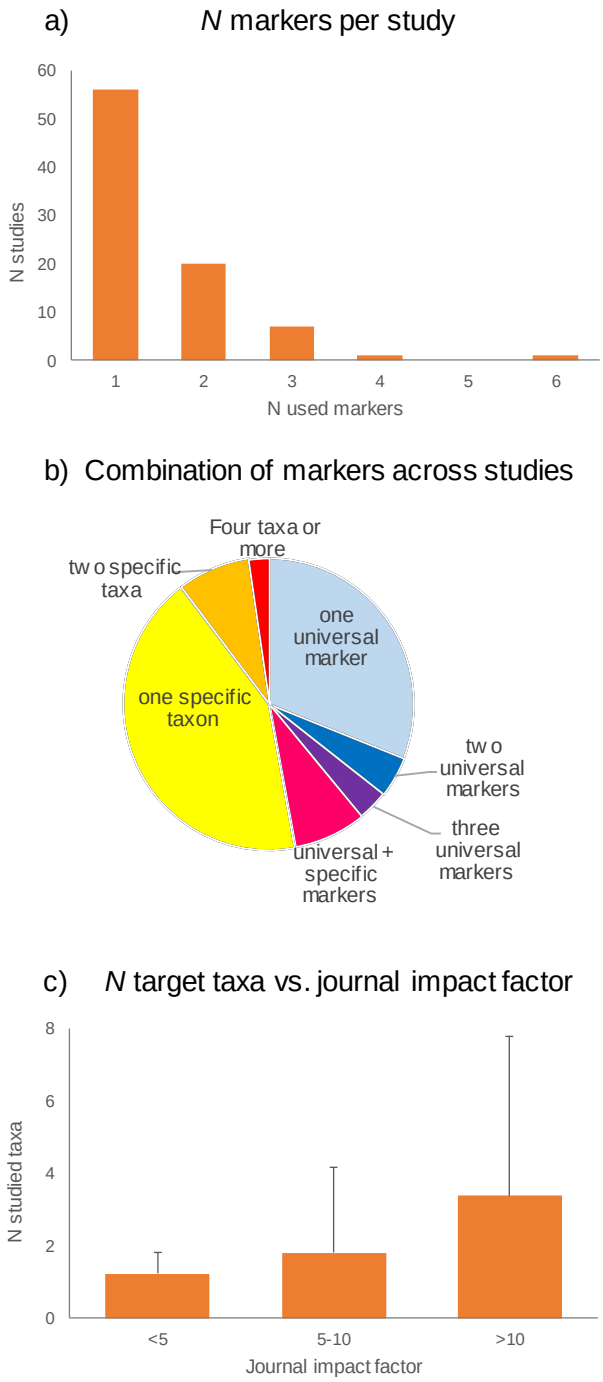

Figure 1. Number and typologies of markers analyzed in papers published during 2021-22 in nine representative scientific journals, using DNA metabarcoding to analyze biodiversity variation. "Universal markers" are markers targeting multiple distantly related phyla and / or an entire domain of life, while studies focusing on "specific taxa" focus on a given taxonomic group (phylum, superphylum or smaller). Note that some studies targeted a specific taxon (e.g. fish), but used more than one marker to improve coverage. In C, error bars represent standard deviation. The complete list of papers is provided in the Supplementary Table S1.

Table 1. Summary of approaches for all-inclusive community ecology, with examples of their strengths and limitations.

Approach	example	pros	cons
Combining many metabarcodes in the same study	F. Li et al. (2023) Analyzed freshwater biodiversity using four primers, focusing on bacteria; micro-eukaryotes; insects and fish	Good coverage of biodiversity Resolution can be high for the selected taxa	Costly Some taxon will always be missing
Universal markers	Holman et al. (2021) performed a joint biogeographical analysis of marine animals, protists and bacteria	Relatively cheap In principle, might cover the whole tree of life	Amplification rate and resolution are often heterogeneous across taxa
Combining universal and specific metabarcodes	Bloor et al. (2021) combined three universal (bacteria, eukaryotes, fungi) and four specific (seed plants, insects, springtails and earthworms) markers for a multi-trophic analysis of soil diversity	Good information on key groups Reduces the number of unrepresented taxa	Costly Resolution can be strongly heterogeneous across taxa
Multiplex of primers	Govender et al. (2022) used six primer cocktails to analyze the diversity of 14 zooplankton taxa	Potentially excellent resolution Potentially excellent coverage of the tree of life Cheaper than analyzing each taxon separately	Methodological developments required to optimize the multiplex Bioinformatics challenges
Shotgun sequencing	Pedersen et al. (2016) used ancient DNA to reconstruct post-glacial colonization patterns of plants, mammals and fish	Bypasses many limitations of metabarcoding (amplification, abundance) Can exploit the whole genomic DNA Can cover the whole tree of life Allows authentication of ancient eDNA	Assignation heavily depends on reference databases Very costly Complex analytical pipelines
Capture enrichment	Murchie et al. (2020) used targeted capture of ancient environmental DNA for the reconstruction of plant and animal communities living in Yukon between the Pleistocene and the Holocene	Better performance than traditional metabarcoding Allows authentication of ancient eDNA Bypasses several limitations of metabarcoding	So far, limited attempts of exhaustive community reconstruction using capture Requires very high-quality reference databases Requires the design of probes