CHAPTER NINE

Advances and prospects of environmental DNA in neotropical rainforests

Lucie Zinger[a,*], Julian Donald[b], Sébastien Brosse[b], Mailyn Adriana Gonzalez[c], Amaia Iribar[b], Céline Leroy[d,e], Jérôme Murienne[b], Jérôme Orivel[e], Heidy Schimann[e], Pierre Taberlet[f,g], Carla Martins Lopes[h]

[a]Ecole Normale Supérieure, PSL Research University, CNRS, Inserm, Institut de Biologie de l'Ecole Normale Supérieure (IBENS), Paris, France
[b]Evolution et Diversité Biologique (EDB), Université Toulouse 3 Paul Sabatier, CNRS, IRD, UMR 5174, Toulouse, France
[c]Programa Ciencias de la Biodiversidad, Instituto de Investigación de Recursos Biológicos Alexander von Humboldt. Bogotá, Colombia
[d]AMAP, Univ Montpellier, CIRAD, CNRS, INRAE, IRD, Montpellier, France
[e]INRAE, CNRS, AgroParisTech, CIRAD, Université de Guyane, Université des Antilles, UMR Ecologie des Forêts de Guyane (EcoFoG), Campus agronomique, Kourou, France
[f]Laboratoire d'Ecologie Alpine (LECA), CNRS, Université Grenoble Alpes, Grenoble, France
[g]UiT—The Arctic University of Norway, Tromsø Museum, Tromsø, Norway
[h]Departamento de Zoologia, Instituto de Biociências, Universidade Estadual Paulista (UNESP), Rio Claro, SP, Brazil
[*]Corresponding author: e-mail address: lucie@zinger.fr

Contents

Advances in Ecological Research, Volume 62
ISSN 0065-2504
https://doi.org/10.1016/bs.aecr.2020.01.001

Abstract

The rainforests of the Neotropics shelter a vast diversity of plant, animal and microscopic species that provide critical ecosystem goods and services for both local and worldwide populations. These environments face a major crisis due to increased deforestation, pollution, and climate change, emphasizing the need for more effective conservation efforts. The adequate monitoring of these ecosystems has proven a complex and time consuming endeavour, which depends on ever dwindling taxonomic expertise. To date, many species remain undiscovered, let alone described, with otherwise limited information regarding known species population distributions and densities. Overcoming these knowledge shortfalls and practical limitations is becoming increasingly possible through techniques based on environmental DNA (eDNA), i.e., DNA that can be obtained from environmental samples (e.g. tissues, soil, sediment, water, etc.). When coupled with high-throughput sequencing, these techniques now enable realistic, cost-effective, and standardisable biodiversity assessments. This opens up enormous opportunities for advancing our understanding of complex and species-rich tropical communities, but also in facilitating large-scale biomonitoring programs in the neotropics. In this review, we provide a brief introduction to eDNA methods, and an overview of their current and potential uses in both terrestrial and aquatic ecosystems of neotropical rainforests. We also discuss the limits and challenges of these methods for our understanding and monitoring of biodiversity, as well as future research and applied perspectives of these techniques in neotropical rainforests, and beyond.

1. Introduction

Faced with the current environmental crisis, there is an ever growing need to accurately assess existing policy and legislation which aims to protect ecosystems, such as the Paris Climate Agreement, the REDD + framework, and the Aichi targets (Marques et al., 2014), as exemplified by the IPBES framework (Díaz et al., 2019). This is particularly true for the neotropical moist broadleaf forests, i.e., those occurring from southern Mexico and Florida to Argentina (Morrone, 2014; Olson et al., 2001). Of these forests, the rainforests occurring across Amazonia are the most substantial, covering 40% of the region, and representing the primary source of biodiversity across most taxa (Antonelli et al., 2018b; Jenkins et al., 2013; Olson et al., 2001).

The biodiversity of neotropical rainforests provides critical ecosystem goods and services for both local and worldwide populations (Chaplin-Kramer et al., 2019; Rice et al., 2018), but these are threatened by increasing human pressures. The region has experienced a 10-fold increase in population densities over the past few decades (Tritsch and Le Tourneau, 2016), coupled with a drastic increase in human activities such as deforestation,

agricultural expansion, mining and infrastructure construction (e.g. roads, damns; Castello et al., 2013; Rice et al., 2018). These unsustainable land transformations considerably modify abiotic conditions across habitats, and lead to species extinctions, resulting in altered ecosystem functioning and service provision (FAO, 2019; Rice et al., 2018). In addition, current predictions for the Amazon basin suggest that climate change will translate to increased droughts, forest-to-savanna transitions, carbon stock losses, and an alteration of the hydrologic and biogeochemical cycles which currently structure this ecosystem (Davidson et al., 2012; Nepstad et al., 2008).

Assessing the fate of biodiversity with global change and the efficiency of management policies relies largely on the measurement of biological variation at genetic, population, community and ecosystem levels. Such measures, termed 'Essential Biodiversity Variables' (EBV; Pereira et al., 2013; Table 1) are most effective when they can be measured in a standardized way that can be employed at varying scales. Currently, these measurements are based on sampling and direct observation of individuals and their description as species by taxonomists. However, obtaining EBVs for neotropical forests is not straightforward. The majority of species occurring in the Neotropics are rare and often exhibit a high level of cryptic diversity (Antonelli et al., 2018a; ter Steege et al., 2013; Zizka et al., 2018), making them difficult to describe. The description of such hyperdiverse ecosystems thus relies on considerable taxonomic expertise, yet these skills are in decline (Paknia et al., 2015).

Such a shortfall inherently affects our understanding of species spatial distribution, abundance, evolutionary history, feeding and habitat preferences, as well as functional properties (Hortal et al., 2015). Even when species are identifiable, uncertainties surrounding their spatial distribution remain considerable for neotropical rainforests, since biodiversity assessments are often spatially restricted and biased towards a limited number of accessible areas. These issues pose major limitations to characterizing these ecosystems, to better anticipating their responses to global change, and ultimately to implementing effective policies of biodiversity conservation across the region.

Environmental DNA (eDNA) based methods (Fig. 1) are now considered as key tools to overcome the aforementioned challenges (Deiner et al., 2017; Taberlet et al., 2012a, 2018; Table 1), providing numerous advantages over classical inventory approaches. Firstly, DNA for taxonomic identification allows an objective analysis of sequence composition, as opposed to more subjective determination using specimen morphology. Secondly,

Table 1 Essential biodiversity variables (EBVs) and potential utility of eDNA-based methods to measure them in neotropical rainforests.

EBV class	EBV candidate	Utility of eDNA	Sections or references
Genetic composition	Co-ancestry	Fairly useful	Sections 2 and 3.4
	Allelic diversity and population genetic differentiation	Fairly useful	Sigsgaard et al. (2016)
	Breed and variety diversity	Unknown	NA
Species populations	Species distribution	Very useful	Sections 1–2, 3.1–3.2, and 4.3–4.4
	Population abundance	Poorly useful	Sections 2, 3.3–3.4
	Population structure by age/size class	Useless	NA
Species traits	Phenology	Fairly useful	Sections 3.1 and 4.1
	Morphology and Reproduction	Useless	NA
	Physiology and movement	Fairly useful	Sections 4.1–4.2
Community composition	Taxonomic diversity	Very useful	Sections 1–3
	Species interactions	Very useful	Sections 1 and 4.3
Ecosystem function	Net Iary or IIary productivity	Poorly useful	NA
	Nutrient retention	Useless	NA
	Disturbance regime	Fairly useful	Sections 3.1–3.2 and 4.5
Ecosystem structure	Habitat structure	Fairly useful	Sections 3.1–3.2 and 4.5
	Ecosystem extent and fragmentation	Fairly useful	Sections 3.1–3.2 and 4.5
	Ecosystem composition by functional type	Useful	Sections 4.1 and 4.3

EBVs are as defined by Pereira et al. (2013). Sections of this review or reference paper discussing such applications, or associated limitations are also indicated. NA: no documentation available yet. Usefulness levels are attributed depending on the biases of eDNA for each EBV candidate, the potential costs, as well as the extent to which eDNA information has to be complemented by other sources (e.g. species functional traits)

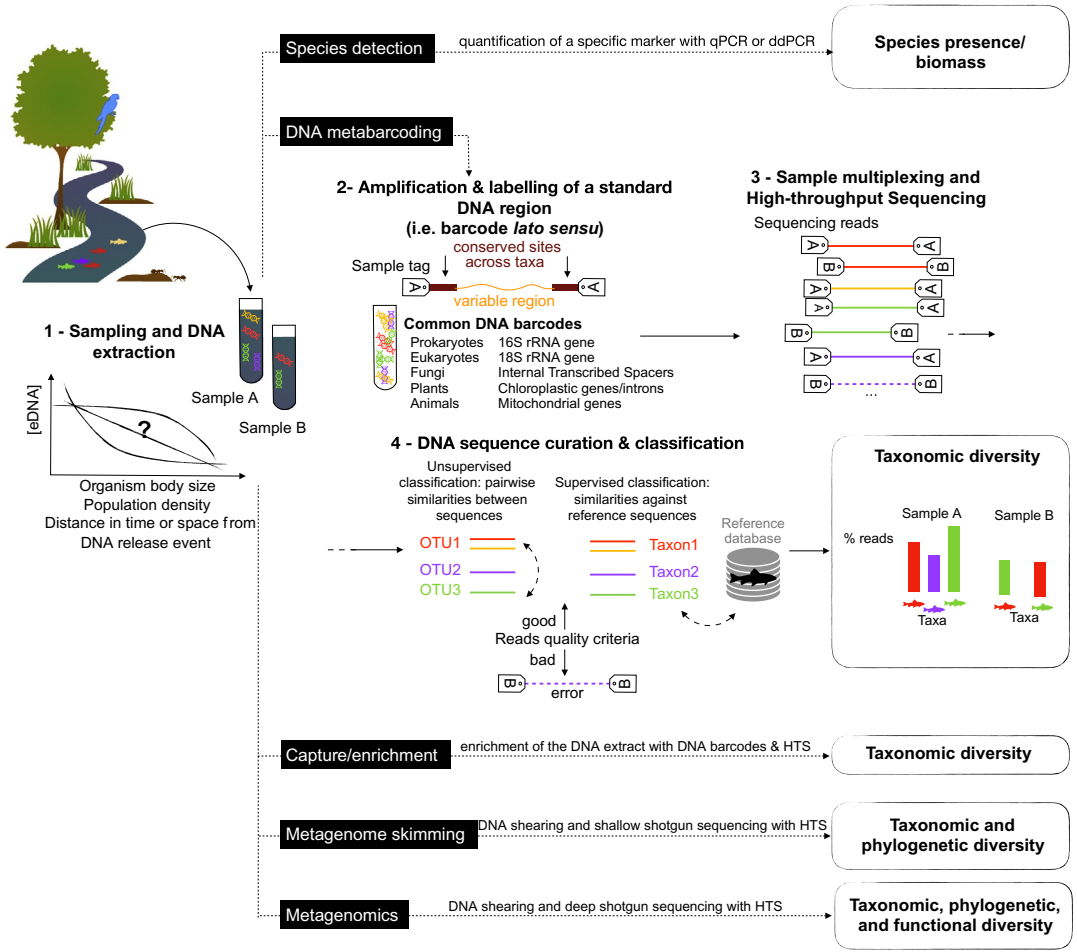

Fig. 1 See legend on next page.

the sampling of the DNA released in the environment by organisms, or environmental DNA (eDNA) is straightforward, due to its prevalence almost everywhere.

In its narrowest sense, eDNA corresponds to the mixture of DNA that can be found in any environmental matrix, whether consisting of soil, sediment or water. This DNA can belong to organisms that are present within the sample in an active or dormant stage (e.g. microbes, spores, pupae, or seeds). Alternatively, it can belong to organisms living in the sample vicinity, since organisms continuously expel DNA into the environment through excretion, secretion, decomposition, or sloughing of tissues. An environmental sample therefore contains a 'metagenome', i.e., a pool of complete or partial genomes from many different species. This metagenome is made up of DNA that can be intracellular or extracellular, dissolved or adsorbed on organic or mineral particles (Nagler et al., 2018).

In its broadest sense, eDNA also corresponds to the DNA that can be extracted from any biological material collected in natural systems, whether it corresponds to a single specimen or a whole community (e.g. bulk samples made of a mass trapping of arthropods or fish larvae). In both cases, the DNA recovered from such a sample does not only contain that of the specimens, but also encompasses the genes/genomes of the specimens symbionts, parasites, or more generally of their microbiota, as well as of their prey (Hacquard et al., 2015; Taberlet et al., 2018).

Fig. 1 Overview of the main eDNA-based methods with a focus on DNA metabarcoding applied to fish diversity assessment. The broad information that can be retrieved through each of these methods is depicted in white boxes. Step 1 corresponds to DNA sampling and extraction, which is common to all eDNA-based methods (black boxes). Each step of DNA metabarcoding is then described: Step 2 depicts the DNA amplification step and which DNA regions are generally used. It also shows how multiple samples can be sequenced in parallel: by adding a small sample-specific nucleotidic label in the 5′ region of each primer (here corresponding to sample A) prior to or after DNA amplification. Step 3 illustrates a multiplex of samples that has been sequenced in a single sequencing run. Between ca. 500–1000 samples can be multiplexed on Illumina sequencers depending on the sample diversity and sequencing technology. The sequencing step can be seen as a sampling process; the more diverse the pool of amplicons (i.e. containing different barcodes), the more sequencing reads are required to appropriately describe the sample diversity and composition. The dashed sequence in sample B illustrates a tag-jump event. Step 4 broadly summarizes the bioinformatic procedures used to curate/annotate the sequencing data and ultimately retrieve a site by OTU/species table.

Thus, the biodiversity retrieved from an eDNA sample is trans-kingdom and multitrophic. Combined with high-throughput sequencing (HTS), environmental DNA-based methods (Section 2; Fig. 1) now make large-scale and multi-taxa surveys possible from material that is easy to collect, requiring minimal taxonomic expertise. So far mostly used in temperate environments, such surveys could considerably speed up the acquisition of EBVs in general (Jetz et al., 2019), and in species-rich and challenging ecosystems such as neotropical rainforests (Table 1).

First, eDNA can provide information on the occurrence of invasive species (Takahara et al., 2013; Valentin et al., 2018), human and agricultural pathogens or pests (Bass et al., 2015; Harwood et al., 2014; Lievens et al., 2006), endangered species or populations (Harper et al., 2018; Tessler et al. 2018) and of wild species in general (Kirshtein et al., 2007; Scibetta et al., 2012). Likewise, it can be used to monitor species that indicate the health of ecosystems (i.e. bioindicators), in particular when these are microbes or invertebrates, of which identification requires advanced and often rare taxonomic skills (Mächler et al., 2014; Pawlowski et al., 2014), especially in tropical ecosystems (Bowles and Courtney, 2018; Rousseau et al., 2013, and references herein).

Second it can provide reliable information on the diversity and community composition of soil or aquatic microbes (e.g. Gilbert et al., 2012; Lauber et al., 2009; Zinger et al., 2011), as well as of invertebrates (Bista et al., 2017; Pansu et al., 2015; Zinger et al., 2019a), fish, amphibian, and mammalian communities (Boussarie et al., 2018; Schnell et al., 2018). eDNA can be further used as a standard impact assessment tool in both aquatic (Chariton et al., 2010; Li et al., 2018) and terrestrial ecosystems (e.g. Drenovsky et al., 2010), or as an evaluation tool for the success of restoration and conservation strategies (Bohmann et al., 2014; Perring et al., 2015). Finally, eDNA can provide information for multiple taxon at the same time (e.g. Li et al., 2018; Zinger et al., 2019a), and thus on biological interactions (Vacher et al., 2016). For example, using the eDNA retrieved from the faeces or gut content of a given species can reveal feeding habits (Pompanon et al., 2012), as well as host-microbiota and the occurrence of potential pathogens/parasites (Bass et al., 2015). This enables the study of full ecological networks across environmental or land disturbance gradients.

The objectives of this review are therefore (i) to provide a brief overview of eDNA-based methods, (ii) to assess their implementation to describe biodiversity in both terrestrial and aquatic ecosystems of neotropical rainforests, (iii) to highlight the limits and challenges of these methods for providing

reliable assessments of EBVs in these environments, and (iv) to propose several avenues for future research in this field.

2. Overview of eDNA methods

The study of eDNA is made possible through the extraction of DNA from its environmental/biological matrix and its separation from any chemicals that can affect DNA amplification or sequencing reactions (e.g. humic substances, polyphenols, etc.). Once the DNA extract is obtained, four main methods are now routinely applied depending on the final objective (Fig. 1). They rely either on the amplification or enrichment of a target genomic region of the metagenome (i.e. species detection, DNA metabarcoding, or capture/enrichment), or on the direct—or 'shotgun'—sequencing of the metagenome (i.e. metagenome skimming or metagenomics). We briefly describe each of these approaches below and in particular emphasize DNA metabarcoding throughout this review, as this method is currently the most widely used in the field, in particular in neotropical rainforests. For more detail regarding the molecular and bioinformatics procedures involved, we refer the reader to dedicated literature (e.g. Bálint et al., 2016; de Bruijn, 2011; Deiner et al., 2017; Taberlet et al., 2018).

The 'species detection' approach consists of detecting/quantifying the amount of a DNA marker that is specific to a single or a small set of species. This approach is most relevant when one aims to detect a species with a high level of sensitivity, including low density populations or dormant/juvenile life forms. The DNA markers used for this approach must correspond to a highly polymorphic locus, enabling the design of primers which are highly species-specific. The approach currently preferred is a direct quantification of the number of copies of the target DNA marker through quantitative PCR (i.e. qPCR, sometimes referred to as real time PCR; Rees et al., 2014) or digital droplet PCR (ddPCR; Doi et al., 2015). These two quantitative methods can help to assess species population density or biomass in the studied area (e.g. Pilliod et al., 2013). This approach is relatively cheap, since it does not rely on sequencing, and is therefore more suitable for large-scale or temporal studies, although is limited to focusing on only one or a reduced set of species.

'DNA metabarcoding' (Taberlet et al., 2012b) is the most popular approach to study eDNA (see Fig. 1 for more detailed information). This approach has also been referred to in the literature as 'amplicon sequencing',

'ecometagenetics', 'metataxogenomics', but should not be confused with 'metagenomics', which we define below. As with species detection, DNA metabarcoding relies on the amplification of a target DNA region by PCR. However in this case, the DNA region targeted is used as a *barcode* to discriminate the species comprising the metagenome under study. A relatively large number of samples processed with DNA metabarcoding can be sequenced in a single HTS run (Fig. 1). The obtained sequencing reads are then processed bioinformatically to retrieve a list of species (or Operational Taxonomic Units, OTUs).

Enrichment capture on eDNA is very similar to DNA metabarcoding in that it consists of sequencing the same targeted regions. However, it differs in that the target DNA to be sequenced is not enriched through PCR amplification, but instead by capturing it with multiple, taxon-specific DNA probes bound to magnetic beads. This approach is often used for the analysis of ancient DNA of single species or simple species assemblages (e.g. Carpenter et al., 2013) and is increasingly used for the analysis of modern eDNA and complex communities (e.g. Shokralla et al., 2016; Wilcox et al., 2018), although the sensitivity and limitations of this approach are yet to be evaluated.

The last alternative relies on shotgun sequencing, i.e., random sequencing of DNA molecules from the environmental sample. 'Metagenomics' is the most direct and comprehensive DNA-based technique, and consists of sequencing as much of the metagenome as possible so as to retrieve organisms taxonomic identity, their phylogenetic relationships, as well as to their metabolic properties. However, it is also the most challenging approach. First, much of the information contained within metagenomes remains undescribed. Second, a metagenome contains a huge diversity of genes and noncoding regions, of which a tiny fraction are highly repeated (e.g. ribosomal RNA genes), and a majority of which are rare. Fully describing this complexity therefore requires substantial sampling, in this case sequencing effort, which today remains costly. Finally, most environmental samples are dominated by microbial DNA, which reduces the probability of detecting larger organisms. Consequently, metagenomics is for now mostly used in environmental microbiology (e.g. de Bruijn, 2011) or for ancient DNA analyses (Thomsen and Willerslev, 2015).

'Metagenome skimming' is a cheap version of metagenomics (Linard et al., 2015; Papadopoulou et al., 2015), albeit more expensive than methods targeting a particular DNA region. In this case, the metagenome is sampled at a shallow sequencing depth so as to sequence only highly repeated DNA

regions, i.e., the ribosomal RNA gene regions and the organelle genomes for eukaryotes. These regions can then be partially or fully reconstructed, and thus used to identify the species present but also their phylogenetic relationships.

3. Current use and challenges of eDNA applications in neotropical rainforests

Current studies of biodiversity in neotropical rainforests that rely on eDNA-based methods mainly describe community composition and diversity changes along environmental or disturbance gradients in order to identify patterns in diversity and their drivers. These studies are reviewed below across ecosystems and focal organisms, and examined to determine what eDNA from different sources can reveal regarding ecological communities from neotropical rainforests and how sampling can be tailored to suit the ecological question. We will restrict our review to contemporary environments, as—to our knowledge—eDNA approaches per se have not been used yet in the neotropical rainforests for palaeoecological purposes. We refer interested readers to dedicated reviews on this particular application (Rawlence et al., 2014; Taberlet et al., 2018; Thomsen and Willerslev, 2015).

3.1 In terrestrial ecosystems

3.1.1 Microbial communities

These have been mostly analysed in the soil environment, with the study of eDNA from soil samples having a relatively long history in soil microbial ecology (Tiedje et al., 1999 for an early review). Available studies for neotropical rainforests have shown that soil prokaryotic and microeukaryotic communities vary across altitudes (Nottingham et al., 2018), soil conditions, forest types and tree species composition (Ritter et al., 2019; Vasco-Palacios et al., 2019). Numerous studies also report steep changes in composition with increased drought (Kivlin and Hawkes, 2016a; Pajares et al., 2018; Waring and Hawkes, 2015), deforestation and reconversion to different types of sylviculture (Carney et al., 2004; Kivlin and Hawkes, 2016a,b; Ndaw et al., 2009), arable farming (Mendes et al., 2015; Paula et al., 2014; Rodrigues et al., 2013; e.g. Franco et al., 2019), and even as a result of pre-columbian activities (Grossman et al., 2010; Kim et al., 2007; Navarrete et al., 2010). Likewise, soil microbial diversity differs between old-growth and secondary forests (Araújo et al., 2014; McGee et al.,

2019). All these studies exemplify the utility of soil eDNA for providing microbial-derived EBVs that are meaningful for monitoring the impact of climate change and land use practices.

3.1.2 Invertebrates

Soil micro- and macroinvertebrates (i.e. nematodes, earthworms, insects and springtails) have seldom been studied with eDNA from neotropical rainforest soil samples (Ritter et al., 2019; Wu et al., 2011; Zinger et al., 2016), and in such cases, rather as part of the whole soil eukaryote diversity, through the use of universal primers. A global-scale analysis suggests that neotropical rainforests are dominated by arthropods and enriched in soil annelids (Wu et al., 2011). Locally, soil micro- and mesofauna communities exhibit primarily random spatial patterns that are more pronounced for the mesofauna as compared to microscopic organisms, as shown at a forest site in French Guiana (Zinger et al., 2019a).

The large majority of studies of soil or aboveground invertebrates have so far rather relied on eDNA extracted from bulk samples and analysed through DNA metabarcoding, which is a fast alternative to time consuming sorting and identification of hundreds to thousands of specimens that are difficult to identify. Using this approach, Porazinska et al. (2012) were able to observe strong variation in soil nematodes communities across sites and habitats of Costa Rican rainforests. This approach has also enabled the description of aboveground terrestrial arthropods, such as sandflies occurring at several sites in French Guiana (Kocher et al., 2017c), or arthropods from a forest canopy in Honduras (Creedy et al., 2019). The latter study also tested the effect of animal size on species detection, with results suggesting such effects are not visible when sequencing depth is sufficient. Enrichment capture has also been used to analyse bulk samples of arthropods sampled with malaise traps in a forest of Costa Rica (Shokralla et al., 2016). This method was found to be more accurate in describing biodiversity than DNA metabarcoding on the same samples and classical observations.

3.1.3 Mammals

Using eDNA from bulk samples of faeces or hematophagous arthropods also seems particularly promising for sampling terrestrial vertebrate diversity as well. For example, DNA extracted from owl pellets in central Brazil provided meaningful information regarding the diversity of small mammals (Rocha et al., 2015). Likewise, vertebrate communities are better described by the DNA contained in blood feeding arthropods collected with Malaise

traps and pitfall traps than with classical or camera trap-based inventories, as shown for forests in Panama and Brazil (Lynggaard et al., 2019; Rodgers et al., 2017). This approach further revealed variation in vertebrate community composition, consistent with a gradient of anthropogenic pressures in French Guiana, with a decline of diversity in the areas experiencing the highest pressures (Kocher et al., 2017b). Alternatively, water samples could also be used to study terrestrial mammals, since water bodies should accumulate and transport material from the whole catchment areas through erosion (Sales et al., 2019a).

3.1.4 Plants

Initial attempts to describe plant diversity with eDNA used bulk samples of dried, fine roots isolated by hand from soil cores that were collected following a grid or regular sampling scheme in the Barro Colorado Island in Panama (Barberán et al., 2015; Jones et al., 2011). With this approach, one soil core exhibited an average diversity of ca. four plant species and the DNA imprint of each tree individual was detectable from 1 to ca. 20 m from the stem. Similar figures can be retrieved by directly using soil as starting material, as shown in a lowland rainforest in French Guiana (Fig. 2A, see also Taberlet et al., 2018; Yoccoz et al., 2012). Thus, root and soil eDNA can offer new insights into plant root distribution in the soil and their functional implications. The aboveground plant community might be better assessed by targeting plant DNA markers on bulk samples of herbivorous arthropods, but to our knowledge, this approach has not been tested yet.

3.1.5 Constraints and limits

The above shows that organisms from terrestrial environments are either studied using environmental DNA extracted from soils, which are noticeable reservoirs of both intra- and extracellular DNA and mostly contain the signature of soil organisms, or using bulk samples of invertebrates. The former material is probably the easiest to sample from a practical point of view, and less biased/variable than different arthropod sampling techniques (Missa et al., 2009). However, one critical aspect when studying diversity using eDNA extracted from soil is the heterogeneous and complex nature of soil substrates themselves, in terms of physical, chemical and biological properties (Bardgett and van der Putten, 2014). This can be an issue when comparing contrasting environments. Typically, the amount of available extracellular DNA, useful for detecting non-microbial organisms, is

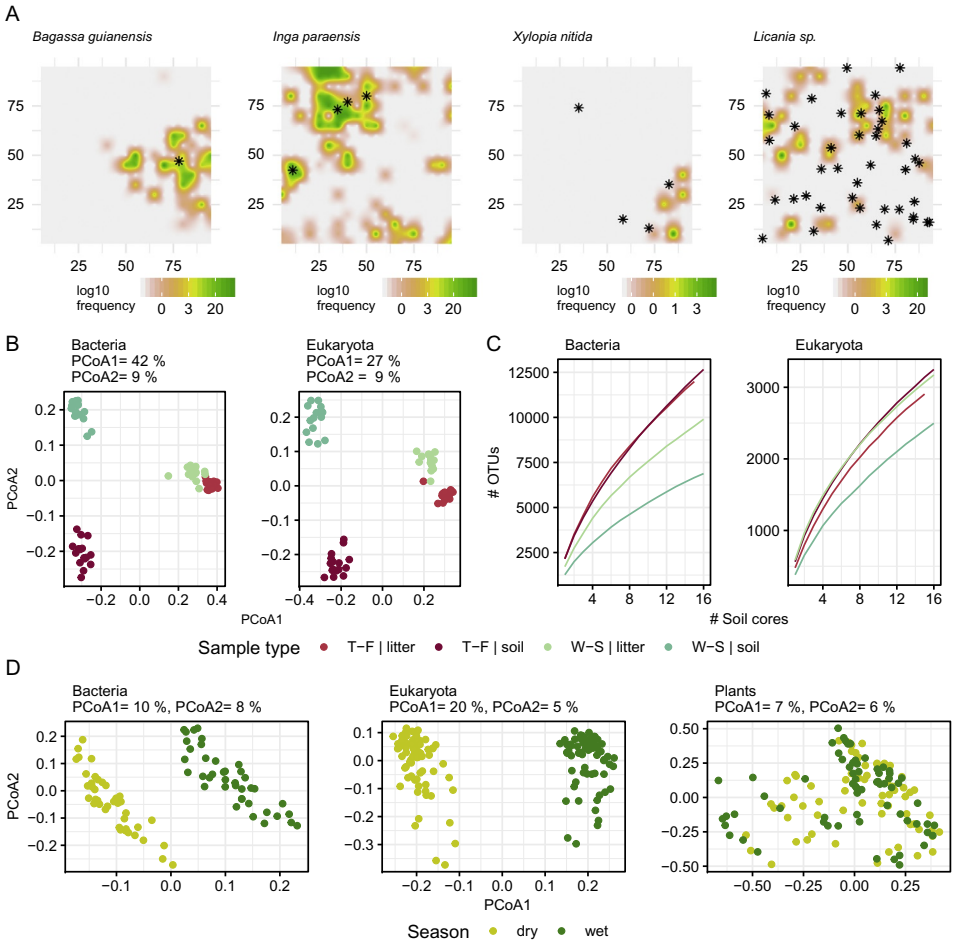

Fig. 2 See legend on next page.

strongly constrained by soil chemical properties. For example, DNA has a much stronger affinity to clay than sand (Levy-Booth et al., 2007), which thus could introduce bias to comparisons between white-sand vs. *terra firme* forest soils. This heterogeneity not only applies horizontally in space but also vertically, with clear differences in prokaryotic and microeukaryotic communities between the organo-mineral horizon and the litter layer from a taxonomic point of view (Fig. 2B), and most likely from a functional one (Basset et al., 2015; Fragoso and Lavelle, 1992; Ritter et al., 2019). This raises the question of if and how one should integrate this vertical dimension.

The same applies for how much soil samples should be collected across space, whatever the taxon targeted. For example, *terra firme* soils/litter and white-sand litter may require higher sampling effort than white-sand soils to estimate the plot-scale diversity, due to higher spatial heterogeneity (Fig. 2C). Alternatively, sampling effort could be reduced when comparing diversity or community turnover across conditions by for example building composite samples at different sampling points to capture local diversity while keeping down experimental costs. At the sample scale, extracting eDNA from volumes of material that are larger than those from most commercial soil DNA extraction kits (typically 250 μg) should best capture local diversity, which is now possible for ≥10 g of starting material, as shown for neotropical rainforest soils (e.g. Zinger et al., 2016). Thus the required

Fig. 2 Examples of soil/litter eDNA signals in 1 ha forest plots of French Guiana. (A) Comparison of the eDNA imprints of different tree species in soil samples collected every 5 m across a 1 ha plot in the Nouragues Reserve, and in the top 10 cm of the soil layer. The colour gradient represents the log 10 relative abundance of sequencing reads from each species. Black stars correspond to the location of tree stems with diameter at breast height ≥10 cm. The two left panels show signatures that are consistent with the locations of conspecific stems. The two right panels show inconsistent trends, where 'false absences' (i.e. absence of DNA when a stem is present) is likely due to deep rooting systems and 'false presences' to roots of small trees not included in the botanical inventory. It is unlikely that they correspond to pollen, seeds or litter, because such material should be present around the other conspecific stems. (B–C) DNA metabarcoding based analyses of bacterial and eukaryotic communities from soils and litter samples (ca. 10 g and 0.5 m^3 each, respectively) collected in 1 ha plots of a *terra firme* forest (Nouragues Reserve) and white-sand forest (Mana). The plots show differences (B) in community composition as measured with the Bray-Curtis index on hellinger-transformed data, summarized with a principal coordinate analysis and (C) in plot-scale diversity and spatial heterogeneity, as depicted with species accumulation curves. (D) Seasonal variations in bacterial, eukaryotic and plant community composition in the same plot as in (A) and retrieved with soil eDNA. The figure has been produced using the same indices and techniques as in (B).

sampling effort is likely to be highly system dependent, and further analyses across habitats will help better define sampling standards for neotropical rainforests when using soil as a starting material.

In any case, soil samples are unlikely to be the most relevant material for sampling the diversity of plants or aboveground animals at the plot scale because eDNA is poorly transported in soils and thus highly patchily distributed (Levy-Booth et al., 2007; Nagler et al., 2018). This patchiness most likely also results from the reduced DNA persistence in tropical rainforest soils due to high demands of the living biomass for phosphorus, which is otherwise highly limitating (Dalling et al., 2016), thus reducing the probability of detecting large organisms. Accordingly, experiments show that dead root DNA is almost totally degraded after 15 days (Bithell et al., 2014). Likewise, microbes and soil fauna communities exhibit marked seasonal and yearly dynamics (Fragoso and Lavelle, 1992; Kivlin and Hawkes, 2016a,b; Pajares et al., 2018), and so most likely does their DNA as compared to that of rooted plants, which continuously release DNA in soils (Fig. 2D).

3.2 In aquatic ecosystems

3.2.1 Microbial communities

Several studies using water eDNA have been conducted across different systems to study microbial communities. For example, Tessler et al. (2017) showed that bacterial communities from Brazilian floodplain lakes were highly distinct from other areas of the globe, while within Brazilian sites, the composition was overall fairly similar. Other studies suggest the opposite for microeukaryotic plankton: Brazilian rivers seem to exhibit marked spatial patterns with relatively high community turnover, even within the same location (Lentendu et al., 2019). These discrepancies raise the question as to whether they arise from biological differences between microeukaryotes and bacteria or from methodological inconsistencies, which emphasize the need for increased efforts in studying aquatic microbial communities in these ecosystems.

Tank bromeliads (Bromeliaceae) accumulate rainwater at the base of each leaf axil and thus represent freshwater islands in a terrestrial matrix. They harbour various aquatic organisms ranging from prokaryotes to macroinvertebrates (Benzing, 2000; Leroy et al., 2016). eDNA methods have provided insights into their community structure through either metagenomics (Rodriguez-Nuñez et al., 2018) or DNA metabarcoding (Louca et al., 2016, 2017), revealing bacterial communities that are substantially

different from freshwater lake sediments and soil, but remarkably similar in functional structure due to an adaptation to oxygen-limited conditions.

3.2.2 Invertebrates

The use of water/sediment eDNA for targeting aquatic invertebrates (aquatic insects, crustaceans) has, to our knowledge, not yet been applied to neotropical rainforest ecosystems. A recent study has shown its usefulness for assessing macroinvertebrates community composition in the tropical freshwaters of Singapore (Lim et al., 2016), suggesting that such an approach could be relevant to neotropical rainforest ecosystems. As for terrestrial environments, the use of bulk samples for aquatic systems is emerging, such as with the study of Talaga et al. (2017), which details the development of DNA reference libraries for Guianese mosquito larvae to distinguish species from bulk samples of freshwater invertebrates. Still, eDNA studies of freshwater invertebrates in neotropical rainforests are currently limited by knowledge deficits related to their taxonomy and ecology and a lack of previously implemented studies. Although several macroinvertebrate indices enabling the biological evaluation of freshwater ecosystems are available (e.g. Couceiro et al., 2012; Dedieu et al., 2016), these are seldom used because to our knowledge, there is currently no environmental law or regulation relying on these in this ecoregion. One exception in that respect is French Guiana, which must comply with the European Water Framework Directive.

3.2.3 Fishes

The potential of water/sediment eDNA has received comparatively much more attention for studying fish communities. This has been particularly stimulated by the strong limitations of traditional sampling methods, which provide biased estimates and/or cause substantial fish mortalities. Indeed, gill nets provide only partial inventories, and ichthyocides such as rotenone, which were widely employed in the past, are increasingly banned. Electric fishing, which is often a good sampling alternative in other environments can be inefficient in neotropical streams because of the very low conductivity of the water (Allard et al., 2014). Hence, fish eDNA has rapidly emerged as the most promising non-invasive alternative to traditional sampling for small streams, rivers, lakes and the sea. Cilleros et al. (2019) compared eDNA and traditional sampling (nets and ichthyocides) both in small streams and rivers across French Guiana. Not only did they find that species assemblages were congruent between eDNA and traditional records, but also that

eDNA results were more efficient in distinguishing the fauna from different river drainages. eDNA also enables the study of fish communities at cryptic life stages, i.e., the ichthyoplankton. Nobile et al. (2019) used DNA metabarcoding on mock communities built from fish eggs and larvae in the Grande River in Brazil, and obtained an average detection rate higher than 95%, and a relatively good estimate of larvae abundances. Likewise, capture enrichment on bulk samples for catfish larvae from the Peruvian Amazon provided a good description of the community in terms of both species and abundance (Maggia et al., 2017; Mariac et al., 2018).

3.2.4 Vertebrates

Several studies have focussed on vertebrates inhabiting aquatic environments for at least a part of their life, such as amphibians. Comparing traditional visual and audio survey techniques with DNA metabarcoding of water samples showed that eDNA accurately reflects the conclusions of the other methods while cutting the length of fieldwork required studying for frog communities in freshwater streams in the Brazilian Atlantic forest (Lopes et al., 2017; Sasso et al., 2017). Likewise, a comparison of cost models suggests that eDNA-based surveys are a cost-efficient alternative to traditional surveys in amphibian species-rich areas such as in the neotropical forest-savannah ecotones of Bolivia (Bálint et al., 2018). All these studies further show that eDNA methods circumvent biases of traditional approaches linked with species abundance and life history traits. Indeed, they not only allow for the detection of species closely associated with streams, but also of frog species at cryptic life stages (e.g. tadpoles or eggs). These are often missed by traditional surveys, but detectable with eDNA since they release DNA into the environment irrespective of their life stage. Likewise, eDNA is also able to detect endangered species in a non-destructive way, such as for the bromeliad inhabiting Trinidad golden tree frog (Brozio et al., 2017). Beyond amphibians, Sales et al. (2019a) also detected eDNA from both aquatic and terrestrial mammals when sampling water in the Amazon's mainstream and tributaries, in addition to a river of the Brazilian Atlantic forest. Comparing these results with camera trapping data confirmed the congruence between the methods (Sales et al., 2019a). Interestingly, some of the species detected using eDNA from water samples belong to strictly terrestrial species such as bats or anteaters, which can be explained by the fact that water conveys DNA from terrestrial to aquatic ecosystems. However, further studies are needed to validate this protocol for capturing terrestrial vertebrate diversity.

3.2.5 Constraints and limits

The above shows that eDNA for studying aquatic ecosystems can be extracted from either water or sediment samples, or bulk samples. For bulk samples, the trapping system is likely to be an important factor, as for traditional observations. For water or sediment samples, the interpretation of eDNA data from these two substrates remains unclear. Apart from microbial communities that highly differ between these two environments due to contrasted oxygen nutrient availability (Thompson et al., 2017; Zinger et al., 2011), the discrepancy between the results obtained from water and sediments when targeting larger organisms has been highlighted by several studies. Some studies have shown that fish eDNA concentration in sediments is higher and detectable over longer timescales than in water (Sales et al., 2019b; Turner et al., 2015). However, other studies found that sediments were less effective than water samples, e.g., allowing the recovery of only 10% of the fish species in an oligotrophic lake in Mexico (Valdez-Moreno et al., 2019). Water remains to date the most commonly used substrate for eDNA studies in neotropical rainforests due to its ease of collection. Sampling of eDNA is mainly conducted using filtration that is either directly performed in the field or subsequently in the laboratory (Lopes et al., 2017; Sales et al., 2019b).

For both water and sediment samples, the concentration of eDNA in the environmental matrix strongly determines how much material should be collected to appropriately sample freshwater diversity. For example, Cantera et al. (2019) sampled up to 340 L of water in streams and rivers in French Guiana to study the impact of sampling effort on fish detection. They showed that with a total filtration of 68 L, 91% of fish diversity could be detected in streams, and 74% in rivers. These results resonate with those obtained by Lopes et al. (2017), showing that filtering larger quantities of water (from 20 to 60 L) increases the detection probability for amphibian species and thus covering local amphibian diversity in the Brazilian Atlantic forest. Nevertheless, according to Cantera et al. (2019), filtering 34 L of water is sufficient for the recovery of 64% of the local fish fauna in Guianese streams and rivers, with a strong redundancy between eDNA replicates. Such a limited sampling effort seems hence sufficient to distinguish fish communities between sites and between ecosystem types (i.e. streams vs. rivers).

The concentration of eDNA in freshwaters is a function of the local living biomass, but also of the transport and degradation rate of eDNA in freshwater ecosystems, which depends on environmental conditions

(Barnes and Turner, 2016; Barnes et al., 2014). These processes require further investigation in both waters and sediments of neotropical rainforest ecosystems, in order to best define the sampling effort required to conduct reliable eDNA studies in these areas. It is now well established that low pH conditions, high oxygen demand and primary production, and high temperatures all accelerate the degradation of aquatic eDNA (Barnes et al., 2014; Strickler et al., 2015), which is likely to strongly vary across neotropical rainforest rivers and streams. As such, a study found an unexpected higher mammal species richness in the Brazilian Atlantic forest compared to the Amazon (Sales et al., 2019a), which is suspected to arise from a higher degradation rate of eDNA due to the low pH of the Amazon waters (pH $\leq$4).

Another important point, strongly linked to the degradation rate, is the transportation of eDNA with water flow. Studies are ongoing on this aspect in neotropical rivers, but Cantera et al. (2019) report that fish species detected from a stream site were no longer detected in eDNA samples collected in a river site located 300 m downstream from the confluence with the river. This suggests either a rapid degradation and hence a relatively short distance of eDNA transportation in neotropical waters, or more generally a high dilution downstream, which should make eDNA detection more difficult at sites distant from where it has been released. Finally, precipitations and stream size should also define local eDNA concentrations. For example, Sales et al. (2019b) reported noticeable compositional differences between samples collected from the same location following a 3-week interval. While this might be due to real variation in species composition, it is also possible that variation in water volume linked to increased precipitation at the time of sampling affected species recovery.

3.3 Common field, wet, and dry lab biases

Besides the clade- and environment-specific considerations mentioned above, the processing of eDNA data typically consists of a series of methodological steps (Fig. 1) that are all subject to various biases (Dickie et al., 2018; Zinger, et al., 2019b). We will briefly outline some of them and their associated solution when crucial for applications in neotropical rainforests ecosystems, as these issues are extensively addressed elsewhere (e.g. Alberdi et al., 2019; Deiner et al., 2017; Taberlet et al., 2018). This discussion will be mostly focused on DNA metabarcoding, as it is the approach the most widely used in eDNA research.

At the sampling step, the extent of the sampling area, sampling point locations, number of biological replicates, sample conditioning and transport, etc. are all important points to critically consider to avoid compromising the results (reviewed in Dickie et al., 2018; Taberlet et al., 2018) and will inherently depend on the particularities of the ecosystem and taxon under study (see above). Appropriate sample conditioning is also critical in tropical climates, in which microbial growth and DNA degradation is faster and more likely to occur during sample transport. Sample cooling in ice can considerably slow down DNA degradation and microbial growth, but this is seldom logistically feasible when working in remote and warm sites. To circumvent this limitation, DNA extraction can be done directly in the field with specified protocols requiring minimal infrastructure (e.g. Zinger et al., 2016; see Taberlet et al., 2018 for a detailed protocol). Alternatively, the sample can be desiccated with *silica gel* for soils or sediments, or more generally conserved with preservation buffers. These are typically used for aquatic eDNA samples, either for conserving water filters on which eDNA has been captured (Cilleros et al., 2019) or for direct addition to water samples, although preservation buffers seems less effective than sample cooling for eDNA recovery and taxon detection (Sales et al., 2019b).

After collection, the molecular processing of samples also has a variety of biases that can reduce the detection or distort the abundance of the taxa retrieved, an important limit for species population EBVs (Table 1). DNA extraction methods are not equally efficient in extracting and purifying DNA, due to variable success of cell lysis for microbes, and more generally to strong variations in the chemical composition of the starting material, with some being noticeable PCR inhibitors (e.g. humic acids). The methods employed for the extraction of DNA should be tailored to the starting material and question, or it may miss or overrepresent certain taxa. Once DNA is extracted, PCR amplification should be done with primers whose specificity-to- and generality-within the clade of interest should have been verified following a thorough literature review, preliminary tests, or the use of in silico PCR softwares (e.g. Elbrecht and Leese, 2017; Ficetola et al., 2010). Use of inappropriate primers will both strongly bias the retrieved taxa abundances and in some cases, their detection altogether.

Both PCR amplification and sequencing can also generate artefactual DNA fragments/sequences, especially when the target DNA is rare (reviewed in Taberlet et al., 2018). These artefacts are generally in low abundance and very similar to genuine fragments (e.g. only one or a few different

nucleotides). They are hence difficult to identify and can artificially inflate taxonomic diversity estimates, this attribute being a community composition EBV candidate (Table 1). Nevertheless, such errors can be reduced by clustering DNA sequences at a certain sequence identity level using supervised or unsupervised approaches (Fig. 1). However, it should be noted that the bioinformatics tools used, as well as their associated parameters (e.g. clustering methods and thresholds, sequences distance indices) are not all equally efficient in reducing this artefactual variability, and can even fail to detect genuine biological variability (Bálint et al., 2016; Coissac et al., 2012; Deiner et al., 2017; Taberlet et al., 2018; Zinger and Philippe, 2016). The same applies when using supervised approaches, as the taxonomic assignment quality of a sequence/OTU inherently relies on the completeness and accuracy of the reference databases. For example, using an incomplete reference database, i.e., without conspecific sequences, can lead to an increase of 20% of erroneous taxonomic assignments as compared to the use of a complete one, as shown for Amazonian mammals (Kocher et al., 2017a).

Diversity estimates can also be inflated through the presence of genuine DNA fragments that are not initially present in the sample. The most obvious source of such a problem is exogenous contamination, which can occur not only at the sampling step, but also at the extraction, PCR, and sequencing steps because labs and reagents all contain a number of contaminants (Salter et al., 2014). Beside this problem, the multiplexing of samples within a single sequencing library or sequencing lane also produces apparent cross-sample contamination. The exact underlying mechanisms remain not well understood, but DNA fragments that are multiplexed seem to exchange the small tags used to identify their sample of origin (Fig. 1), a bias often referred to as 'tag-switches', 'tag-jumps', or 'cross-talks' (e.g. Esling et al., 2015; Schnell et al., 2015). Although this bias produces contaminants at usually low abundances, it can have strong consequences if downstream analysis relies on presence/absence and occurrences.

Given the different artefacts mentioned above, the reader should now be aware that the inclusion of negative and positive controls at the sampling, extraction, amplification and sequencing steps as well as technical replicates is critically important to ensure not only data reliability but also to optimize the processing and curation procedures of the obtained sequences through bioinformatics pipelines. The problem of false positives can be reduced by using PCR-independent methods, such as metagenomics/metagenome skimming, or capture enrichment. However both approaches still require

substantial developments and cost reductions to be applicable in large-scale studies. In addition, these approaches are not error-free. They still include tag-jumps or sequencing errors (Taberlet et al., 2018; Wilcox et al., 2018) that remain difficult to detect and filter out.

Artefactual signals can have dramatic effects on estimates and patterns of alpha, and to a lesser extent beta diversity (Calderón-Sanou et al., 2019), as well as on model parameters inference such as for Hubbell's neutral model (Sommeria-Klein et al., 2016). Since these artefacts are generally low in frequency, end-users should also be careful when focusing on rare taxa. This corresponds to the majority of species in neotropical rainforest ecosystems (Antonelli et al., 2018a; ter Steege et al., 2013; Zizka et al., 2018), which suggests that it is unlikely that current eDNA-based approaches provide reliable estimates of species richness, i.e., the number of species being present in the ecosystem studied. Nevertheless, these approaches can still provide meaningful information on alpha or beta diversity patterns by using diversity indices penalizing low-abundance OTUs or taxa such as those based on Hill numbers, which includes well known indices such as Shannon or Simpson diversity (Chao et al., 2014). These have been shown to provide more reliable ecological inferences (Calderón-Sanou et al., 2019), and should be favoured over other indices where singletons (e.g. Chao, ACE, Fisher's alpha indices) or rare species have a strong weight (e.g. inferences based on species abundance distribution or on presence-absence data). Nevertheless, new occupancy models able to detect both false negative and false positives are currently emerging (Ficetola et al., 2016; Guillera-Arroita et al., 2017), and their inclusion in current data curation procedures will certainly allow overcoming the above-mentioned limitations.

3.4 Biological interpretation of eDNA

Beyond the methodological considerations raised above, eDNA has specific intrinsic properties which must be considered when interpreting derived results. Even if eDNA data resembles a traditional species abundance table, the abundances correspond to sequencing read counts and species correspond to species, genera, or to OTUs defined at a given level of sequence similarity. This difference can have strong implications for the type of EBV that eDNA can actually measure (Table 1), as well as on ecological inferences depending on the question addressed and types of inference tools used, in particular when they involve theoretical frameworks and models that rely heavily on species and abundances (e.g. niche or neutral models, species abundance distributions).

A first uncertainty is on the extent to which sequences or OTUs can be used as a proxy for species. In most eDNA studies, species or OTUs are defined by using a threshold of 97% of sequence similarity. This threshold has been historically defined for full-length barcode genes (e.g. Hebert et al., 2003; Schoch et al., 2012; Stackebrandt and Goebel, 1994). However, current eDNA studies target small regions within these barcodes (Fig. 1) in order to comply with both the sequencing limits of current HTS instruments and, when applicable, with the fragmented nature of extracellular DNA. This constraint inherently comes with a loss of taxonomic resolution, which may have consequences for subsequent ecological inferences. The 'Amplicon-' or 'Exact Sequence Variant' concept (ASV or ESV, Callahan et al., 2017) has been recently proposed to, amongst other reasons, circumvent this problem, yet this remains sensitive to some molecular artefacts. Sometimes interpreted as intraspecific variability, which can be a desirable output of eDNA (Table 1), ASVs may also yield ecological signals that differ from what one should expect when considering species. Finally, eDNA markers do not have the same taxonomic resolution across clades. For example, the fungal Internal Transcribed Spacers, or the metazoan cytochrome oxidase subunit I (COI) can exhibit some intraspecific variability for certain groups, and only genus to family level variability for others (Schoch et al., 2012). Phylogenetic-based approaches can to a certain extent deal with these limitations. However, while these can be employed with metagenomics or metagenome skimming data (Andújar et al., 2015; Papadopoulou et al., 2015), the short and hypervariable nature of most classical DNA markers used for DNA metabarcoding do not enable making robust phylogenetic inferences, which limits the use of such data to retrieve co-ancestry relationships (Table 1). For such data, the phylogenetic diversity should be retrieved through phylogenetic placement methods, provided that a robust backbone phylogenetic tree is available (e.g. Czech et al., 2019; Matsen et al., 2010), which remains challenging for neotropical taxa (see Section 4.1.).

The other uncertainty of eDNA data relates to the meaning of sequencing reads counts. As mentioned in Section 3.3, a DNA extract is subjected to a suite of molecular manipulations that can distort the original distribution of DNA fragment abundances. Adding spiked DNA of known composition and concentrations in environmental samples could allow for the retrieval of absolute values of eDNA molecules (e.g. Smets et al., 2015; Thomas et al., 2016). However, while the abundance (relative or not) of eDNA molecules has been found to correlate with organism biomass in simple

experimental set ups (e.g. Nobile et al., 2019) or when quantifying single species *in natura* with qPCR (reviewed in Taberlet et al., 2018), several factors can alter this relationship, and hence, assessment of population abundance (Table 1). First, eDNA persistence and transport in the environment makes it difficult to know whether this biomass is local and contemporary. This is likely to be especially true for soils or sediments as compared to water, the latter being more exposed to high temperature and UV radiations, which favour DNA degradation (Barnes and Turner, 2016; Nagler et al., 2018). Even if this bias is limited, relating eDNA abundance to population abundance per se remains challenging. Indeed, the number of DNA marker gene copies depends on the taxon, on the tissues from which eDNA is released, the biomass/size of the organisms, but also its life stage (Maruyama et al., 2014). To our knowledge, there is no tool which can retrieve individual counts from sequencing reads or eDNA molecules at the scale of the biological community. These uncertainties have often led researchers to prefer presence-absence metrics over abundance-based ones. However, unless the representativeness of the data curation procedure can be proven, we advocate against such reasoning due to the high error rate of PCR and sequencing based approaches (see Section 3.3.).

Given the above-mentioned differences in the intrinsic nature of eDNA data as compared to traditional species abundance tables, this raises the question of whether one can draw ecological inferences with classical tools. Typically, it remains largely uncertain whether inferring community diversity and related characteristics from eDNA-based species abundance distribution or using process models involving explicitly species and individuals is a correct approach. For example, adaptation of Hubbell's model to account for body size or biomass could be more appropriate (O'Dwyer et al., 2009; Sommeria-Klein et al., 2016). There is hence a need for development of related tools and theories in ecology that would better comply with the nature of eDNA data.

4. Future directions and perspectives

The past decade has seen enormous advances in the development and extension of eDNA-based approaches, as well as a large number of potential applications in various environments, including neotropical rainforests. However, these applications remain largely underused in this part of the world when compared with other far less diverse regions (this paper; Mulatu et al., 2017; Belle et al., 2019). This is because countries harbouring

lower diversity are in general more developed economically: infrastructure for molecular-based research is accessible, with associated personnel now relatively well trained for eDNA data generation and analysis. On the other hand, the Nagoya Protocol on Access and Benefit Sharing restricts the access of genetic resources to the country where the sample has been collected, protecting local countries, which are often less economically developed, from unethical practices by collectors outside of and within the scientific community. We argue that current efforts to develop eDNA-based research in neotropical countries should be encouraged and strengthened through international collaborations between researchers from Neotropical countries and researchers from countries that have already overcome issues relating to methodological application, technical infrastructure and skill acquisition. Such efforts will enable the acquisition of EBVs related to taxonomic diversity, but also beyond to provide information such as species distributions or biotic interactions (Table 1), as well as associated underlying processes. In this final section of the review, we will explore how eDNA can be better used to improve research methods and their subsequent applications, and in doing so ultimately contribute to improving conservation programs and management strategies for these hyperdiverse ecosystems.

4.1 Making better sense of eDNA data with better reference databases

A key limit to current eDNA studies in neotropical rainforests is the provision of relatively poor taxonomic information. This drawback arises in part from the limitations of eDNA-based methods mentioned above, but is further exacerbated when dealing with neotropical taxa in that they are largely underrepresented in current DNA reference databases, and/or they have an unresolved taxonomy. This is particularly true for microeukaryotes, for which a significant proportion of OTU and sequencing reads remain unassigned to a taxon, even at the phylum level (Ritter et al., 2019; Zinger et al., 2019a). The deficit in DNA references also applies to less cryptic organisms. For example, only 58% of the São Paulo tree flora has genetic records in international DNA reference databases (de Lima et al., 2018). While eDNA does facilitate the identification of challenging taxa at gross taxonomic levels, it is therefore unlikely to provide a satisfactory solution for resolving the Linnean shortfall and provide on its own information on EBVs related to species evolutionary history and functional traits. We hence argue that the future of eDNA remains inherently intertwined with the continued efforts of taxonomists and naturalists to sample, identify and store

physical specimens in order to complement DNA reference databases, but also to describe their morphology, evolutionary history, functional traits, and to solve taxonomic problems (Dormontt et al., 2018; Pinheiro et al., 2019; Sheth and Thaker, 2017).

Augmenting the completeness of DNA reference databases is crucial not only to facilitate the assignment of unknown sequences. It is also essential to ensure, or verify the plausibility of the retrieved signal, which can be extremely noisy as discussed above. However, one of the difficulties in improving DNA reference databases is the current lack of consensus when choosing the DNA regions to be used across studies. Indeed, these may differ from the ones used in curated reference databases linked to voucher specimens such as the BOLD system for animals and plants (Ratnasingham and Hebert, 2007) or databases dedicated to the ribosomal clusters for microorganisms (e.g. UNITE, Abarenkov et al., 2010; SILVA, Quast et al., 2013), which only contain gold standard barcoding genes (i.e. COI for animals, *rbc*L or *mat*K for plants, and ITS for fungi). This is because gold standard barcodes are not necessarily compatible with all applications of eDNA, which often require DNA primers that target broad taxonomic groups and DNA markers that are short to suit existing sequencing technologies or the degraded state of eDNA. Conserved priming sites across broad taxonomic groups are often absent within these gold standard barcodes, an issue highlighted for animals (Deagle et al., 2014) and plants (Hollingsworth et al., 2011). As a consequence, existing primer sets targeting classical barcode subregions are often biased towards certain taxa or on the contrary lack of specificity because they contain too many degenerate bases (Collins et al., 2019; Deagle et al., 2014). Alternative DNA markers fulfilling these conditions are often located in mitochondrial or chloroplastic introns or ribosomal genes (Fig. 1) which are better conserved. However these regions also often exhibit lower taxonomic resolution and are much less referenced in DNA databases. When choosing a DNA marker, the end-user must hence usually compromise between more precise taxonomic information versus unbiased sampling of biodiversity. These considerations go beyond the scope of this review and we refer interested users to dedicated literature on the subject (Deagle et al., 2014; Hollingsworth et al., 2011; Taberlet et al., 2018).

As stated above, the choice of a given DNA marker strongly relies on the biological question to be addressed, the starting material used and because current reference databases have large deficits in neotropical organisms. Therefore, we encourage the construction of custom reference databases for the targeted DNA region from local taxa that are likely to be detected

with the eDNA analysis, as done for example in studies using the mt 12S rRNA gene of neotropical mammals (Kocher et al., 2017a) and of Guianese fishes (Cilleros et al., 2019), or for the ITS1 region for the Basidiomycota of French Guiana (Jaouen et al., 2019). Although often considered as a costly endeavour, it can be achieved at relatively low expense (as low as ca. 5 $USD/specimen) by using freshly collected specimens, or herbarium/museum collections (e.g. Dormontt et al., 2018) and by multiplexing thousands specimens in a single HTS run. Another promising alternative that will alleviate the lack of standard DNA markers across studies lies in the building of 'marker-free' DNA reference databases. This is now possible with genome skimming (Dodsworth, 2015), which is similar to metagenome skimming but relies on a single specimen. This approach produces sequences usable for both gold standard and other barcodes as it generates sequences of the complete organelle genomes and full nuclear ribosomal regions (Coissac et al., 2016). Although this remains relatively expensive (as low as ca. 100 $USD/specimen), it is likely to become more affordable with continued decreases in sequencing costs.

In addition to compiling DNA information across species, reference databases could complement taxonomic data with ecophysiological characteristics, such as foliar, root or seed traits for plants, and morphological characteristics such as body size for animals. Such information would be extremely valuable, allowing eDNA studies to go beyond the simple description of taxonomic and phylogenetic diversity of the studied system (Table 1). For example, inferring taxon function or gross ecological traits from eDNA data is now possible for bacteria and fungi through databases that compile both metabolic, life history traits, or broad lifestyle types (e.g. PiCrust, Langille et al., 2013; FUNGuild, Nguyen et al., 2016). To our knowledge, such tools are currently not directly available for macroorganisms, although several databases compiling taxonomic and functional information in a number of groups have been developed (e.g. FishBase, Froese and Pauly, 2019; TRY, Kattge et al., 2019; Atlantic Bird Traits, Rodrigues et al., 2019; or the Global Ants Database, Gibb et al., 2019). Their coupling with DNA reference databases would certainly help advance the field of eDNA studies to include more process-based approaches.

4.2 Toward eDNA-based occurrence portals for the neotropics?

The greatest strength of eDNA-based approaches is their relative ease of implementation for both long-term and large-scale monitoring of complex

communities. Even if these data are not necessarily well resolved at the species level, they still constitute invaluable occurrence data and thereby provide more information on species distributions, another EBV (Table 1), that is currently largely lacking for neotropical rainforest taxa (Antonelli et al., 2018a).

To date, eDNA data and metadata reporting the location, time and exact protocol of the sampling are disseminated individually using study specific web repositories, as in data papers (e.g. Murienne et al., 2019) or more general repositories (e.g. Dryad, https://datadryad.org, Zenodo, https://zenodo.org; or the Short Read Archives from GenBank, https://www.ncbi.nlm.nih.gov/sra). However, the construction of dedicated portals compiling eDNA-based taxa occurrence can now be envisioned for all neotropical rainforests and beyond following the examples of the occurrence portal GBIF (https://www.gbif.org), the BOLD system (http://www.boldsystem.org) which integrates DNA data with occurrence, or the EMP (http://www.earthmicrobiome.org) which compiles occurrence and diversity of microbial taxa across the globe. The success of such an endeavour depends on the adequate standardization of data, a challenge given that ecological signal from eDNA data is influenced by the technique used, the DNA region targeted, and the protocols of molecular biology and bioinformatics chosen. While defining standards for such purposes will certainly facilitate the integration of data across studies, it is also likely that this will be difficult to apply to all desired situations, which may ultimately undermine scientific advances. Several alternatives have been proposed to circumvent this issue. The first is to adopt sequence taxonomy classification as a standard unit (Ramirez et al., 2018). As highlighted above, such an approach heavily depends on taxonomic expertise and enriched DNA reference databases to make the best use of eDNA data. The second is the implementation of 'eDNA biobanking', i.e., the development of storage facilities for eDNA samples that could be reused with different technologies (Jarman et al., 2018).

Although less precise than traditionally collected occurrence data, which are limited in other ways, Sections 3.1.2 demonstrate how eDNA-based studies can unveil the abiotic determinants of neotropical diversity. Increasing eDNA-based taxonomic inventories across environmental gradients will provide insights into taxa environmental/physiological tolerances/preferences (Table 1), information which remains scarce in neotropical rainforests. From a more applied perspective, increasing eDNA sampling across land use gradients will enable the identification of indicator taxa for

environmental impacts or umbrella taxa that are specific to this ecoregion. However, this application currently remains limited by the difficulty in retrieving population size information from eDNA as discussed above. Without significant developments for this particular aspect, eDNA-based approaches will likely remain of limited utility when assessing the conservation status of neotropical taxa.

4.3 Shedding new light on biotic interactions

The increasing use of eDNA will also certainly fill the current gap of knowledge on species interactions (Table 1) by improving the description of complex and multitrophic communities for both well studied taxa and more elusive organisms. Such assessments are urgently needed at a time where environmental changes already cause direct species loss and cascading extinction via bottom-up or top-down effects, especially in tropical ecosystems, including neotropical rainforests, where biotic interactions are often expected to be highly specific (Barnes et al., 2017).

It is now possible to analyse the diet of a particular species by collecting faeces, gut contents or even the DNA traces herbivores or pollinators leave on plants (Thomsen and Sigsgaard, 2019). These applications are routinely used in temperate ecosystems (Alberdi et al., 2019; Bohmann et al., 2014; Taberlet et al., 2018). By contrast, only few diet studies have been performed on neotropical organisms, i.e., on tapirs from French Guiana (Hibert et al., 2013), on white-face capuchins from Costa Rica (Mallott et al., 2018), on neotropical vampire bats (Bohmann et al., 2018) and rodents (Lopes et al., 2015), and on particular arthropods (Kocher et al., 2017b; Paula et al., 2016; Rodgers et al., 2017). New protocols of diet assessment based on faeces or gut contents are now available and optimized to reduce host DNA concentration in DNA extracts (e.g. Krehenwinkel et al. 2017). Such improvements considerably reduce the costs associated with molecular treatments and sequencing and hence allow for the implementation of large-scale studies of full food-webs composed of understudied and hyperdiverse taxa. This will certainly enable improved characterization of trophic niche and breadth for many neotropical taxa, thereby improving documentation of feeding behaviour in relation to species functional traits and competitive interactions.

Likewise, eDNA can be used to unravel plant-pollinator networks. Pollinators yield substantial amounts of pollen on their bodies, and conversely the surfaces of leaves and flower petals also harbour traces of DNA belonging

to visiting pollinators. This material can be used to build reliable plant-pollinator insect interactions, as shown in temperate ecosystems (Pornon et al., 2016; Thomsen and Sigsgaard, 2019). The applicability of the methods has, to our knowledge, not yet been tested in neotropical rainforests and remains to be critically assessed due to the particular climatic conditions, much greater richness, and also the greater amount of vertebrate pollinators in these ecosystems, which can be more challenging to sample than arthropods.

Similarly, improved understanding of host-microbiota interactions can have important implications for threatened species conservation (West et al., 2019). This can be done by studying microbial communities occurring at the surface or within larger organisms in a more comprehensive way than before. So far, existing studies have principally aimed to describe microbial communities and, in some cases, their assembly mechanisms. This has been done mostly for leaf or root endophytes in trees (Bonfim et al., 2016; Donald et al., 2020; Kembel et al., 2014; Schroeder et al., 2019), palms (Donald et al., 2019), grasses (Higgins et al., 2014) or fern species (Del Olmo-Ruiz and Elizabeth Arnold, 2017) and for the microbiota of frogs to assess its potential role in the resistance to the chytrid fungus *Batrachochytrium dendrobatidis* (Catenazzi et al., 2018; Hughey et al., 2017). To our knowledge the microbiota associated with neotropical mammals has been only assessed for the endangered Andean Bear (Borbón-García et al., 2017), and the same holds true for the microbiota of invertebrates, which has been so far mostly studied on emblematic arthropods such as ants (Pringle and Moreau, 2017; Sapountzis et al., 2015). Although few studies have shown experimentally that the plant microbiota can promote the growth and survival of seedlings (Christian et al., 2017; Leroy et al., 2019), much remains to be done to understand the functional contribution of the microbiota to host health, and how this can affect community level distribution or diversity patterns (e.g. Janzen-Connell effects accounting for the whole microbiota).

The approaches discussed above mostly enable reconstructing bi- or tri-partite networks, but future applications are likely to span the whole ecological network to advance our understanding of the resistance and resilience of biological communities to disturbance. Indeed, eDNA can provide co-occurrence data for multiple taxonomic, functional and trophic groups retrieved from soil, sediments or water. While these co-occurrences do not represent biological interactions per se, these can assist in the discovery of a large variety of interactions at larger temporal/spatial scales, provided that these inferences are evaluated with a priori knowledge of the system or statistical tools (Vacher et al., 2016).

4.4 Epidemiology and healthcare

Neotropical rainforests ecosystems harbours many emerging infectious diseases, and use of eDNA for monitoring their agents or vectors has enormous implications for human health. Most parasites and pathogens are usually only detected when aggregating on or in their hosts and without eDNA, their detection remains challenging in the environment (Bass et al., 2015). Recent results from Sengupta et al. (2019) indicate that free living larval aquatic phases of *Schistosoma* can be detected with eDNA from water samples, opening an avenue to the control of this neglected tropical disease affecting >250 million people worldwide, mainly in Africa, but with human infestations in several regions of South and Central America. Although using eDNA as diagnostic evidence for pathogens or parasites requires extensive validation before it is used in notification procedures or detection programs (Bass et al., 2015), developments of such methods in the region would considerably improve the monitoring and fight against agents of tropical diseases.

A number of human diseases require a vector, typically an insect, to transmit the pathogen and surveillance programs usually rely on monitoring potential vector populations. Such a task can prove daunting given a single night of trapping using a standard CDC trap (Center for Disease and Control) could yield thousands of mosquitoes/sandflies that need to be identified to species level. eDNA-based approaches greatly reduce the time and costs related to these identifications (Kocher et al., 2017c; Talaga et al., 2017), and could be used for routine monitoring of vector species and help in the control of vector-borne diseases.

Classical epidemiological monitoring programs largely focused on pathogens or their vectors, yet it is increasingly recognized that the prediction of transmission risk should include a better understanding of the ecosystem as a whole. This is particularly true in a context of biodiversity erosion and habitat degradation, which could be connected to the emergence of diseases as a result of trophic food-web modifications. For example, deforestation has been suggested to lead to the emergence of diseases such as malaria (Vittor et al., 2009) or Buruli ulcer (Morris et al., 2016), through reductions in diversity and modifications to the species composition of aquatic food-webs. Because eDNA-based methods can provide not only rapid information on pathogens and vectors, but also a broad characterization of the whole ecological network, we believe they will strongly modify our approach to epidemiology and understanding of disease emergence in the next few years.

4.5 Conservation and impact assessments in neotropical rainforests and beyond

Managing ecosystems and biodiversity requires efficient detection of the species of interest, but also standard, cost- and time-effective protocols that can be implemented repeatedly across large spatial scales and through time, with low, or limited impact on organisms. Such protocols are currently not available for monitoring neotropical rainforests and, more generally, neotropical ecosystems. This review shows that eDNA-based methods fulfil these criteria while enabling characterization of the taxonomic composition of multiple trophic communities, and could even constitute proxies of other EBVs. These methods complement remote sensing tools since eDNA provides information at a much finer taxonomic resolution, thereby better complying with some of the Aichi Targets that focus on endangered and invasive species (Bush et al., 2017; Marques et al., 2014). Their use could hence greatly facilitate the establishment of Rapid Biodiversity Assessment programs.

eDNA-based rapid biodiversity assessments hold great potential for the evaluation of environmental impacts, in particular for the ever increasing unsustainable use of land in neotropical rainforests, as exemplified with soil organisms (e.g. Franco et al., 2019). Likewise, eDNA-based methods will be able to help evaluate the success of different restoration and conservation strategies (Fernandes et al., 2018). However, the use of eDNA for informing management and political decisions will inherently require the development of quick and standardized sampling protocols that work across varying environment types and can be easily applied by practitioners. Beyond standardization, which we show here to be a challenging issue, such an application implies the development of biotic integrity indices that are easily transferable to stakeholders, resource managers, and policy makers, and eDNA research is still in its infancy on this particular matter (Cordier et al., 2019). Nevertheless, we are confident that these limits can and will be overcome in the near future.

Aside from rainforests, the Neotropics holds large areas of other biomes that face threats that are not necessarily the same as for tropical rainforests but whose diversity remains poorly described with both traditional and eDNA methods (Antonelli et al., 2018a). For example, eDNA could be particularly relevant to describe and monitor white-sand ecosystems which harbour a unique flora and fauna (Fine and Baraloto, 2016), but which are currently threatened by increases in cattle ranching, deforestation for firewood or

mining for sand (Ferreira et al., 2013). Likewise, it could be used for savanna and dry forest conservation, habitats which currently experience greater pressures than other neotropical biomes, typically as deforestation, localized human disturbance and increasing drought frequency and intensity (González-M et al., 2018; Strassburg et al., 2017). For example, the revision of Brazil's Forest Code in 2012, the Cerrado (Brazilian savanna) indirectly encouraged Brazilian agribusiness to invest in this biome (Soares-Filho et al., 2014; Strassburg et al., 2017). Estuaries, including mangrove forests, also represent neglected and threatened habitats in the Neotropics, while harbouring rich communities and serving as a nursery for many fish and crustaceans (Mumby et al., 2004). In these environments, the turbidity and strong water currents make species inventories difficult, a limit that could be circumvented with eDNA (Belle et al. 2019). A last example is the Pantanal biome, a savanna wetland which hosts a unique diversity, supports essential ecosystem services, and is currently under strong human pressure (Alho, 2008). Descriptive and monitoring studies using eDNA analysis in these neglected yet important ecosystems would therefore help to better characterize their diversity and how they respond to various pressures.

From a more basic perspective, the possibility to implement comprehensive, large-scale and long-term biodiversity observatories will certainly help to gain insights into the origin and maintenance of neotropical biodiversity, and its singularity in many ecosystems. Reconstructing past ecosystems from ancient DNA (Thomsen and Willerslev, 2015) would be extremely valuable in such a case, and would further improve our understanding of the long-term dynamics of neotropical ecosystems, and hence better predict their future. However, it remains unclear whether eDNA can persist in the long term in tropical ecosystems and further studies are required in this area. Nevertheless, long-term dynamics can be assessed through monitoring initiatives along transitions between different biomes. For example, savannas and dry forests constitute transitory or alternative stable states of rainforests in response to global changes (Dexter et al., 2018; Nepstad et al., 2008), and monitoring these sites through eDNA should provide useful information on their dynamics, enabling the identification of early warning markers of major ecological transitions. Acquisition of such data will prove valuable for anticipating the status of these environments and prioritizing corresponding conservation or restoration actions to mitigate such transitions.

Acknowledgements

Some of the research presented in this paper received funding from the French Agence Nationale de la Recherche (ANR) (METABAR: ANR-11-BSV7-0020; GlobNets: ANR-16-CE02-0009; DEBIT: ANR-17-CE02-0007-01), from 'Investissement d'Avenir' grants managed by the ANR (CEBA: ANR-10-LABX-25-01; TULIP: ANR-10-LABX-0041), and from São Paulo Research Foundation (FAPESP #2013/50741-7).

References

Abarenkov, K., et al., 2010. The UNITE database for molecular identification of fungi—recent updates and future perspectives. New Phytol. 186 (2), 281–285.

Alberdi, A., et al., 2019. Promises and pitfalls of using high-throughput sequencing for diet analysis. Mol. Ecol. Resour. 19 (2), 327–348.

Alho, C.J.R., 2008. Biodiversity of the Pantanal: response to seasonal flooding regime and to environmental degradation. Braz. J. Biol. 68 (Suppl. 4), 957–966.

Allard, L., et al., 2014. Electrofishing efficiency in low conductivity neotropical streams: towards a non-destructive fish sampling method. Fish. Manag. Ecol. 21 (3), 234–243.

Andújar, C., et al., 2015. Phylogenetic community ecology of soil biodiversity using mitochondrial metagenomics. Mol. Ecol. 24 (14), 3603–3617.

Antonelli, A., Zizka, A., et al., 2018a. Amazonia is the primary source of Neotropical biodiversity. Proc. Natl. Acad. Sci. U. S. A. 115 (23), 6034–6039.

Antonelli, A., Ariza, M., et al., 2018b. Conceptual and empirical advances in Neotropical biodiversity research. PeerJ 6, e5644.

Araújo, A.S.F., et al., 2014. Soil bacterial diversity in degraded and restored lands of Northeast Brazil. Antonie Van Leeuwenhoek 106 (5), 891–899.

Bálint, M., et al., 2016. Millions of reads, thousands of taxa: microbial community structure and associations analyzed via marker genes. FEMS Microbiol. Rev. 40 (5), 686–700.

Bálint, M., et al., 2018. Accuracy, limitations and cost efficiency of eDNA-based community survey in tropical frogs. Mol. Ecol. Resour. 18 (6), 1415–1426.

Barberán, A., et al., 2015. Relating belowground microbial composition to the taxonomic, phylogenetic, and functional trait distributions of trees in a tropical forest. Ecol. Lett. 18 (12), 1397–1405.

Bardgett, R.D., van der Putten, W.H., 2014. Belowground biodiversity and ecosystem functioning. Nature 515 (7528), 505–511.

Barnes, M.A., Turner, C.R., 2016. The ecology of environmental DNA and implications for conservation genetics. Conserv. Genet. 17, 1–17.

Barnes, M.A., et al., 2014. Environmental conditions influence eDNA persistence in aquatic systems. Environ. Sci. Technol. 48 (3), 1819–1827.

Barnes, A.D., et al., 2017. Direct and cascading impacts of tropical land-use change on multitrophic biodiversity. Nat. Ecol. Evol. 1 (10), 1511–1519.

Bass, D., et al., 2015. Diverse applications of environmental DNA methods in parasitology. Trends Parasitol. 31 (10), 499–513.

Basset, Y., et al., 2015. Arthropod distribution in a tropical rainforest: tackling a four dimensional puzzle. PLoS One 10 (12), e0144110.

Belle, C.C., Stoeckle, B.C., Geist, J., 2019. Taxonomic and geographical representation of freshwater environmental DNA research in aquatic conservation. Aquat. Conserv. Mar. Freshwat. Ecosyst. 29 (11), 1996–2009.

Benzing, D.H., 2000. Bromeliaceae: Profile of an Adaptive Radiation. Cambridge University Press.

Bista, I., et al., 2017. Annual time-series analysis of aqueous eDNA reveals ecologically relevant dynamics of lake ecosystem biodiversity. Nat. Commun. 8, 14087.

Bithell, S.L., et al., 2014. DNA analysis of soil extracts can be used to investigate fine root depth distribution of trees. AoB Plants 7, lu091.
Bohmann, K., et al., 2014. Environmental DNA for wildlife biology and biodiversity monitoring. Trends Ecol. Evol. 29, 358–367.
Bohmann, K., et al., 2018. Using DNA metabarcoding for simultaneous inference of common vampire bat diet and population structure. Mol. Ecol. Resour. 18 (5), 1050–1063.
Bonfim, J.A., et al., 2016. Dark septate endophytic fungi of native plants along an altitudinal gradient in the Brazilian Atlantic forest. Fungal Ecol. 20, 202–210.
Borbón-García, A., et al., 2017. Captivity shapes the gut microbiota of Andean bears: insights into health surveillance. Front. Microbiol. 8, 1316.
Boussarie, G., et al., 2018. Environmental DNA illuminates the dark diversity of sharks. Sci. Adv. 4 (5), eaap9661.
Bowles, D.E., Courtney, G.W., 2018. Advances in aquatic insect systematics and biodiversity in the Neotropics: introduction. Aquat. Insects 39 (2-3), 89–93.
Brozio, S., et al., 2017. Development and application of an eDNA method to detect the critically endangered trinidad golden tree frog (*Phytotriades auratus*) in bromeliad phytotelmata. PLoS One 12 (2), e0170619.
Bush, A., et al., 2017. Connecting Earth observation to high-throughput biodiversity data. Nat. Ecol. Evol. 1 (7), 176.
Calderón-Sanou, I., et al., 2019. From environmental DNA sequences to ecological conclusions: how strong is the influence of methodological choices. J. Biogeogr. 47 (1), 193–206.
Callahan, B.J., McMurdie, P.J., Holmes, S.P., 2017. Exact sequence variants should replace operational taxonomic units in marker-gene data analysis. ISME J. 11 (12), 2639–2643.
Cantera, I., et al., 2019. Optimizing environmental DNA sampling effort for fish inventories in tropical streams and rivers. Sci. Rep. 9 (1), 3085.
Carney, K.M., Matson, P.A., Bohannan, B.J.M., 2004. Diversity and composition of tropical soil nitrifiers across a plant diversity gradient and among land-use types. Ecol. Lett. 7 (8), 684–694.
Carpenter, M.L., et al., 2013. Pulling out the 1%: whole-genome capture for the targeted enrichment of ancient DNA sequencing libraries. Am. J. Hum. Genet. 93, 852–864.
Castello, L., et al., 2013. The vulnerability of Amazon freshwater ecosystems. Conserv. Lett. 6 (4), 217–229.
Catenazzi, A., et al., 2018. Widespread elevational occurrence of antifungal bacteria in Andean amphibians decimated by disease: a complex role for skin symbionts in defense against Chytridiomycosis. Front. Microbiol. 9, 465.
Chao, A., Chiu, C.-H., Jost, L., 2014. Unifying species diversity, phylogenetic diversity, functional diversity, and related similarity and differentiation measures through Hill numbers. Annu. Rev. Ecol. Evol. Syst. 45, 297–324.
Chaplin-Kramer, R., et al., 2019. Global modeling of nature's contributions to people. Science 366 (6462), 255–258.
Chariton, A.A., et al., 2010. Ecological assessment of estuarine sediments by pyrosequencing eukaryotic ribosomal DNA. Front. Ecol. Environ. 8 (5), 233–238.
Christian, N., et al., 2017. Exposure to the leaf litter microbiome of healthy adults protects seedlings from pathogen damage. Proc. R. Soc. B Biol. Sci. 284 (1858), 20170641.
Cilleros, K., et al., 2019. Unlocking biodiversity and conservation studies in high-diversity environments using environmental DNA (eDNA): a test with Guianese freshwater fishes. Mol. Ecol. Resour. 19 (1), 27–46.
Coissac, E., Riaz, T., Puillandre, N., 2012. Bioinformatic challenges for DNA metabarcoding of plants and animals. Mol. Ecol. 21, 1834–1847.
Coissac, E., et al., 2016. From barcodes to genomes: extending the concept of DNA barcoding. Mol. Ecol. 25 (7), 1423–1428.

Collins, R.A., et al., 2019. Non-specific amplification compromises environmental DNA metabarcoding with COI. Methods Ecol. Evol. 10 (11), 1985–2001.
Cordier, T., et al., 2019. Embracing environmental genomics and machine learning for routine biomonitoring. Trends Microbiol. 27 (5), 387–397.
Couceiro, S.R.M., et al., 2012. A macroinvertebrate multimetric index to evaluate the biological condition of streams in the Central Amazon region of Brazil. Ecol. Indic. 18, 118–125.
Creedy, T.J., Ng, W.S., Vogler, A.P., 2019. Toward accurate species-level metabarcoding of arthropod communities from the tropical forest canopy. Ecol. Evol. 9 (6), 3105–3116.
Czech, L., Barbera, P., Stamatakis, A., 2019. Methods for automatic reference trees and multilevel phylogenetic placement. Bioinformatics 35 (7), 1151–1158.
Dalling, J.W., et al., 2016. Nutrient availability in tropical rain forests: the paradigm of phosphorus limitation. In: Tropical Tree Physiology. Springer, Cham, pp. 261–273.
Davidson, E.A., et al., 2012. The Amazon basin in transition. Nature 481 (7381), 321–328.
de Bruijn, F.J. (Ed.), 2011. Handbook of Molecular Microbial Ecology I: Metagenomics and Complementary Approaches. John Wiley & Sons.
de Lima, R.A.F., et al., 2018. Can plant DNA barcoding be implemented in species-rich tropical regions? A perspective from São Paulo State, Brazil. Genet. Mol. Biol. 41 (3), 661–670.
Deagle, B.E., et al., 2014. DNA metabarcoding and the cytochrome c oxidase subunit I marker: not a perfect match. Biol. Lett. 10 (9), 20140562.
Dedieu, N., et al., 2016. A multimetric macroinvertebrate index for the implementation of the European Water Framework Directive in French Guiana, East Amazonia. River Res. Appl. 32 (3), 501–515.
Deiner, K., et al., 2017. Environmental DNA metabarcoding: transforming how we survey animal and plant communities. Mol. Ecol. 26 (21), 5872–5895.
Del Olmo-Ruiz, M., Elizabeth Arnold, A., 2017. Community structure of fern-affiliated endophytes in three neotropical forests. J. Trop. Ecol. 33 (1), 60–73.
Dexter, K.G., et al., 2018. Inserting tropical dry forests into the discussion on biome transitions in the tropics. Front. Ecol. Evol. 6, 104.
Díaz, S., et al., 2019. Pervasive human-driven decline of life on Earth points to the need for transformative change. Science 366 (6471), eaax3100.
Dickie, I.A., Boyer, S., Buckley, H.L., 2018. Towards robust and repeatable sampling methods in eDNA-based studies. Mol. Ecol. 18 (5), 940–952.
Dodsworth, S., 2015. Genome skimming for next-generation biodiversity analysis. Trends Plant Sci. 20 (9), 525–527.
Doi, H., et al., 2015. Droplet digital polymerase chain reaction (PCR) outperforms real-time PCR in the detection of environmental DNA from an invasive fish species. Environ. Sci. Technol. 49 (9), 5601–5608.
Donald, J., et al., 2019. Tropical palm endophytes exhibit low competitive structuring when assessed using co-occurrence and antipathogen activity analysis. Front. Forests Glob. Chang. 2, 86.
Donald, J., et al., 2020. A test of community assembly rules using foliar endophytes from a tropical forest canopy. J. Ecol. Available at: https://doi.org/10.1111/1365-2745.13344.
Dormontt, E.E., et al., 2018. Advancing DNA barcoding and metabarcoding applications for plants requires systematic analysis of herbarium collections-an Australian perspective. Front. Ecol. Evol. 6, 134.
Drenovsky, R.E., et al., 2010. Land use and climatic factors structure regional patterns in soil microbial communities. Glob. Ecol. Biogeogr. 19 (1), 27–39.
Elbrecht, V., Leese, F., 2017. PrimerMiner: an r package for development and in silico validation of DNA metabarcoding primers. Methods Ecol. Evol. 8 (5), 622–626.

Esling, P., Lejzerowicz, F., Pawlowski, J., 2015. Accurate multiplexing and filtering for high-throughput amplicon-sequencing. Nucleic Acids Res. 43 (5), 2513–2524.
FAO, 2019. The state of the world's biodiversity for food and agriculture. In: Bélanger, J., Pilling, D. (Eds.), FAO Commission on Genetic Resources for Food and Agriculture Assessments, Rome, pp. 572.
Fernandes, K., et al., 2018. DNA metabarcoding-a new approach to fauna monitoring in mine site restoration. Restor. Ecol. 26 (6), 1098–1107.
Ferreira, L.V., et al., 2013. A extração ilegal de areia como causa do desaparecimento de campinas e campinaranas no Estado do Pará, Brasil. Pesquisas (Botânica) 64, 157–173.
Ficetola, G.F., et al., 2010. An in silico approach for the evaluation of DNA barcodes. BMC Genomics 11, 434.
Ficetola, G.F., Taberlet, P., Coissac, E., 2016. How to limit false positives in environmental DNA and metabarcoding? Mol. Ecol. Resour. 16, 604–607.
Fine, P.V.A., Baraloto, C., 2016. Habitat endemism in white-sand forests: insights into the mechanisms of lineage diversification and community assembly of the neotropical flora. Biotropica 48 (1), 24–33.
Fragoso, C., Lavelle, P., 1992. Earthworm communities of tropical rain forests. Soil Biol. Biochem. 24 (12), 1397–1408.
Franco, A.L.C., et al., 2019. Amazonian deforestation and soil biodiversity. Conserv. Biol. 33 (3), 590–600.
Froese, R., Pauly, D., 2019. FishBase. World Wide Web electronic publication. Available at: https://www.fishbase.org.
Gibb, H., et al., 2019. The Global Ants Database. Available at: http://globalants.org/.
Gilbert, J.A., et al., 2012. Defining seasonal marine microbial community dynamics. ISME J. 6 (2), 298–308.
González-M, R., et al., 2018. Disentangling the environmental heterogeneity, floristic distinctiveness and current threats of tropical dry forests in Colombia. Environ. Res. Lett. 13 (4), 045007.
Grossman, J.M., et al., 2010. Amazonian anthrosols support similar microbial communities that differ distinctly from those extant in adjacent, unmodified soils of the same mineralogy. Microb. Ecol. 60 (1), 192–205.
Guillera-Arroita, G., et al., 2017. Dealing with false-positive and false-negative errors about species occurrence at multiple levels. Methods Ecol. Evol. 8 (9), 1081–1091.
Hacquard, S., et al., 2015. Microbiota and host nutrition across plant and animal kingdoms. Cell Host Microbe 17 (5), 603–616.
Harper, L.R., et al., 2018. Needle in a haystack? A comparison of eDNA metabarcoding and targeted qPCR for detection of the great crested newt (Triturus cristatus). Ecol. Evol. 8 (12), 6330–6341.
Harwood, V.J., et al., 2014. Microbial source tracking markers for detection of fecal contamination in environmental waters: relationships between pathogens and human health outcomes. FEMS Microbiol. Rev. 38 (1), 1–40.
Hebert, P.D.N., et al., 2003. Biological identification through DNA barcodes. Proc. R. Soc. Lond. B Biol. Sci. 270, 313–321.
Hibert, F., et al., 2013. Unveiling the diet of elusive rainforest herbivores in next generation sequencing era? The tapir as a case study. PLoS One 8, e60799.
Higgins, K.L., et al., 2014. Communities of fungal endophytes in tropical forest grasses: highly diverse host- and habitat generalists characterized by strong spatial structure. Fungal Ecol. 8, 1–11.
Hollingsworth, P.M., Graham, S.W., Little, D.P., 2011. Choosing and using a plant DNA barcode. PLoS One 6 (5), e19254.
Hortal, J., et al., 2015. Seven shortfalls that beset large-scale knowledge of biodiversity. Annu. Rev. Ecol. Evol. Syst. 46 (1), 523–549.

Hughey, M.C., et al., 2017. Skin bacterial microbiome of a generalist Puerto Rican frog varies along elevation and land use gradients. PeerJ 5, e3688.
Jaouen, G., et al., 2019. Fungi of French Guiana gathered in a taxonomic, environmental and molecular dataset. Sci. Data 6 (1), 206.
Jarman, S.N., Berry, O., Bunce, M., 2018. The value of environmental DNA biobanking for long-term biomonitoring. Nat. Ecol. Evol. 2 (8), 1192–1193.
Jenkins, C.N., Pimm, S.L., Joppa, L.N., 2013. Global patterns of terrestrial vertebrate diversity and conservation. Proc. Natl. Acad. Sci. U. S. A. 110 (28), E2602–E2610.
Jetz, W., et al., 2019. Essential biodiversity variables for mapping and monitoring species populations. Nat. Ecol. Evol. 3 (4), 539–551.
Jones, F.A., et al., 2011. The roots of diversity: below ground species richness and rooting distributions in a tropical forest revealed by DNA barcodes and inverse modeling. PLoS One 6, e24506.
Kattge, J., et al., 2019. TRY plant trait database—enhanced coverage and open access. Glob. Chang. Biol. 26, 119–188. Available at: https://doi.org/10.1111/gcb.14904.
Kembel, S.W., et al., 2014. Relationships between phyllosphere bacterial communities and plant functional traits in a neotropical forest. Proc. Natl. Acad. Sci. U. S. A. 111 (38), 13715–13720.
Kim, J.-S., et al., 2007. Bacterial diversity of terra preta and pristine forest soil from the Western Amazon. Soil Biol. Biochem. 39 (2), 684–690.
Kirshtein, J.D., et al., 2007. Quantitative PCR detection of *Batrachochytrium dendrobatidis* DNA from sediments and water. Dis. Aquat. Organ. 77 (1), 11–15.
Kivlin, S.N., Hawkes, C.V., 2016a. Temporal and Spatial Variation of Soil Bacteria Richness, Composition, and Function in a Neotropical Rainforest. PLoS One 11 (7), e0159131.
Kivlin, S.N., Hawkes, C.V., 2016b. Tree species, spatial heterogeneity, and seasonality drive soil fungal abundance, richness, and composition in Neotropical rainforests. Environ. Microbiol. 18 (12), 4662–4673.
Kocher, A., de Thoisy, B., Catzeflis, F., et al., 2017a. Evaluation of short mitochondrial metabarcodes for the identification of Amazonian mammals. Methods Ecol. Evol. 8 (10), 1276–1283.
Kocher, A., de Thoisy, B., Catzeflis, F., et al., 2017b. iDNA screening: disease vectors as vertebrate samplers. Mol. Ecol. 26 (22), 6478–6486.
Kocher, A., Gantier, J.-C., et al., 2017c. Vector soup: high-throughput identification of Neotropical phlebotomine sand flies using metabarcoding. Mol. Ecol. Resour. 17 (2), 172–182.
Krehenwinkel, H., et al., 2017. A cost-efficient and simple protocol to enrich prey DNA from extractions of predatory arthropods for large-scale gut content analysis by Illumina sequencing. Methods Ecol. Evol. 8 (1), 126–134.
Langille, M.G.I., et al., 2013. Predictive functional profiling of microbial communities using 16S rRNA marker gene sequences. Nat. Biotechnol. 31 (9), 814–821.
Lauber, C.L., et al., 2009. Pyrosequencing-based assessment of soil pH as a predictor of soil bacterial community structure at the continental scale. Appl. Environ. Microbiol. 75, 5111–5120.
Lentendu, G., et al., 2019. Protist biodiversity and biogeography in lakes from four Brazilian river—floodplain systems. J. Eukaryot. Microbiol. 66 (4), 592–599.
Leroy, C., et al., 2016. The contribution of microorganisms and metazoans to mineral nutrition in bromeliads. J. Plant Ecol. 9 (3), 241–255.
Leroy, C., et al., 2019. How significant are endophytic fungi in bromeliad seeds and seedlings? Effects on germination, survival and performance of two epiphytic plant species. Fungal Ecol. 39, 296–306.

Levy-Booth, D.J., et al., 2007. Cycling of extracellular DNA in the soil environment. Soil Biol. Biochem. 39 (12), 2977–2991.
Li, F., et al., 2018. Application of environmental DNA metabarcoding for predicting anthropogenic pollution in rivers. Environ. Sci. Technol. 52 (20), 11708–11719.
Lievens, B., et al., 2006. Real-time PCR for detection and quantification of fungal and oomycete tomato pathogens in plant and soil samples. Plant Sci. 171 (1), 155–165.
Lim, N.K.M., et al., 2016. Next-generation freshwater bioassessment: eDNA metabarcoding with a conserved metazoan primer reveals species-rich and reservoir-specific communities. R. Soc. Open Sci. 3 (11), 160635.
Linard, B., et al., 2015. Metagenome skimming of insect specimen pools: potential for comparative genomics. Genome Biol. Evol. 7 (6), 1474–1489.
Lopes, C.M., et al., 2015. DNA metabarcoding diet analysis for species with parapatric vs sympatric distribution: a case study on subterranean rodents. Heredity 114, 525–536.
Lopes, C.M., et al., 2017. eDNA metabarcoding: a promising method for anuran surveys in highly diverse tropical forests. Mol. Ecol. Resour. 17 (5), 904–914.
Louca, S., et al., 2016. High taxonomic variability despite stable functional structure across microbial communities. Nat. Ecol. Evol. 1 (1), 15.
Louca, S., et al., 2017. Functional structure of the bromeliad tank microbiome is strongly shaped by local geochemical conditions. Environ. Microbiol. 19 (8), 3132–3151.
Lynggaard, C., et al., 2019. Vertebrate diversity revealed by metabarcoding of bulk arthropod samples from tropical forests. Environ. DNA 56, 1637.
Mächler, E., et al., 2014. Utility of environmental DNA for monitoring rare and indicator macroinvertebrate species. Freshwat. Sci. 33 (4), 1174–1183.
Maggia, M.E., et al., 2017. DNA metabarcoding of amazonian ichthyoplankton swarms. PLoS One 12 (1), e0170009.
Mallott, E.K., Garber, P.A., Malhi, R.S., 2018. trnL outperforms rbcL as a DNA metabarcoding marker when compared with the observed plant component of the diet of wild white-faced capuchins (*Cebus capucinus*, Primates). PLoS One 13 (6), e0199556.
Mariac, C., et al., 2018. Metabarcoding by capture using a single COI probe (MCSP) to identify and quantify fish species in ichthyoplankton swarms. PLoS One 13 (9), e0202976.
Marques, A., et al., 2014. A framework to identify enabling and urgent actions for the 2020 Aichi Targets. Basic Appl. Ecol. 15 (8), 633–638.
Maruyama, A., et al., 2014. The release rate of environmental DNA from juvenile and adult fish. PLoS One 9, e114639.
Matsen, F.A., Kodner, R.B., Armbrust, E.V., 2010. pplacer: linear time maximum-likelihood and Bayesian phylogenetic placement of sequences onto a fixed reference tree. BMC Bioinf. 11, 538.
McGee, K.M., et al., 2019. Determinants of soil bacterial and fungal community composition toward carbon-use efficiency across primary and secondary forests in a Costa Rican conservation area. Microb. Ecol. 77 (1), 148–167.
Mendes, L.W., et al., 2015. Land-use system shapes soil bacterial communities in Southeastern Amazon region. Appl. Soil Ecol. 95, 151–160.
Missa, O., et al., 2009. Monitoring arthropods in a tropical landscape: relative effects of sampling methods and habitat types on trap catches. J. Insect Conserv. 13 (1), 103.
Morris, A.L., et al., 2016. Deforestation-driven food-web collapse linked to emerging tropical infectious disease, Mycobacterium ulcerans. Sci. Adv. 2 (12), e1600387.
Morrone, J.J., 2014. Biogeographical regionalisation of the Neotropical region. Zootaxa 3782, 1–110.
Mulatu, K.A., et al., 2017. Biodiversity monitoring in changing tropical forests: a review of approaches and new opportunities. Remote Sens. (Basel) 9 (10), 1059.

Mumby, P.J., et al., 2004. Mangroves enhance the biomass of coral reef fish communities in the Caribbean. Nature 427 (6974), 533–536.
Murienne, J., et al., 2019. Aquatic eDNA for monitoring French Guiana biodiversity. Biodivers. Data J. 7, e37518.
Nagler, M., et al., 2018. Extracellular DNA in natural environments: features, relevance and applications. Appl. Microbiol. Biotechnol. 102 (15), 6343–6356.
Navarrete, A.A., et al., 2010. A molecular survey of the diversity of microbial communities in different Amazonian agricultural model systems. Diversity 2 (5), 787–809.
Ndaw, S.M., et al., 2009. Relationships between bacterial diversity, microbial biomass, and litter quality in soils under different plant covers in northern Rio de Janeiro State, Brazil. Can. J. Microbiol. 55 (9), 1089–1095.
Nepstad, D.C., et al., 2008. Interactions among Amazon land use, forests and climate: prospects for a near-term forest tipping point. Philos. Trans. R. Soc. B 363 (1498), 1737–1746.
Nguyen, N.H., et al., 2016. FUNGuild: an open annotation tool for parsing fungal community datasets by ecological guild. Fungal Ecol. 20, 241–248.
Nobile, A.B., et al., 2019. DNA metabarcoding of Neotropical ichthyoplankton: enabling high accuracy with lower cost. Metabarcod. Metagenom. 3, e35060.
Nottingham, A.T., et al., 2018. Microbes follow Humboldt: temperature drives plant and soil microbial diversity patterns from the Amazon to the Andes. Ecology 99 (11), 2455–2466.
O'Dwyer, J.P., et al., 2009. An integrative framework for stochastic, size-structured community assembly. Proc. Natl. Acad. Sci. U. S. A. 106 (15), 6170–6175.
Olson, D.M., et al., 2001. Terrestrial ecoregions of the world: a new map of life on earth. Bioscience 51 (11), 933.
Pajares, S., et al., 2018. Environmental controls on soil microbial communities in a seasonally dry tropical forest. Appl. Environ. Microbiol. 84 (17), e00342–18.
Paknia, O., Rajaei, S., H. & Koch, A., 2015. Lack of well-maintained natural history collections and taxonomists in megadiverse developing countries hampers global biodiversity exploration. Org. Divers. Evol. 15 (3), 619–629.
Pansu, J., et al., 2015. Landscape-scale distribution patterns of earthworms inferred from soil DNA. Soil Biol. Biochem. 83 (0), 100–105.
Papadopoulou, A., Taberlet, P., Zinger, L., 2015. Metagenome skimming for phylogenetic community ecology: a new era in biodiversity research. Mol. Ecol. 24 (14), 3515–3517.
Paula, F.S., et al., 2014. Land use change alters functional gene diversity, composition and abundance in Amazon forest soil microbial communities. Mol. Ecol. 23 (12), 2988–2999.
Paula, D.P., et al., 2016. Uncovering trophic interactions in arthropod predators through DNA shotgun-sequencing of gut contents. PLoS One 11 (9), e0161841.
Pawlowski, J., et al., 2014. Environmental monitoring through protist next-generation sequencing metabarcoding: assessing the impact of fish farming on benthic foraminifera communities. Mol. Ecol. Resour. 14, 1129–1140.
Pereira, H.M., et al., 2013. Ecology. essential biodiversity variables. Science 339 (6117), 277–278.
Perring, M.P., et al., 2015. Advances in restoration ecology: rising to the challenges of the coming decades. Ecosphere 6 (8), 1–25.
Pilliod, D.S., et al., 2013. Estimating occupancy and abundance of stream amphibians using environmental DNA from filtered water samples. Can. J. Fish. Aquat. Sci. 70, 1123–1130.
Pinheiro, H.T., et al., 2019. Will DNA barcoding meet taxonomic needs? Science 365 (6456), 873–874.
Pompanon, F., et al., 2012. Who is eating what: diet assessment using next generation sequencing. Mol. Ecol. 21, 1931–1950.

Porazinska, D.L., et al., 2012. Nematode spatial and ecological patterns from tropical and temperate rainforests. PLoS One 7 (9), e44641.
Pornon, A., et al., 2016. Using metabarcoding to reveal and quantify plant-pollinator interactions. Sci. Rep. 6, 27282.
Pringle, E.G., Moreau, C.S., 2017. Community analysis of microbial sharing and specialization in a Costa Rican ant–plant–hemipteran symbiosis. Proc. R. Soc. B Biol. Sci. 284 (1850), 20162770.
Quast, C., et al., 2013. The SILVA ribosomal RNA gene database project: improved data processing and web-based tools. Nucleic Acids Res. 41 (Database issue), D590–D596.
Ramirez, K.S., et al., 2018. Detecting macroecological patterns in bacterial communities across independent studies of global soils. Nat. Microbiol. 3 (2), 189–196.
Ratnasingham, S., Hebert, P.D.N., 2007. bold: the barcode of life data system (http://www.barcodinglife.org). Mol. Ecol. Notes 7 (3), 355–364.
Rawlence, N.J., et al., 2014. Using palaeoenvironmental DNA to reconstruct past environments: progress and prospects. J. Quat. Sci. 29, 610–626.
Rees, H.C., et al., 2014. The detection of aquatic animal species using environmental DNA—a review of eDNA as a survey tool in ecology. J. Appl. Ecol. 51 (5), 1450–1459.
Rice, J., et al., 2018. Summary for policymakers of the regional assessment report on biodiversity and ecosystem services for the Americas of the Intergovernmental Science-Policy Platform on Biodiversity and Ecosystem Services. In: IPBES book. Available at: https://www.fs.usda.gov/treesearch/pubs/56826.
Ritter, C.D., et al., 2019. Locality or habitat? Exploring predictors of biodiversity in Amazonia. Ecography 42 (2), 321–333.
Rocha, R.G., et al., 2015. DNA from owl pellet bones uncovers hidden biodiversity. Syst. Biodivers. 13 (4), 403–412.
Rodgers, T.W., Xu, C.C.Y., Giacalone, J., 2017. Carrion fly-derived DNA metabarcoding is an effective tool for mammal surveys: evidence from a known tropical mammal community. Mol. Ecol. 17 (6), e133–e145.
Rodrigues, J.L.M., et al., 2013. Conversion of the Amazon rainforest to agriculture results in biotic homogenization of soil bacterial communities. Proc. Natl. Acad. Sci. U. S. A. 110 (3), 988–993.
Rodrigues, R.C., et al., 2019. ATLANTIC BIRD TRAITS: a data set of bird morphological traits from the Atlantic forests of South America. Ecology 100 (6), e02647.
Rodriguez-Nuñez, K.M., Rullan-Cardec, J.M., Rios-Velazquez, C., 2018. The metagenome of bromeliads phytotelma in Puerto Rico. Data Brief 16, 19–22.
Rousseau, L., et al., 2013. Soil macrofauna as indicators of soil quality and land use impacts in smallholder agroecosystems of western Nicaragua. Ecol. Indic. 27, 71–82.
Sales, N.G., et al., 2019a. Assessing the Potential of Environmental DNA Metabarcoding for Monitoring Neotropical Mammals: A Case Study in the Amazon and Atlantic Forest, Brazil. bioRxiv, p. 750414. Available at: https://www.biorxiv.org/content/biorxiv/early/2019/08/31/750414.full.pdf.
Sales, N.G., et al., 2019b. Influence of preservation methods, sample medium and sampling time on eDNA recovery in a neotropical river. Environ. DNA 1 (2), 119–130.
Salter, S.J., et al., 2014. Reagent and laboratory contamination can critically impact sequence-based microbiome analyses. BMC Biol. 12, 87.
Sapountzis, P., et al., 2015. Acromyrmex leaf-cutting ants have simple gut microbiota with nitrogen-fixing potential. Appl. Environ. Microbiol. 81 (16), 5527–5537.
Sasso, T., et al., 2017. Environmental DNA characterization of amphibian communities in the Brazilian Atlantic forest: potential application for conservation of a rich and threatened fauna. Biol. Conserv. 215, 225–232.
Schnell, I.B., Bohmann, K., Gilbert, M.T.P., 2015. Tag jumps illuminated—reducing sequence-to-sample misidentifications in metabarcoding studies. Mol. Ecol. Resour. 15 (6), 1289–1303.

Schnell, I.B., et al., 2018. Debugging diversity—a pan-continental exploration of the potential of terrestrial blood-feeding leeches as a vertebrate monitoring tool. Mol. Ecol. Resour. 18 (6), 1282–1298.
Schoch, C.L., et al., 2012. Nuclear ribosomal internal transcribed spacer (ITS) region as a universal DNA barcode marker for Fungi. Proc. Natl. Acad. Sci. U. S. A. 109 (16), 6241–6246.
Schroeder, J.W., et al., 2019. Host plant phylogeny and abundance predict root-associated fungal community composition and diversity of mutualists and pathogens. R. Shefferson, ed J. Ecol. 107 (4), 1557–1566.
Scibetta, S., et al., 2012. A molecular method to assess Phytophthora diversity in environmental samples. J. Microbiol. Methods 88 (3), 356–368.
Sengupta, M.E., et al., 2019. Environmental DNA for improved detection and environmental surveillance of schistosomiasis. Proc. Natl. Acad. Sci. U. S. A. 116 (18), 8931–8940.
Sheth, B.P., Thaker, V.S., 2017. DNA barcoding and traditional taxonomy: an integrated approach for biodiversity conservation. Genome 60 (7), 618–628.
Shokralla, S., et al., 2016. Environmental DNA barcode sequence capture: targeted, PCR-free sequence capture for biodiversity analysis from bulk environmental samples. bioRxiv. Available at: https://doi.org/10.1101/087437.
Sigsgaard, E.E., et al., 2016. Population characteristics of a large whale shark aggregation inferred from seawater environmental DNA. Nat. Ecol. Evol. 1, 0004.
Smets, W., et al., 2015. A method for simultaneous measurement of soil bacterial abundances and community composition via 16S rRNA gene sequencing. PeerJ Prepr. 3, e1622, e1622.
Soares-Filho, B., et al., 2014. Land use. Cracking Brazil's forest code. Science 344 (6182), 363–364.
Sommeria-Klein, G., et al., 2016. Inferring neutral biodiversity parameters using environmental DNA data sets. Sci. Rep. 6, 35644.
Stackebrandt, E., Goebel, B.M., 1994. Taxonomic note: a place for DNA-DNA reassociation and 16S rRNA sequence analysis in the present species definition in bacteriology. Int. J. Syst. Evol. Microbiol. 44 (4), 846–849.
Strassburg, B.B.N., et al., 2017. Moment of truth for the Cerrado hotspot. Nat. Ecol. Evol. 1 (4), 99.
Strickler, K.M., Fremier, A.K., Goldberg, C.S., 2015. Quantifying effects of UV-B, temperature, and pH on eDNA degradation in aquatic microcosms. Biol. Conserv. 183, 85–92.
Taberlet, P., Coissac, E., Hajibabaei, M., et al., 2012a. Environmental DNA. Mol. Ecol. 21 (8), 1789–1793.
Taberlet, P., Coissac, E., Pompanon, F., et al., 2012b. Towards next-generation biodiversity assessment using DNA metabarcoding. Mol. Ecol. 21 (8), 2045–2050.
Taberlet, P., et al., 2018. Environmental DNA: For Biodiversity Research and Monitoring. Oxford University Press.
Takahara, T., Minamoto, T., Doi, H., 2013. Using environmental DNA to estimate the distribution of an invasive fish species in ponds. PLoS One 8, e56584.
Talaga, S., et al., 2017. DNA reference libraries of French Guianese mosquitoes for barcoding and metabarcoding. PLoS One 12 (6), e0176993.
ter Steege, H., et al., 2013. Hyperdominance in the Amazonian tree flora. Science 342 (6156), 1243092.
Tessler, M., et al., 2017. A global eDNA comparison of freshwater bacterioplankton assemblages focusing on large-river floodplain lakes of Brazil. Microb. Ecol. 73 (1), 61–74.
Tessler, M., et al., 2018. Bloodlines: mammals, leeches, and conservation in southern Asia. Syst. Biodivers. 16 (5), 488–496.
Thomas, A.C., et al., 2016. Quantitative DNA metabarcoding: improved estimates of species proportional biomass using correction factors derived from control material. Mol. Ecol. Resour. 16 (3), 714–726.

Thompson, L.R., et al., 2017. A communal catalogue reveals Earth's multiscale microbial diversity. Nature 551 (7681), 457–463.
Thomsen, P.F., Sigsgaard, E.E., 2019. Environmental DNA metabarcoding of wild flowers reveals diverse communities of terrestrial arthropods. Ecol. Evol. 9 (4), 1665–1679.
Thomsen, P.F., Willerslev, E., 2015. Environmental DNA—an emerging tool in conservation for monitoring past and present biodiversity. Biol. Conserv. 183, 4–18.
Tiedje, J.M., et al., 1999. Opening the black box of soil microbial diversity. Appl. Soil Ecol. 13 (2), 109–122.
Tritsch, I., Le Tourneau, F.-M., 2016. Population densities and deforestation in the Brazilian Amazon: new insights on the current human settlement patterns. Appl. Geogr. 76, 163–172.
Turner, C.R., Uy, K.L., Everhart, R.C., 2015. Fish environmental DNA is more concentrated in aquatic sediments than surface water. Biol. Conserv. 183, 93–102.
Vacher, C., et al., 2016. Learning ecological networks from next-generation sequencing data. Adv. Ecol. Res. 54, 1–39.
Valdez-Moreno, M., et al., 2019. Using eDNA to biomonitor the fish community in a tropical oligotrophic lake. PLoS One 14 (4), e0215505.
Valentin, R.E., et al., 2018. Early detection of invasive exotic insect infestations using eDNA from crop surfaces. Front. Ecol. Environ. 16 (5), 265–270.
Vasco-Palacios, A.M., et al., 2019. Carbon content and pH as important drivers of fungal community structure in three Amazon forests. Plant and Soil. Available at: https://doi.org/10.1007/s11104-019-04218-3.
Vittor, A.Y., et al., 2009. Linking deforestation to malaria in the Amazon: characterization of the breeding habitat of the principal malaria vector, Anopheles darlingi. Am. J. Trop. Med. Hyg. 81 (1), 5–12.
Waring, B.G., Hawkes, C.V., 2015. Short-term precipitation exclusion alters microbial responses to soil moisture in a wet tropical forest. Microb. Ecol. 69 (4), 843–854.
West, A.G., et al., 2019. The microbiome in threatened species conservation. Biol. Conserv. 229, 85–98.
Wilcox, T.M., et al., 2018. Capture enrichment of aquatic environmental DNA: a first proof of concept. Mol. Ecol. Resour. 18 (6), 1392–1401.
Wu, T.H., et al., 2011. Molecular study of worldwide distribution and diversity of soil animals. Proc. Natl. Acad. Sci. U. S. A. 108, 17720–17725.
Yoccoz, N.G., et al., 2012. DNA from soil mirrors plant functional and structural diversity. Mol. Ecol. 21, 3647–3655.
Zinger, L., Philippe, H., 2016. Coalescing molecular evolution and DNA barcoding. Mol. Ecol. 25 (9), 1908–1910.
Zinger, L., et al., 2011. Global patterns of bacterial beta-diversity in seafloor and seawater ecosystems. PLoS One 6, e24570.
Zinger, L., et al., 2016. Extracellular DNA extraction is a fast, cheap and reliable alternative for multi-taxa surveys based on soil DNA. Soil Biol. Biochem. 96, 16–19.
Zinger, L., Taberlet, P., et al., 2019a. Body size determines soil community assembly in a tropical forest. Mol. Ecol. 28 (3), 528–543.
Zinger, L., Bonin, A., et al., 2019b. DNA metabarcoding—need for robust experimental designs to draw sound ecological conclusions. Mol. Ecol. 28 (8), 1857–1862.
Zizka, A., et al., 2018. Finding needles in the haystack: where to look for rare species in the American tropics. Ecography 41 (2), 321–330.