

Review

Environmental DNA Time Series in Ecology

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Ecological communities change in time and space, but long-term dynamics at the century-to-millennia scale are poorly documented due to lack of relevant data sets. Nevertheless, understanding long-term dynamics is important for explaining present-day biodiversity patterns and placing conservation goals in a historical context. Here, we use recent examples and new perspectives to highlight how environmental DNA (eDNA) is starting to provide a powerful new source of temporal data for research questions that have so far been overlooked, by helping to resolve the ecological dynamics of populations, communities, and ecosystems over hundreds to thousands of years. We give examples of hypotheses that may be addressed by temporal eDNA biodiversity data, discuss possible research directions, and outline related challenges.

The Importance of Temporal Data in Ecology

Although ecological processes can be studied with spatial patterns in populations, communities, and ecosystems, there are particular advantages of temporal data for ecology [1]. Because temporal data are unidirectional (environmental changes must occur before their impacts are visible), they provide some of the best means of identifying causal relations involved with environmental change and ecological dynamics [1,2]. This is especially so for large-scale and long-term changes where experimental manipulation is not feasible. Temporal data are also important for making predictions about future patterns and trends in biodiversity because temporal fluctuations in population, community, and genetic structures can provide warning signs of species with a high extinction risk and of possible sudden changes of ecosystem functions, so-called ‘regime shifts’ [3].

Conservation and environmental management can also benefit from long-term data and especially from knowledge on the past states of ecosystems before human impacts, including the widespread expansion of agriculture and the Industrial Revolution [4,5]. This knowledge helps to better define conservation goals and improve their implementation, for example, in discussions about the possible rewilding of landscapes [6].

However, current data sources for temporal ecology often come with important limitations, especially with regard to time span (Box 1). Consequently, there is a recognized gap between the timescales at which biodiversity is studied by ecologists and the scales at which the underlying biological processes take place [7].

eDNA as a Source of Temporal Ecological Data

Here, we argue that eDNA could be a major, but so far underutilized, source of temporal ecological data (Figure 1). While eDNA is increasingly promoted as a tool for monitoring, for example [18,19], its value for hypothesis-driven temporal ecological research can prove just as important.

Highlights

eDNA fills an important data gap for temporal ecology: it provides data over long time periods with high taxonomic breadth, permitting tests of hypotheses and models of biodiversity.

Dynamic biodiversity data obtained with eDNA have the potential to reveal the long-term dynamics of populations, species, communities, and ecosystems in changing environments.

eDNA records both ecological and evolutionary changes and thus may be used to understand eco-evolutionary feedbacks.

Models of how historical environmental changes impact biodiversity may be hindcast validated with eDNA.

Important challenges remain concerning the deposition and preservation of eDNA, the nature of different eDNA archives, and the interpretation of the resulting biodiversity data.

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Box 1. Current Sources of Temporal Biodiversity Data

Several sources of temporal data are currently used by ecologists, each of which has important limitations (Figure 2).

Long-Term Monitoring

Examples of long-term programs include the Continuous Plankton Recorder surveys in the North Atlantic (since 1931) and the Christmas Bird Count (since 1900) in the USA. The importance of these data sets is evident from the insights that they have provided about biodiversity dynamics. For instance, data on the long-term declines of farmland birds have highlighted the impacts of agricultural intensification [8]. Other surveys usually cover shorter time periods, for example, European bird, butterfly, and plant monitoring programs, beginning in the 1980s and 1990s [9]. However, 'little' organisms such as insects (except butterflies), fungi, microbes, soil fauna, etc., are generally undersampled or totally neglected. Overall, biodiversity data from monitoring schemes are heterogeneous, taxonomically strongly biased, often cover only a few decades or less, and come mostly from developed countries [10,11]. Because many candidate drivers of biodiversity (e.g., pollution and habitat change) are collinear on decadal timescales, this constrains our ability to identify the causes of ecological change.

Historical Documents and 'Legacy' Data

People have collected various kinds of quantitative biodiversity data for centuries, providing snapshots of historical ecological communities that can be used to assess changes between the past and present [12]. For instance, many sites that were surveyed by plant and animal ecologists of the early and mid-20th century have been revisited by contemporary researchers to document the changes that have occurred since then [13,14]. However, such data are available for only a limited number of places and times, and their value is affected by uncertainties concerning the comparability of historical and contemporary data.

Paleoecology

Recent calls have been made to combine contemporary ecology and paleoecological data [15,16]. This expands the time span of temporal ecology enormously. As an example, an evaluation of ancient (fossil-based) and modern biodiversity data revealed that large-bodied animals are particularly threatened by extinction in modern oceans, while ancient mass extinctions were either random or had stronger effects on small taxa [17]. However, paleoecological data are severely limited by the availability and discovery of well-preserved and identifiable organismic remains, such as pollen, bones, and shells. In addition, the temporal and/or spatial grain of paleodata is usually larger than those of ecological community dynamics.

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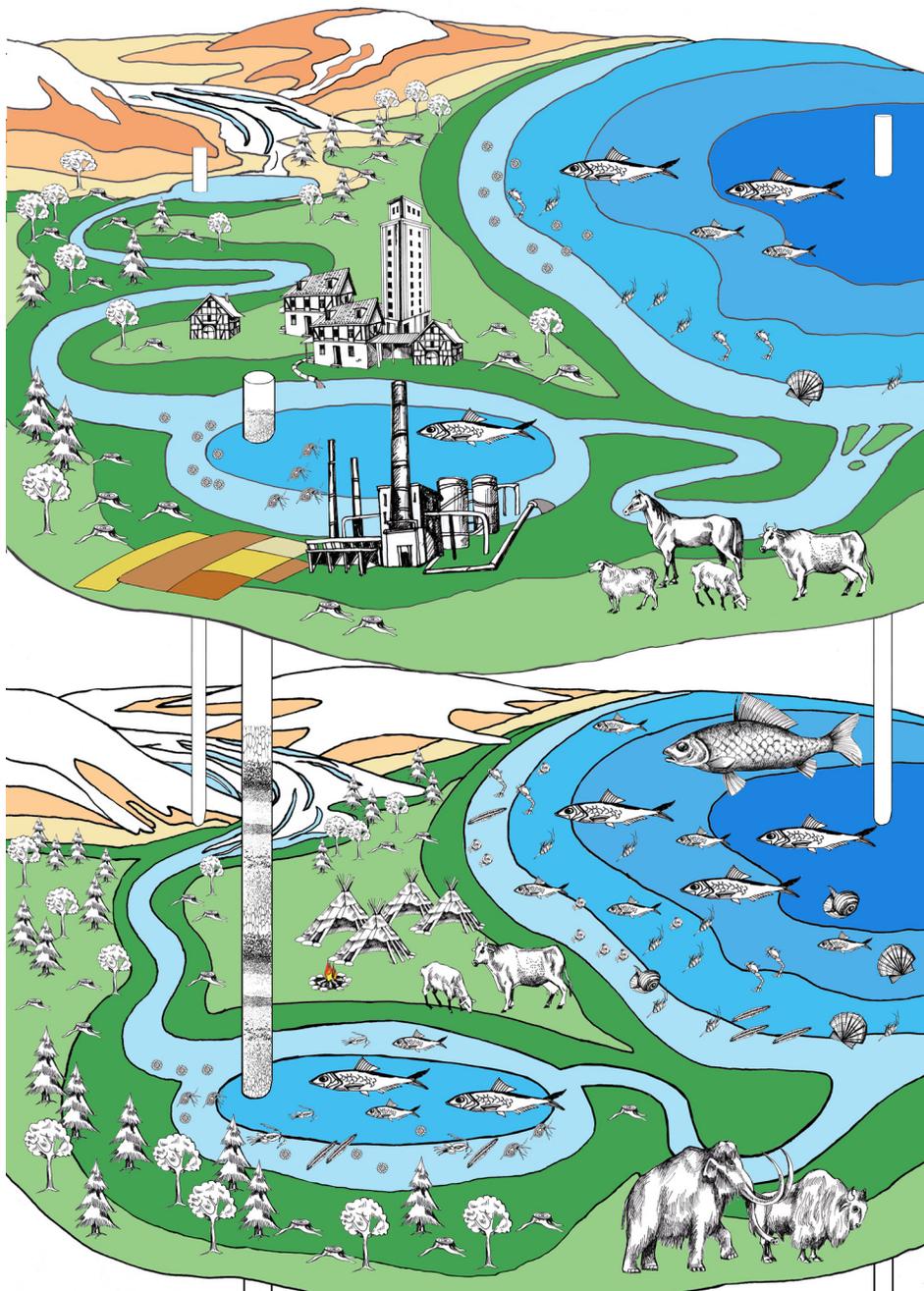
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eDNA can provide continuous temporal data on biodiversity over long time periods for a broader range of species and a wider array of geographic contexts than most other sources of temporal data (Box 2). A unique feature of eDNA data is its potential taxonomic breadth, allowing simultaneous investigation of many taxonomic groups of organisms. Using eDNA, time series can be compared for organisms of different trophic levels and functional groups, potentially with species-level resolution [20,21]. In short, eDNA data fills a gap between paleoecology and paleontology, meta-analysis of modern studies, and long-term experiments (Figure 2). It can be used to test ecological theories, hypotheses, and models that need both long timescales and broad taxonomic coverage. This can assist a deeper understanding of biodiversity dynamics that underlie crucial ecosystem processes and services.

Here, we review examples showing how eDNA was successfully used to resolve research questions in ecology that needed dynamic, taxonomically inclusive biodiversity time series data. We also highlight a broader range of possible future applications and outstanding questions that we think are particularly promising, but for which we are not aware of published examples (Box 4). Compared to previous reviews that largely focus on important technical and methodological issues [20,22], we outline topical ecological and evolutionary questions that can be addressed using these methods, which might be of interest to researchers still unfamiliar with eDNA as a temporal data source. Moreover, we explicitly focus on testing hypotheses, particularly those concerning the roles of natural and anthropogenic environmental changes



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Figure 1. Environmental DNA as a Source of Spatio-Temporal Biodiversity Data. eDNA can be sampled from biological archives, such as sediment cores within lake and marine sediments. The figure shows a representation of human pressures and biodiversity changes that can be studied with eDNA. By providing continuous data on biodiversity patterns between the present day (top) and the past (bottom) over centuries and even millennia, eDNA can inform the tests of hypotheses on (i) the dynamics of single species, such as colonization or extinction (🐟); (ii) community dynamics, such as shifts in community composition and richness (🐞🐛); and (iii) human pressures on ecosystems, such as the impacts of industry (🏭) or agriculture (🌾).

Glossary

Coprolite: fossilized feces.

DNA metabarcoding: high-throughput sequencing of PCR-amplified taxonomic marker genes.

Eco-evolutionary dynamics: mutual feedback between evolutionary and ecological processes occurring on similar timescales.

High-throughput sequencing: the simultaneous sequencing of millions of DNA fragments

Midden: concentrations of waste (e.g., shells, urine, feces) produced by humans or other animals.

Shotgun sequencing: the random sequencing of DNA fragments.

Taphonomy: the deposition, preservation, transport, etc., of biological materials in the eDNA archive.

Taxonomic marker genes: genes regularly used in taxonomic identification [e.g., cytochrome oxidase (*COI*), ribosomal RNA (*16S*, *18S*, *ITS*), ribulose biphosphate carboxylase (*rbcL*)].

Varved sediments: sediments with layers distinguishable at an annual resolution.

Box 2. Temporal Environmental DNA

DNA is frequently extracted from environmental sources and is then referred to as 'environmental DNA,' or eDNA [23]. In a temporal context, sources of eDNA are biological archives, for example, lake and marine sediments (Figure 1) [24,25], soil [26,27], scat [28], dental calculus [29], and feco-urinary middens [30]. Environmental DNA can potentially provide continuous temporal data, while 'ancient DNA' is extracted from discrete sources, such as single bones, seeds, or scales, that rather provide temporal time slices. Paleoecology studies that extract eDNA from sediments often make this distinction by referring to sedaDNA (sediment aDNA), a special type of eDNA [20,22,24]. Upon extraction, genomic regions selected for taxonomic identification (e.g., RNA-coding gene fragments) may be enriched with PCR or DNA capture. Alternatively, the entire sample of eDNA can be sequenced [31], although this is challenging at the data analysis step. Since eDNA fragments tend to be very short [23], there is generally no reason to use long-read sequencing platforms (i.e., from Pacific Biosciences or Oxford Nanopore). Currently, the sequencing is most frequently performed on Illumina platforms: these have huge sequence yield and are generally cost efficient. eDNA requires considerable technical expertise and equipment. Probably the most important prerequisites are access to a clean lab and experience with contamination control procedures (see [22]). Excellent overviews of these requirements, methods, and workflows are already available [20,22,32–34].

The time span covered by eDNA data depends on the survival time of deposited DNA, which can be more than 1 million years under favorable conditions [25,35]. Dating methods try to establish the deposition of the biological archive strata where the DNA is preserved. They rely on decay of isotopes (e.g., ^{14}C and ^{210}Pb), detectable rare dated events (e.g., volcanic eruptions, atomic weapon tests), or the presence of annual lamina in sediments. The temporal resolution of eDNA time series varies from annual (**varved sediments**, [36]) to decadal-centennial, depending on sedimentation conditions and limitations in the precision and resolution of dating.

Unlike much of paleontology, the taxonomic breadth of eDNA data does not depend on the discovery of morphologically identifiable organismal remains; thus, any and all organisms with DNA left behind can be recorded. Biases do occur: for example, not all organisms shed equal amounts of DNA, and DNA may degrade differently in different tissues/species and under different environmental conditions (see also Box 3). Standardized and **high-throughputsequencing** methods allow many samples to be simultaneously processed. For example, Willerslev *et al.* inferred 50 000 years of vegetation history, nematodes, and megaherbivores with 242 samples from 21 Arctic sites in a single study [27].

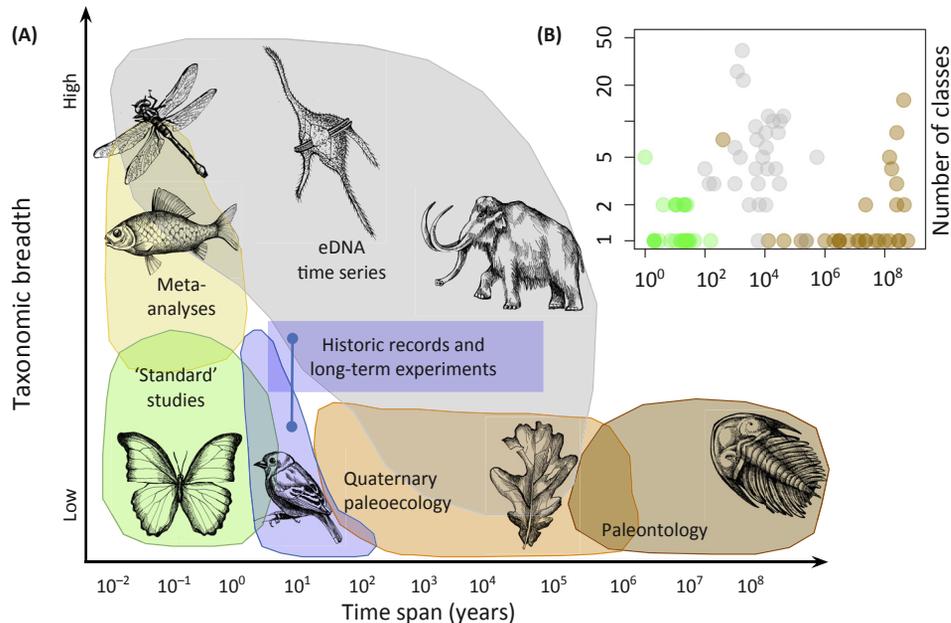
There are many challenges associated with eDNA (Box 3). PCR primers might miss entire taxa [32,34], or DNA concentrations of target taxa may be too low for detection [31]. Processing old eDNA is particularly challenging given the potential for contamination, nucleotide damage, and the difficulties of taxonomic assignment (reviewed by [20,22,33,37]). In Box 3, we provide a brief discussion of the challenges that have received relatively little attention in the literature to date.

on populations, communities, and ecosystems, in contrast to descriptive paleoenvironmental reconstructions.

Applications and Perspectives

Dynamics of Single Species

eDNA can provide a new data source on changes in spatial patterns of occurrence of species and may even potentially show changes in relative abundances through time. So far, this has been especially useful to understand processes related to colonizations and invasions. For example, the colonization route of the Americas by humans after the Last Glacial Maximum is a long-debated issue [31]. **Shotgun sequencing** (see Glossary) of eDNA from lake sediments (combined with radiocarbon dating, plant fossils, and pollen; Box 2) showed that the 1500-km corridor between the retreating Laurentide and Cordilleran ice sheets (along the present-day border between British Columbia and Alberta, Canada) became ecologically suitable for megaherbivores only after 12 600 years BP, considerably later than the first appearance of human settlements south of the ice sheets. This indicates that the first human colonizers used an alternative route along the Pacific coast. Similarly, Olajos *et al.* [38] showed with a continuous, 10 000-year-long eDNA time series from lake sediment cores that colonization by whitefish of a Swedish lake happened immediately after deglaciation from the Baltic sea, and not at a later time point.



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Figure 2. A Niche for eDNA Time Series in Temporal Ecology

For a Figure360 author presentation of Figure 2, see the figure legend at <https://doi.org/10.1016/j.tree.2018.09.003>

(A) Study types (colored fields) in temporal ecology with time spans and taxonomic breadths. Key references anchor study types along the time and taxonomy axes: eDNA time series [27,45], meta-analyses [51], historic records and long-term experiments [106], 'standard' studies [105], quaternary paleoecology [16], and paleontology [107]. (B) Results of a Web of Science literature search along the same axes (y axis in logscale), using the number of classes reported in each study to represent taxonomic breadth. Papers published between 2006 and 2017 were randomly selected (30 for each field): temporal ecology (green), eDNA (gray) and paleoecology & paleontology (brown). Web of Science literature search criteria (A niche for eDNA time series in temporal ecology) Ecology: ("time-series" OR "time series" OR "temporal" OR "long-term" OR "long term") AND year AND (population* OR communit*) AND PUBLICATION NAME: (ecology OR ecology letters); eDNA: ("ancient DNA" AND environment*); paleoecology & paleontology: (fossil* AND ecology* AND (palaeo* OR paleo*)). The paleoecological study with the broadest taxonomic coverage (15 classes) is a meta-analysis of monographic works [108].

The 'native' versus 'non-native' status of species is often a key consideration in conservation, but it is not easily defined over time frames longer than a few decades. Over periods of hundreds to thousands of years, the definition of (non)-native can actually be ambiguous, since human activities have had a variety of effects on species distributions [39]. There is clear potential for eDNA to contribute temporal data critical to establishing more subtle human influences on species distributions, and the impact of introduced species. Ficetola *et al.* used sediment eDNA to refine the invasion history of rabbits introduced to the Kerguelen island, and to show that their impact on the local vegetation exceeds the impact of recent climate change [40]. Beyond placing species on the native-to-non-native continuum, spatially and temporally explicit eDNA data can be used to address important questions about invasion ecology, such as the frequency of introduction and establishment events [41,42].

Abundance data contain more information on the dynamics of populations than presence/absence data and hence potentially allow for more powerful tests of the factors affecting populations. However, there are many challenges regarding the extraction of abundance

information from eDNA. At the very least, eDNA could provide information on changes in species' relative abundances through time by using the number of sequenced eDNA reads as a quantitative index (see [Box 3](#) for discussion of problems and solutions on abundance or biomass estimation). Long-term monitoring is occasionally able to capture the influence of extreme events on populations [\[43\]](#). However, with the longer time series permitted by eDNA, the effect of extreme events on population abundances could be potentially better quantified. Population viability analysis (PVA) aims to estimate extinction risks of endangered species and assess the role of catastrophic events that cause sharp reductions in

Box 3. Challenges

Numerous challenges associated with eDNA have already received considerable attention: biases associated with PCR primers [\[32,34\]](#), detection issues [\[31\]](#) for contamination, nucleotide damage, and taxonomic assignment. These problems are relatively well reviewed (see, e.g., [\[20,22,32,33,37\]](#)). Here, we aim to discuss additional challenges that are less commonly mentioned in the literature.

Taphonomy

eDNA observations depend on preserving substrates. Particles that bind DNA may be transported and reworked by erosion [\[81\]](#). The vertical movement of DNA (leaching) may confound the interpretation of eDNA data [\[37\]](#). Some studies on soils find evidence of such movement [\[82,83\]](#), while others do not [\[84\]](#). Leaching is less likely to be an issue in aquatic sediments [\[24\]](#).

Imperfect Sampling of eDNA

A species may be present at a site, but not detected (a false negative, [\[85\]](#)), or a species may be erroneously recorded due to contamination (a false positive). Imperfect eDNA sampling, particularly the issue of false positives, is challenging [\[86\]](#), but it is increasingly addressed with species occupancy models [\[38\]](#). It is increasingly advocated to use positive standards (i.e., artificial samples with known taxonomic composition) and negative controls in all laboratory steps [\[87\]](#). The sequence information from these may inform the parametrization of occupancy models.

Organismic Abundance/Biomass

The use of eDNA to examine changes in species abundance is in its infancy, but it holds great potential [\[58,88\]](#). The most serious problems are (i) variation in the copy numbers of marker genes among and within species, (ii) temporal variation in DNA deposition and preservation, and (iii) differential detection probabilities of operational taxonomic units (OTUs) due to primer bias during the PCR (specific to **metabarcoding**: it makes cross-species comparisons potentially unreliable [\[89\]](#)). We expect that increase in genome data availability [\[67\]](#) will help to solve the first problem. A potential strategy from paleoecology that has been proposed to address the second problem is to standardize the read numbers of each OTU by the cumulative sum of OTU reads in a given sample. This standardization could help cancel out effects of temporal variation in sedimentation and decaying rates [\[90\]](#). However, the robustness of this approach requires further investigation. A solution to the third, metabarcoding-specific problem might be to exploit the relationship between occurrence and abundance [\[91\]](#) with technical replicates (e.g., replicated PCRs): if a species occurs in more technical replicates of the same sample, it implies that this species also contributed more DNA to this sample [\[85\]](#). This theoretically may not completely solve the problem since occurrence is also influenced by primer bias, although to a smaller extent than read abundances [\[89\]](#). Many of the techniques that address similar issues for direct monitoring data (differential detection probabilities of species due to differences in behavior, morphology, or habitat) might also be applicable for eDNA [\[86\]](#). Quantitative PCR [\[24\]](#) and digital droplet PCR [\[92\]](#) are further options to get better abundance/biomass data for single, or a few, species.

Dead or Alive?

Ecosystem functions can be inferred from microbial genes, but many microorganisms actually live in sediments. DNA is continuously released from recently dead cells and contributes to the eDNA pool [\[93\]](#). For temporal inferences, it is important to separate long-dead versus recently dead DNA. There are three emerging solutions. (i) DNA may be extracted from both extracellular and intracellular fractions. Reads from the intracellular fraction are assumed to originate from alive organisms and removed [\[94\]](#). A similar approach might be to co-extract DNA and RNA, and then remove RNA-reads from DNA reads since RNA must originate from living organisms [\[94\]](#). (ii) DNA degrades with age and this may help authentication [\[95\]](#). (iii) Studies may focus on microbes that require oxygen or light: their DNA must originate from dead organisms since they cannot live in sediments [\[25,96,97\]](#).

abundance [44]. However, catastrophic scenarios used in current PVA models are often arbitrary because the frequency distribution of catastrophes is usually unknown [44]. Single-species time series of relative abundances may also be used to study the value of early warning signals, such as slower recovery and increased variance, for predicting the switch to an alternative stable state [3].

Community Dynamics

eDNA sampling generally provides simultaneous information on multiple taxa and hence is particularly valuable for questions concerning community dynamics. Many temporal eDNA studies have provided evidence for historical environmental changes driving shifts in community composition and hence information on the relative importance of specific environmental drivers over long timescales. For example, changes in microbial eukaryotic communities over two thousand years in lakes of the French Alps and Greenland reflect major climate variations; however, impacts of anthropogenic eutrophication became relatively more important beginning in the 1940s [45]. Stoof-Leichsenring *et al.* [46] used lake sediment eDNA to test whether the composition of diatom communities along Siberian environmental gradients are shaped by the environment or by geography. Their results emphasize the importance of environmental conditions (particularly vegetation), at least during the past 7000 years.

The investigation of biotic interactions in community assembly is one of the most exciting applications of eDNA in ecology [47], since the large taxonomic breadth increases the opportunity to obtain simultaneous data for different trophic levels (e.g., plant–herbivore or predator–prey level). Given these successes, eDNA is clearly promising to characterize changes in food web structure through time [24]. Bohan *et al.* used machine learning to reconstruct food webs from pit trap–sampled time series of invertebrate communities [48], and we are not aware of a study that reconstructs all parts of an interaction network exclusively from eDNA time series. Probably the closest to this is the reconstruction of vegetation and megafaunal diet in the Arctic for the past 50 000 years by using **taxonomic marker genes** that were PCR amplified from permafrost-preserved eDNA [27]. A higher occurrence of mammoth, woolly rhino, horse, reindeer, and elk in forb-dominated environments suggested a preference for forbs in these species.

eDNA may also permit tests of hypotheses about specific resource use of organisms. Welker *et al.* [49] used eDNA from **coprolites** to reconstruct the diet composition of the extinct Balearic mountain goat (in combination with pollen and plant macrofossil analyses). The results showed that the species' extinction 4000–5000 years ago was linked to the climate-driven drastic decrease of its main food plant and not necessarily to hunting by humans. eDNA extracted from dental calculus was used to reconstruct the food sources of distinct Neanderthal communities, with results showing that these humans were not strictly carnivorous but that they used a variety of locally available foods [29].

Beyond predator–prey relationships, eDNA may permit historical studies of other biotic interactions, such as mutualisms. Zobel *et al.* [50] used the Arctic vegetation data of Willerslev *et al.* [27] to show that plant–heterotroph mutualist traits (i.e., associations with mycorrhizae or nitrogen-fixing bacteria) predicted plant responses to major climatic events over the past 50 000 years. Their approach relied on the assumption that present-day relationships among plants and mutualists are stable over time. A novel application would be to test this assumption by investigating long-term occurrence patterns for both plants and mutualists with eDNA.

Human Pressures on Ecosystems

Although a few data sets extend further back [14,51], surveys to monitor the state of biodiversity and its changes mostly began towards the end of the 20th century [9]. However, human impacts via land use, especially relating to agriculture, urbanization, and climate change, began much earlier. The lack of pre-impact data makes the full consequences of human activities for biodiversity difficult to determine. Attempts have been made to provide older baseline data by using a combination of historical documents [52–56] or other sources (such as paleo, archeo, recent monitoring, living memory). eDNA so far has been rarely used [57] in these frameworks, although it holds great potential for providing better baseline biodiversity data. Moreover, avoiding the notion of a single set-in-stone baseline, eDNA could help characterize the historical range of variability of biodiversity, with relevance for the definition of conservation goals.

Several studies have already taken advantage of the potential for eDNA to cover long timescales to quantify human impacts on biological communities. Time series of sediment eDNA from Canadian lakes were used to test hypotheses about the causes of an increased frequency of algal blooms [58]. The incidence of cyanobacteria was compared before and after the establishment of a national park, within which land-use change was minimal. No difference was found in the cyanobacterial trends between sites within and outside the park, suggesting that the cause of more frequent blooms was not land-use change, but rather a large-scale driver, most likely climate change. Studies have even begun to reconstruct earlier human impacts, such as the effects of livestock farming after the Neolithic Period [59,60]. A particular concern in conservation is that land-use activities can cause critical and lasting transitions in ecosystems. eDNA has been used to demonstrate that cyanobacteria communities did not return to pre-eutrophic stages after the reversal of lake eutrophication, at least on a timescale of 100 years [61].

Biotic homogenization of communities by human activities has been documented in many studies using traditional long-term monitoring data [51] and has become a major concern for conservation. However, eDNA permits quantification of biotic homogenization over longer timescales and may potentially help to identify the drivers. A recent study linked the 20th century homogenization of peri-Alpine cyanobacterial communities to climate change through the selection of taxa that thrive in lakes with strong thermal stratification of the water column [62], a phenomenon associated with rising temperatures in mountain areas.

eDNA can be particularly helpful for inferring the impacts of human activities in parts of the world where other sources of temporal biodiversity data are scarce (Box 1), for instance, in the tropics. In a study in tropical African lowlands, eDNA was used to record shifts in agriculture through the detection of both sweet potato and the invasive water hyacinth in an area where they were introduced in the 16th century [63]. Such applications may be possible also on soils to track historically cultivated plants [64], although DNA **taphonomy** in soils needs further examination (Box 3).

Evolutionary Dynamics in Ecology

It is increasingly recognized that evolution may occur on ecological timescales, resulting in feedback loops between selective forces, evolutionary responses, and ecological dynamics [65]. Both neutrally evolving genes and genes under selection can be used to study these processes, but there are very few examples where eDNA was used to this end. In a pioneering study, Sigsgaard *et al.* used modern eDNA fragments of neutrally evolving mitochondrial genes to infer population genetic characteristics from a whale shark aggregation [66]. eDNA can simultaneously provide both ecological (occurrence, abundance, community composition) and evolutionary (genetic) information. In principle, it seems promising to obtain both neutral and adaptive evolutionary information through time by capturing or PCR amplifying of candidate genes (Box 4)

Box 4. Key Temporal Ecology Questions for eDNA

Here, we identify prominent ecological research areas for which eDNA could prove to be a new data source for more rigorous testing of some classic hypotheses.

Species Richness Patterns

Species richness often monotonically increases with productivity on regional scale [98,99]. Two hypotheses have been proposed to explain this increase: (i) priority effects causing multiple alternate stable states and (ii) 'rock-paper-scissors' (RPS) scenarios (intransitive competition). Priority effects have been found in ecosystems dominated by long-lived organisms [100], while RPS scenarios have been suggested for short-lived organisms such as plankton [101]. Since only priority effects predict no temporal community change (at least in the absence of environmental change), temporal data, such as from eDNA, are essential to separate these hypotheses and assess the role of species' life histories.

Metacommunity Dynamics

The dynamics of any local community is linked to the dynamics of nearby communities through dispersal, with potentially ecosystem-wide effects [102]. This means that the broader regional biota may be affected by the cumulative effects of species interactions across local communities. Current work has almost exclusively focused on purely spatial patterns due to lack of suitable temporal-spatial data (Box 1), but this may be solved by eDNA.

Anthropogenic Impacts

eDNA is likely to permit quantification of population trends for taxa that are poorly represented by standard temporal data sources (Box 1). Insect abundance is suspected to have declined in recent times in western Europe based on data from German protected areas [103]. Although such data are rare, eDNA may allow studies of the relationship between insect declines and particular land management practice (e.g., use of neonicotinoid pesticides).

Evolutionary Dynamics

eDNA may be used to target candidate genes under selection through time (e.g., temperature or drought tolerance), increasingly identified in extant genomic studies [104]. Accordingly, temporal evolutionary processes that involve physiology may become accessible through eDNA, in a major step forward compared to current approaches that study long-term evolutionary change through fossils and subfossils: the stability of morphological characters often emphasizes stasis, while physiological adaptation is often highly dynamic.

Testing Biodiversity Models to Improve Future Predictions

Scenarios describing biodiversity responses to expected changes in anthropogenic drivers are increasingly being used to inform management decisions. The relevance of scenario models could be tested through hindcasting, but this is rarely done, mainly because contemporary time series data have limited taxonomic, temporal, and geographic coverage [10,11]. The situation can be improved through the use of eDNA, as such data provide long enough time series with relevant taxonomic and geographic scope.

from eDNA, but we are not aware of any published studies to date. Targeting candidate genes from eDNA underlines the importance of high-quality reference genomes, which are becoming increasingly available from several emerging biodiversity genome initiatives [67].

Beyond the study of *a priori*-identified genes (neutrally or non-neutrally evolving), further improvements in access to sequencing throughput and computational power might enable eDNA-based, genome-wide environmental association studies. Such an approach will require three elements. First, high-quality reference genomes of the organisms of interest are needed (see above; [67]). Second, massive sequencing throughput and computing power are needed. We estimate that it would take about 15 Tb of sequenced eDNA to obtain the genome of an abundant eukaryotic organism (DNA from the species representing 0.1% of the total eDNA extracted from a sediment sample) with 30X sequencing coverage (assuming a genome size of 0.5 Gb). If only a single or few species are targeted, enrichment techniques may substantially lower the sequencing needs. Third, data about relevant environmental change are needed, which can be gained from proxy data or long-term historic surveys.

In the proposed genome-wide environmental association studies, the reference genome(s) can be used to map species-specific reads from the pool of massively sequenced eDNA. As most reads likely originate from different individuals, the resulting mapping yields genome-wide estimates of SNP allele frequencies of a population. The structure of the data is thus similar to that of a PoolSeq approach [68]. The allele frequency changes through time are then associated to environmental changes to identify regions of selective change in the genome. With linear regression, Bayesian methods, and latent factor mixed modeling, several analysis frameworks for the association of genomic and environmental change are available [69]. Such a brute force approach would yield genome-wide information on many selective and neutral processes through time, including gene-flow estimates [70] and answers to questions about **eco-evolutionary dynamics** such as whether changes in community composition are preceded by population-level 'attempts' to adapt.

Ecosystem Functioning Responses

Studies regarding the impacts of human activities on Earth have increasingly emphasized ecosystem services (e.g., the Intergovernmental Science-Policy Platform on Biodiversity and Ecosystem Services). Already, patterns in temporal data indicate that some key ecosystem services are being threatened by contemporary global change, while others (e.g., food production) have increased [71,72]. However, ecosystem functions and services are relatively recent concepts, and existing temporal biodiversity data (Box 1) are not sufficient to understand how community composition and diversity relate to resilience [73] and ecosystem function [74].

The connections of biodiversity and ecosystem functions are frequently studied with assumptions about species traits and their roles in given ecosystems. For microorganisms, the links between traits and certain ecosystem functions are frequently inferred from taxonomic identity (but see Box 3 for challenges). As an example, methanotrophy in lakes is caused by phylogenetically distinct aerobic and anaerobic bacteria [75]. A quantitative PCR-based application from the French Jura (combined with stable isotope analyses) showed that these methanotrophic bacteria were an important food source for chironomid midges over the past 1500 years [76]. Furthermore, potential functional genes often allow the inference of functional traits of bacteria and archaea (but not necessarily of eukaryotes, [77]). Sedimentary eDNA was used to link the presence of genes involved in aerobic ammonia oxidation (an important source of internal nitrate loading of lakes) to variations in nutrient levels and salinity during the past 18 500 years in a large Tibetan lake [78]. Functional genes from eDNA in lake sediments have also been used to infer the dynamics of ecosystem services, such as the presence of genes responsible for microcystin synthesis (cyanobacterial toxins, threatening drinking water supplies and irrigation) in times of increased human impact during the past 200 years [79]. The approach can be easily extended to other genes that encode ecologically important functions, for example, genes involved in nitrogen- and phosphorus-uptake systems, antibiotics, and pollutant resistance [80].

Concluding Remarks

eDNA increasingly receives attention as a source of spatial and temporal biodiversity data to reconstruct community compositions at particular time points in the past as well as for monitoring schemes for the future. Moreover, we suggest that the ability of eDNA to provide continuous time series data, simultaneously across many taxa, can be exploited to a much greater extent than it is at present (see Outstanding Questions). Specifically, temporal data from eDNA may permit explicit tests of ecological hypotheses concerning the drivers of long-term biodiversity change. These tests are important to understand global change, but they are

Outstanding Questions

What is the taphonomy of DNA in different archives? The physico-chemical processes that affect the deposition and preservation of DNA are poorly understood, even in the best-known non-frozen archives: aquatic sediments. Understanding these processes is, however, important for an informed interpretation of eDNA biodiversity data.

To what extent and under what conditions can soils be used as eDNA archives? Closely related to the question above, the processes that influence eDNA in soils are particularly interesting since soils are probably the most common archives of terrestrial environments. A whole array of new possibilities arises in ecology if soil-preserved eDNA provides reliable access to temporal biodiversity data.

How representative is eDNA sampling? eDNA archives are mostly samples at a single point in space, for example, lake communities are described based on horizons in a single sediment core. Sampling of multiple cores will help evaluate the amount of sampling variation and determine whether single cores are sufficient.

Differential preservation of species in eDNA? It is likely that detection of a given species in eDNA is influenced by properties such as biomass, lifestyle, habitat, etc. Although for plants it is relatively clear that eDNA mostly originates from vegetative tissues (rather than pollen), other taxon groups are much less studied in this regard.

How does temporal data from eDNA differ from that collected via other methods? Comparing eDNA data with other data sources could validate the use of eDNA as a temporal data source. The use of hierarchical models to account for false positives as well as false negatives may be verified by using taxon lists from other sources. Abundance data from direct observation could be compared with eDNA-based estimates of biomass to assess the similarities and differences in how they capture biodiversity change.

difficult or impossible to perform with traditional temporal data sources. While challenges in methods development remain, eDNA has the potential to further expand biodiversity research into the temporal dimension.

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