Biomonitoring for the 21st Century: Integrating Next-Generation Sequencing Into Ecological Network Analysis

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Abstract

Ecological network analysis (ENA) provides a mechanistic framework for describing complex species interactions, quantifying ecosystem services, and examining the impacts of environmental change on ecosystems. In this chapter, we highlight the importance and potential of ENA in future biomonitoring programs, as current biomonitoring indicators (e.g. species richness, population abundances of targeted species) are mostly descriptive and unable to characterize the mechanisms that underpin ecosystem functioning. Measuring the robustness of multilayer networks in the long term is one way of integrating ecological metrics more generally into biomonitoring schemes to better measure biodiversity and ecosystem functioning. Ecological networks are nevertheless difficult and labour-intensive to construct using conventional approaches, especially when building multilayer networks in poorly studied ecosystems (i.e. many tropical regions). Next-generation sequencing (NGS) provides unprecedented opportunities to rapidly build highly resolved species interaction networks across multiple trophic levels, but are yet to be fully exploited. We highlight the impediments to ecologists wishing to build DNA-based ecological networks and discuss some possible solutions. Machine learning and better data sharing between ecologists represent very important areas for advances in NGS-based networks. The future of network ecology is very exciting as all the tools necessary to build highly resolved multilayer networks are now within ecologists reach.

1. INTRODUCTION

Traditionally, community ecology tends to focus on patterns of species richness and community composition, while ecosystem ecology focuses on fluxes of energy and materials. Ecological networks (sometimes called food webs for trophic interactions), however, provide a quantitative framework to combine these approaches and unify the study of biodiversity and ecosystem function (Thompson et al., 2012). Ecological networks, which describe which species are interacting with which (i.e. qualitative networks) as well as the strength of their interactions (i.e. quantitative networks), are now routinely used to understand ecosystem 'robustness' to species extinctions (Evans et al., 2013; Säterberg et al., 2013), quantify ecosystem services (Derocles et al., 2014a; Macfadyen et al., 2009) or examine the impacts of environmental change (Morris et al., 2015; Thompson and Gonzalez, 2017; Tylianakis et al., 2007). By using a burgeoning range of metrics to describe network structure, complexity and stability (see Arnoldi et al., 2016; Bersier et al., 2002; Donohue et al., 2013; Dunne et al., 2002a,b), ENA is considerably improving our understanding of ecology and evolution, with a growing number of applications for biomonitoring (Bohan et al., 2017; Gray et al., 2014). Indeed, ENA is increasingly being used to assess ecosystem response to environmental changes (e.g. climate change, pollution, invasive species; Aizen et al., 2008; Blanchard, 2015; Bohan et al., 2017; Thompson et al., 2016). There is consequently a growing shift in biodiversity monitoring away from conventional species and community-level descriptions towards a more comprehensive and mechanistic approach using species interaction networks (Bohan et al., 2013; Derocles et al., 2014a; Evans et al., 2013; Fontaine et al., 2011; Gray et al., 2014; Ings et al., 2009; Kéfi et al., 2012; Macfadyen et al., 2009; Pocock et al., 2012; Wirta et al., 2014).

Nevertheless, ecological networks can be difficult to construct with conventional approaches and suffer some major pitfalls mainly centred on sampling issues, taxonomic misidentification and/or incorrect species interactions (Evans et al., 2016; Gibson et al., 2011). Major errors occurring in either of these steps could ultimately affect network-level structural metrics and thus our understanding of ecosystem functioning (Novak et al., 2011). DNA-based methods (based on combined taxonomic identification and interaction data from DNA sequences) have the potential to overcome many of these issues, providing large, highly resolved, phylogenetically structured networks suitable for rapid and reliable biomonitoring (Bohan et al., 2017; Evans et al., 2016; Vacher et al., 2016; Valentini et al., 2009b).

Today, next-generation sequencing (NGS) or high-throughput sequencing (see Goodwin et al., 2016 for a review) can rapidly generate millions of DNA sequences. Sequences can describe, very precisely, not only the biodiversity present within an ecosystem, but also species interactions, the data from which can then be used to construct ecological networks (Evans et al., 2016). Recently, ecological network studies have taken advantage of NGS to successfully construct networks (e.g. Toju et al., 2014 for a plant–fungus network). Advances in statistical modelling and machine learning approaches bring a new opportunity to predict species interactions and rapidly build multilayer ecological networks from DNA sequences data generated with NGS (Vacher et al., 2016).

Despite species identification from DNA sequences commonly being seen as a universal way to identify species (Hebert et al., 2003), the NGS technology to build food webs is not applied uniformly in network ecology. Experimental designs (field sampling and molecular protocols) and the construction of ecological networks are heavily dependent on the ecosystem studied, and particularly on the type of interactions (see Box 1). Here, we

BOX 1 Species interactions in ecological networks

Species interactions are a major component of ecosystem functioning. In ecological communities, a wide range of interactions can be described and visualized as ecological networks. These include direct and indirect interactions. Direct interactions relate to cases where a species directly affects another (i.e. species A impacts species B). Indirect interactions refer to cases where the impact of a species on another is mediated or transmitted by a third species (i.e. a first species A affects a second species B through an intermediary species C).

In ecological networks, direct interactions are usually described and collectively shape the structure of the networks. However, this does not mean that indirect interactions are ignored as ecological networks are also used as a framework to study indirect interactions such as resource competition (Tilman, 1982), apparent competition (interactions through shared natural enemies; Derocles et al., 2014a; Holt, 1997; Morris et al., 2004; van Veen et al., 2006) or trophic cascades (Hairston et al., 1960; Oksanen et al., 1981). Indeed, indirect interactions result from the cooccurrence of several direct interactions. Hence, because the purpose of ecological networks is to describe the set of (direct) species interactions in an ecosystem, building networks constitutes a powerful approach to identify potential indirect interactions. Within a network it is, for example, possible to detect shared natural enemies when searching for cases of apparent competition—a particular instance of three-node network motifs (Stouffer et al., 2007).

Table 1 summarizes a general classification of direct ecological interactions. Although a wide range of interactions occur in nature, ecological network

BOX 1 Species interactions in ecological networks—cont'd

Table 1 Direct Interactions Between Two Species (According to Lidicker, 1979; see Faust and Raes, 2012)

Type of Interaction	Effect on Species A	Effect on Species B	Nature of Interaction	Examples in Ecological Networks
Mutualism	**Positive**	**Positive**	**Mutual benefits of the species**	Plant–pollinator, plant–ant, plant–seed disperser, plant–fungi
Interference competition	Negative	Negative	Species have negative effect on each other	
Trophic/ predation	**Positive**	**Negative**	**Predator gains at the expense of the prey, which is killed. We include here prey–predator, plant–consumer and host–parasitoid interactions**	Host–parasitoid, prey–predator, plant–herbivore
Parasitism	**Positive**	**Negative**	**Parasite develops at the expense of the host, which is not killed**	Host–parasite, host–pathogen
Commensalism	Positive	Null	Species A is benefited, species B is not affected	
Amensalism	Null	Negative	Species A has a negative effect on species B, but species A is not affected	
Neutralism	Null	Null	Neither species is affected	

The interaction types studied in depth in network ecology are in *bold*.

Continued

BOX 1 Species interactions in ecological networks—cont'd

studies to date have tended to focus on three types of interactions: parasitism, mutualism and trophic interactions. Other types of interactions have been studied (see Allesina and Levine, 2011; Coyte et al., 2015; Mougi, 2016 for networks with competitive interactions), but they are relatively rare in comparison with the large majority of studies dealing with trophic and mutualist networks. A complementary classification was established by Pantel et al. (2017) accounting for the degree of interaction immediacy: whether the interaction takes place over a short or long part of an organism's life cycle. This distinguishes, for example, parasitism from predation, scramble competition from contest competition and mutualistic symbiosis from external mutualism. However, when discussing species interactions in this chapter, we will be referring to parasitism, mutualism and trophic interactions.

distinguish two cases in particular. First, NGS can be directly used to build quantitative ecological interactions between organisms by resolving species interactions (e.g. Evans et al., 2016; Kitson et al., 2016; Piñol et al., 2014; Toju et al., 2013, 2014). This use of NGS data is, however, only possible in ecosystems in which relationships between organisms can clearly be established, such as host–parasitoid interactions where the parasitoid can be detected within the host (Derocles et al., 2014a, 2015; Wirta et al., 2014), prey–predator interactions by detecting prey in gut contents (e.g. Piñol et al., 2014; Tiede et al., 2016) or faeces (Clare et al., 2014; Zeale et al., 2011; see Symondson and Harwood, 2014) and plant–pollinator interactions by using high-throughput sequencing to identify the pollen carried (Bell et al., 2017; Galimberti et al., 2014; Pornon et al., 2016; Sickel et al., 2015). Second, there are systems in which it is impossible (or logistically very problematic) to detect interactions between organisms and assessing whether these interactions are positive or negative, such as those within microbial (Jakuschkin et al., 2016) or planktonic communities (Lima-Mendez et al., 2015). For these systems, NGS approaches can only identify cooccurring species and their relative abundance. NGS data then need to be combined with theoretical approaches, including statistical modelling (Faust and Raes, 2012) or machine learning (Bohan et al., 2011a), to predict species interactions from their abundance patterns and finally to build ecological networks (Bohan et al., 2017; Kamenova et al., 2017; Vacher et al., 2016). These two ways of building ecological networks have their own specificities and challenges to overcome but also share common problems. These problems are

related to (1) the qualitative and quantitative reliability of NGS data (i.e. polymerase chain reaction (PCR) bias and errors, sequencing bias and estimation of species abundances and frequency of interactions with number of NGS reads; Sommeria-Klein et al., 2016); (2) the identification of nodes and interactions in the network (inferring species interactions with statistical models when interactions are not directly resolved by molecular tools); (3) the costs of the sequencing technology and the expertise needed to process the data (Toju et al., 2013, 2014; Vacher et al., 2016).

Here, we bring new insights on how to integrate NGS and ENA into biomonitoring (Fig. 1). We first consider why ecological networks provide a suitable framework for a better understanding of biodiversity and ecosystem functioning and how they can be used to complement or supersede conventional biomonitoring approaches. Second, we underline the challenges that ecologists face in building ecological networks when DNA-based tools are not available (which represent the vast majority of food web studies in the literature). Third, we demonstrate how molecular methods, NGS in particular, can overcome (at least partially) the numerous constraints inherent in conventional network construction methodologies (e.g. taxonomic identification, insect rearing, fieldwork issues), while considering the challenges of using NGS tools for building networks. Fourth, we give insights on how to overcome NGS data issues and efficiently build networks through machine learning and data sharing. Finally, we discuss new areas of research and development centred on ENA of multilayer networks to ultimately create more resilient ecosystems.

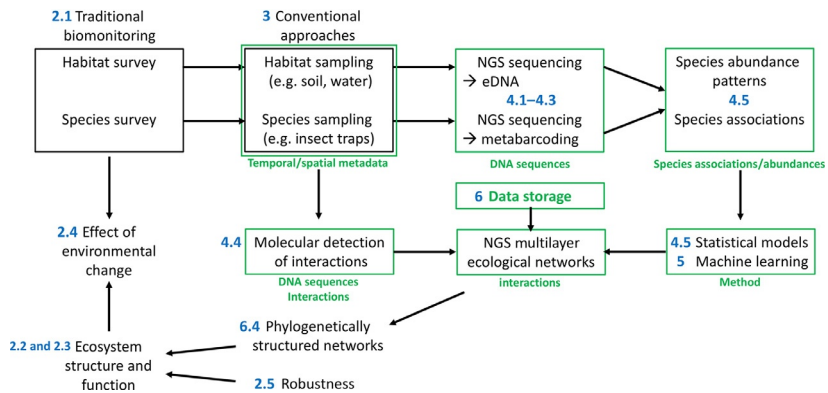

Fig. 1 A road map to integrate NGS and ENA into biomonitoring. The successive steps are discussed in this chapter, and the corresponding sections are indicated in *blue*. Data stored and shared for an efficient biomonitoring are indicated in *green*.

2. HOW ARE ECOLOGICAL NETWORKS USEFUL FOR BIOMONITORING?

2.1 Traditional Biomonitoring Is Typically Descriptive and Rarely Provides an Understanding of the Underlying Mechanisms Behind Ecosystem Functions

Biomonitoring of change lies at the core of ecosystem conservation, management and restoration. As biomonitoring is an obligation today, biomonitoring programs are framed by government organizations (e.g. European Commission, Joint Nature Conservation Committee in the United Kingdom). In its simplest form, biomonitoring consists of recording species diversity and abundances across different locations and times using a range of ecological census techniques and taxonomic identification. Most biomonitoring sampling methodologies were developed in the middle of the 20th century (Bohan et al., 2017) and were selected for entirely pragmatic reasons that reflected the current state of knowledge, simplicity and cost. Indicators are sampled to evaluate risks to human health and the environment for communication to the public or government policy makers. These include pesticide residues, elements and metabolites as pollution indicators, while abundances of target species or community descriptors are used to assess the ecological condition of ecosystems. However, these established methodologies are often of low generality. They are also often limited to particular ecosystems of species and communities of study and may not allow comparison between different systems. The evaluation of the myriad of changes in ecosystems that can occur is simply too costly, time-intensive and not necessarily captured by current biomonitoring indicators.

Consequently, biomonitoring of the full diversity of species and their interactions within an ecosystem is rarely, if ever, attempted (Bohan et al., 2017). While traditional biomonitoring is useful for simple conservation purposes such as identifying biodiversity hot spots or mapping 'functional gaps' in ecological communities (Forest et al., 2007; Myers et al., 2000; Raxworthy et al., 2003), such an approach is clearly not suited to the task of predicting the consequences of human actions that specifically target particular species or habitats. This is due to the fact that these human actions can have unintended consequences that spread through the network of species interactions at different spatial and temporal scales (Estes et al., 1998; Polis et al., 1997). For instance, traditional biomonitoring schemes have repeatedly failed at predicting the consequences of species

introductions and have only just begun to look for guidance in interaction network approaches (David et al., 2017; Médoc et al., 2017; Pantel et al., 2017).

2.2 Ecological Networks Provide a Framework to Describe and Monitor Ecological Processes and Ecosystem Functions

Networks have become a prominent tool for studying community and ecosystem ecology, as they serve as a generic, conceptual framework for undertaking research across a broad range of ecological systems. Ecological networks, famously described by Darwin as the 'tangled bank', describe the interactions between species, the underlying structure of communities and the function and stability of ecosystems (Montoya et al., 2006). Historically, the ecologist Charles Elton pioneered the concepts of food chains and food webs, organizing species into functional groups (Elton, 1927; see also Cousins, 1987; Polis, 1991). These concepts formed the basis for ecologist Raymond Lindeman's classic and landmark paper on trophic dynamics (Lindeman, 1942). The examination of networks has then been spurred by now classic studies such as the keystone predation experiments and theory (Paine, 1966, 1969, 1974), the complexity–stability debate (Gilpin, 1975; MacArthur, 1955; May, 1972, 1973a,b) and the search for invariant patterns linking, for example, species diversity with the number of links in food webs (Briand and Cohen, 1984; Cohen and Briand, 1984; Cohen and Newman, 1985; Cohen et al., 1990; Pimm, 1980; Stenseth, 1985; Williams and Martinez, 2000, 2004). The past decade in particular has seen significant advances in the theoretical understanding, construction, analysis and application of complex species interactions networks (see Fontaine et al., 2011; Kéfi et al., 2012 for reviews). This area of ecology has been marked by two trends: (i) the building of more sophisticated models aimed at predicting and/or explaining the structure of ecological networks based on a variety of mechanisms (e.g. Allesina et al., 2008; Bascompte et al., 2003; Canard et al., 2012; Dalla Riva and Stouffer, 2016; Eklöf et al., 2013; Jordano, 1987; Jordano et al., 2003; Lewis and Law, 2007; Rohr et al., 2016; Williams and Martinez, 2000; reviewed in Kamenova et al., 2017) and (ii) the search for more precise data (in particular, taxonomic identification), and practical methods to obtain them, that has chiefly been done to counteract the tendency to lump together insufficiently described species that reduces the ability to make predictions and identify food web invariants (Novak et al., 2011; Solow and Beet, 1998; Yodzis, 1998). More recently,

Thompson et al. (2012) proposed using ecological networks as a conceptual framework to reconcile biodiversity and ecosystem function studies.

A network approach can be built on current biomonitoring schemes: if interaction data is collected alongside conventional monitoring of biodiversity, then it is possible to start monitoring both biodiversity and ecosystem functioning (see Mulder et al., 2006 for an example for soil microbial communities). For example, plant surveys could be complemented with insect flower visitation data to create plant–flower–visitor networks. Conversely, when pollinators are targeted by biomonitoring programs, the pollen carried by the species could be identified and used to create pollen-transport networks. These complementary approaches could be implemented in traditional biomonitoring methodologies and would give a better understanding of ecological processes through the construction of networks. Taking a step further, a combination of NGS and ENA together could provide a radically new approach to understand how environmental change affects ecosystems.

2.3 Ecological Network Structure Characterizes Ecosystem Properties

To measure changes in ecosystems, a wide range of metrics have been developed to encapsulate the emergent architecture of the networks (see Bersier et al., 2002). ENA relies on a wide range of network descriptors to assess the effect of environmental changes on ecosystem function. Ma et al. (2017) discuss descriptors of network complexity, such as connectance (a measure of network complexity), modularity (representing compartmentalization within the network) and nestedness (i.e. nodes with few connections linked to a subset of nodes interacting with more connected nodes) and their importance for detecting changes occurring in ecosystems (see Fortuna et al., 2010; Poisot and Gravel, 2014 for a critical view of some network metrics). Metrics of consumer–prey asymmetries are, in addition, very important to consider. The effect of environmental changes may vary across a food web (Thompson et al., 2012), and a change in an ecosystem may go undetected using measures of network complexity but nevertheless can affect consumer–prey asymmetries, with consequences on ecosystem function and services. Such asymmetries can be described as 'vulnerability' and 'generality' introduced by Schoener (1989) as, respectively, the mean number of consumers per prey and the mean number of prey per consumer within a food web. These consumer–prey asymmetry metrics are particularly well suited to the study of host–parasitoid networks (Derocles et al.,

2014a; Wirta et al., 2014). Other metrics of ecological network structure have also been proposed as determinants of ecosystem properties, such as the existence of fast and slow energy channels (Rooney et al., 2006), negative relations between interaction strength and the length (Neutel et al., 2002) of the trophic loop it is part of, or the frequency of network motifs (Stouffer et al., 2007).

Ecological processes such as pollination, pest control and seed dispersal are historically and still currently well studied in network ecology. These processes rely on mutualist and antagonist interactions with structural properties that can be characterized with network descriptors. Mutualist networks are, for example, often described as nested structures (Bascompte et al., 2003; Thébault and Fontaine, 2010). Network structure thus constitutes an efficient indicator of pollination quality (Kaiser-Bunbury et al., 2017). Similarly, a compartmentalized (or modular) structure often emerges from antagonist networks (Derocles et al., 2014a; Ma et al., 2017). Compartmentalized networks have important implications for natural pest control as they suggest a high specificity between the pest species and their natural enemies. With the current threat to food security (Godfray et al., 2010), ENA could help in our understanding of the underlying mechanisms involved in pest control and provide indicators to help agroecosystem management.

Nevertheless, characterizing ecosystem properties through ENA must be done with caution. Most networks metrics are highly dependent on sampling completeness (see Blüthgen et al., 2006; Jordano, 2016; Rivera-Hutinel et al., 2012). Consequently, the effort spent to sample and characterize an environment may directly affect the structure highlighted. Since DNA is ubiquitous in ecosystems, NGS constitutes a promising way to overcome the sampling completeness issues in ENA.

2.4 Knowledge of Ecological Networks Helps to Assess the Effect(s) of Environmental Changes on Ecosystem Processes and Associated Services

Ecological networks are increasingly (but not systematically) used to assess the effects of environmental changes on ecosystems as they provide a more complete description of ecological processes than conventional community or species-oriented approaches. For instance, Tylianakis et al. (2007) demonstrated that habitat modification altered the structure of networks of cavity-nesting bees, wasps and their parasitoids. The altered network structure had effects on parasitism rate, with consequences on ecosystem services such as pollination and biological control. A striking result from this study

was that, despite only little observed variation in species richness, marked changes arose in network structure. Evans et al. (2013) demonstrated in an organic farm model system that two particular seminatural habitats (representing less than 5% of total area of the farm) were disproportionately important to maintain the integrity of the overall network, and thus of the associated ecosystem services (i.e. natural pest control, pollination). More recently, Kaiser-Bunbury et al. (2017) showed that ecosystem restoration in mountaintop communities affects the network structure in a positive way with a higher functional redundancy in restored communities. This modification of network architecture had direct and positive effects on the reproductive performance of the most abundant plant species. Thus, the development and application of ENA represent a paradigm shift in the biomonitoring of ecosystems (Kaiser-Bunbury and Blüthgen, 2015). However, empirical studies of this sort are still relatively rare in the literature, mainly because of the underlying network construction process. In particular, theoretical links between network structure and ecological function need to be better established. In this context, ecological network modelling has made some impressive progress in the understanding of ecosystem functioning. For example, the allometric food web model designed by Schneider et al. (2016) established the link between the diversity of animal communities and primary productivity. They demonstrated that diverse animal communities are more exploitative on plants but do not reduce plant biomass because this communities are composed of energetically more efficient plant and animal species. Network modelling such as the allometric food web model can therefore complement empirical studies. Consequently, more collaborative research between empirical and theoretical network ecologists is urgently needed and could be especially useful in helping to address a number global challenges, such as climate change, biodiversity loss and food security.

2.5 The Robustness of Networks of Ecological Networks: Applications for Understanding Species and Habitat Loss, Restoration and Building Ecosystem Resilience

The study of network 'robustness' (Dunne et al., 2002a,b; Memmott et al., 2004) has grown rapidly in recent years, partly driven by advances in computational modelling (Kaiser-Bunbury et al., 2010; Staniczenko et al., 2010), but mostly by the objective of understanding the threat of biodiversity loss to ecosystem services and functioning (Astegiano et al., 2015; Pocock et al., 2012). Studies have progressed from simple qualitative, bipartite mutualistic

networks (Memmott et al., 2004), to investigations of patterns across ecosystems (Srinivasan et al., 2007) and to current quantitative approaches that take into account species abundance (Kaiser-Bunbury et al., 2010).

Pocock et al. (2012) constructed and analysed a 'network of ecological networks' (i.e. 11 groups of animals interacting with shared plants on farmland), providing new analytical tools for understanding both the consequences of species extinctions across multiple animal groups, and the potential for ecological restoration. The study provided a method to calculate the relative importance of plants, and thus identified some plants that were disproportionately important in the network of networks (i.e. common agricultural plants such as clover *Trifolium* and thistle *Cirsium* spp.). Although yet to be tested empirically, one application of this approach is that important plants could be targets for conservation and restoration that would benefit multiple animal groups. By examining the robustness of the joined networks, the study found that animal groups varied in their robustness to sequences of plant extinction, with the plant–pollinator network exhibiting much lower robustness than the seed-feeding bird network. Therefore, using a network approach, it should be possible to identify more sensitive groups for targeted conservation effort and/or assessment for biomonitoring rather than spending limited funds on charismatic species. Evans et al. (2013) developed this approach further by modelling the cascading effects of habitat loss, driven by plant extinctions, on the robustness of multiple animal groups. Habitat robustness analysis identified two seminatural habitats (i.e. waste ground and hedgerows together comprising $<5\%$ of the total area of the farm) as disproportionately important to the integrity of the overall network. This provides another tool for directing the management of multiple-habitat sites and landscape restoration, although it is yet to be tested empirically. Field and landscape-scale manipulations are required to both test and improve robustness models as a way of increasing the resilience of ecosystems.

More recently, Pilosof et al. (2017) demonstrated that the multilayer network from Pocock et al. (2012) provides much more realistic information on the stability and robustness of ecological communities than the examination of a single disconnected monolayer network (e.g. a bipartite host–parasitoid network). Parasitoid extinctions (representing a major aspect for the natural pest control) differ between scenarios purely based on the plant–parasitoid network and more comprehensive scenarios considering a multilayer network of both plant–parasitoid and plant–flower–visitor interactions. As flower visitors are involved in plant pollination, pollinator extinctions lead

to secondary plant extinctions and tertiary parasitoid loss. This demonstrates that the biomonitoring of ecosystems cannot be realized reliably without considering the myriad of interactions occurring between organisms, as everything is connected in an ecosystem (Evans et al., 2017).

The robustness of interactions calculated from multilayer networks represents a powerful indicator of the ecological condition of an ecosystem and should therefore be developed further in the context of biomonitoring programs. Multilayer network approaches allow the long-term monitoring of the fragility of key components of ecological processes and ecosystem services such as plant–flower visitor networks (i.e. pollination) or insect pest–parasitoid networks (i.e. natural pest control) across spatial scales. With the development of ENA and the availability of NGS, we foresee a complementary use of traditional biomonitoring indicators (i.e. species richness, population surveys) with new indicators based on the architecture of ecological networks (in particular, the robustness) which are ultimately much more intimately linked to ecological processes.

3. ECOLOGICAL NETWORKS CAN BE CHALLENGING TO BUILD USING CONVENTIONAL APPROACHES

Despite their proven value in ecological research, networks are nevertheless limited by the difficulties of building them. These difficulties are centred around three major issues: (i) the sampling effort required to capture a significant range of species interactions; (ii) the reliable identification of specimens; and (iii) the adequate description of interactions between the organisms (see Box 1).

First, detecting the majority of species and their interactions within a network requires monumental effort. The challenges increase with the species richness in the ecosystem, the spatial scale of the habitat/ecosystem of interest and the temporal scale over which interactions are being considered. For example, the biodiversity of tropical ecosystems is much more difficult to assess accurately than its equivalent in arctic environments or temperate agroecosystems (Lewinsohn and Roslin, 2008; Morris et al., 2004), even if the latter is not trivial to study either (Derocles et al., 2014a, 2015; Evans et al., 2013; Macfadyen et al., 2009; Pocock et al., 2012; Wirta et al., 2014). Moreover, quantifying any aspect of species diversity in order to monitor environmental changes in biodiverse regions runs into major issues of scale-dependency (e.g. Dumbrell et al., 2008), raising further logistical challenges associated with repeatedly monitoring species diversity,

while environmental changes modifying the spatial (and most likely temporal) scaling properties of species within these systems. These problems all arise from the sampling effort and associated logistical constraints required to detect a representative and significant proportion of the species living in the ecosystem (Gotelli and Chao, 2013; Gotelli and Colwell, 2011; Jordano, 2016). Furthermore, all species are not sampled equitably (and some of them simply cannot be sampled at all; Valentini et al., 2009b). For example, temporally transient species (e.g. due to migration or phenology), which when present may have a disproportional influence on network interactions, are almost always ignored. Thus, these issues all lead to a biased view of biodiversity, which favours reporting the presence of species that are the most conspicuous and easiest to sample. As sampling effort and completeness greatly impact the inferred structure of ecological networks, it is now usual to quantify network sampling completeness (see Costa et al., 2016) using estimators such as Chao 2 (Chao, 1984; Colwell and Coddington, 1994). This approach partially alleviates the sampling issues (assuming high sampling completeness is attained), but ecologists still need new tools for a more exhaustive detection of species and interactions.

Second, accurate species identification remains a major challenge, with two separate but related issues. The first issue is that accurate and reliable species identification requires specific taxonomic expertise for the studied group (Derocles et al., 2012a; Evans et al., 2016). Consequently, if multiple taxonomic groups are studied, many taxonomists may be required to assess the biodiversity within a network (Valentini et al., 2009a). Hence, reliable morphological identification may not always be possible for all taxa. For example, the existence of cryptic species (i.e. hard-to-identify species using morphological criteria) may lead to an underestimation of the species richness within ecosystems, resulting in biases at the network level (Derocles et al., 2016; Hebert et al., 2004; Kaartinen et al., 2010; Smith et al., 2006, 2007, 2008) and inaccurate model predictions (Novak et al., 2011). The second issue is that numerous taxa cannot be identified in situ (e.g. microbes) and require additional laboratory processing that is often limited due to financial constraints. In the case of microbial species, this is further hindered by the need to culture them in order to provide sufficient numbers for identification. This provides a major identification bias as most microbial taxa are not readily cultivable in the laboratory. In network ecology, misidentifications can be very problematic as they may bias the structure and distribution of interactions. As species may interact with numerous other organisms at different network levels, each identification error is compounded

with each interaction across the network. Consequently, sufficient sampling and accurate identification are crucial steps in the construction of highly resolved ecological networks.

Third, exhaustively describing the possible range of species interactions that structure ecosystems is an onerous task (Bohan et al., 2013) and sampling all interactions of even a single type is conditioned by the number of observations (Blüthgen et al., 2008). While most species interactions are hard to identify in the field, some of them simply cannot be detected or observed with traditional sampling methods (see Jordano, 2016). As discussed by Gotelli and Colwell (2011), sampling biodiversity is very labour-intensive and often fails to detect most of the species in an ecosystem. For example, the construction of mutualist networks (e.g. plant–pollinator and plant–flower visitor interactions in Pocock et al., 2012) requires laborious and time-consuming field observations: it is therefore very hard to exhaustively capture mutualist interactions. Similarly in food webs built from host–parasitoid interactions (Derocles et al., 2012b; Gariepy et al., 2008), specimen sampling and rearing in the laboratory are imperfect for most taxonomic groups (Derocles et al., 2012b, 2015; Evans et al., 2016). For instance, rearing hosts sampled in the field until the emergence of adult parasitoids is a very challenging task. Indeed, both hosts and parasitoids have a high risk of dying during the rearing process, hence compromising the identification of host–parasitoid interactions. In webs based on prey–predator interactions, a 'Russian doll' effect may lead to the detection of false interactions from morphological (or molecular) identification of gut contents (Woodward et al., 2012). In this latter case, the prey is not directly consumed by the predator from which the gut content was analysed, but in the gut content of an intermediate consumer present in the focal predators gut. Consequently, overlooking these cases of secondary predation may lead to unrepresentative ecological networks.

Finally, some interactions cannot realistically be observed in the field despite providing valuable information on ecosystem services, such as seed–ground beetle interactions associated with weed regulation by Carabids (Bohan et al., 2011b), or belowground plant–microbe interactions, such as arbuscular mycorrhizae that influence terrestrial ecosystem productivity (Fitter et al., 2005). When relying purely on classic approaches (i.e. field observations, specimen rearing, morphological identification) to build ecological networks, the construction of networks becomes risky.

In order to limit the complexities and costs of describing complete ecological networks for a given ecosystem, most ecological network studies to

date have assessed ecosystem function and services by studying a subsample of the network and focusing on particular types of interactions (e.g. mutualist or trophic interactions). The choice of subsampling (according to the question addressed) makes sampling, field observation, specimen rearing and taxonomic identification logistically possible. Focussing on a subset of interactions also illustrates the a priori expectations ecologists have on the underlying role of some taxonomic groups on ecosystem functions and services. Many ecological networks studies to date may better reflect the interactions which are easy to study or that ecologists think more important, rather than an actual representation of ecosystem functioning. This can lead to key aspects of networks being overlooked: there are still vast numbers of as yet 'unknown' interactions that need to be described and their role in ecosystem function evaluated. For example, in an agricultural network, a machine learning approach discovered an unexpected role for predatory spiders as prey (Bohan et al., 2011a; Tamaddoni-Nezhad et al., 2013), a finding confirmed by subsequent gut content analyses (Davey et al., 2013), giving a new mechanistic insight into the role of spiders in agroecosystems. The application of combined approaches, such as machine learning and NGS, that are less limited by the a priori expectations and assumptions of ecologists could greatly expand and speed up the discovery of links to build a more holistic and exhaustive view of ecological networks (Bohan et al., 2017).

Pocock et al. (2012) were among the first to assess multiple types of interactions that were pooled in a 'network of ecological networks' in the context of farmland ecosystem services and functioning, providing new insights into the robustness of these interconnected networks (Evans et al., 2013). These networks of networks were built using conventional methodologies that rely on field observations or rearing specimens followed by morphological identification by taxonomists. Although species interactions were highly resolved and well quantified for many of the subnetworks (e.g. plant–insect pollinators), others were potentially subject to bias (e.g. plant–leafminer–parasitoids) because of the limitations of taxonomically selective rearing success and the reliance on accurate morphological identification. Given that the construction of such networks is labour-intensive, building larger, highly resolved ecological networks in a wide range of ecosystems is likely to be hindered until more cost-effective methodologies can be developed. The application of NGS technology is one such method that is likely to revolutionize network ecology.

Advances in DNA-sequencing technologies are answering previously intractable questions in functional and taxonomic biodiversity and provide

enormous potential to determine hitherto difficult to observe species interactions. Thus, DNA-based approaches, NGS in particular, hold the potential to provide many of the solutions to the problems described earlier (Bohan et al., 2017; Evans et al., 2016; Vacher et al., 2016). Combining DNA-barcoding technologies with ENA offers important new opportunities for understanding large-scale ecological and evolutionary processes (such as invasive species, see Kamenova et al., 2017), as well as providing powerful tools for building ecosystems that are resilient to environmental change (see Evans et al., 2016 for a conceptual framework). Until recently, ecological networks represented therefore a powerful but challenging approach to establish and were consequently difficult to integrate in biomonitoring. As discussed in the next section, NGS technologies together with the prediction of interactions with statistical modelling and machine learning represent now an exciting opportunity to include ENA more systematically into biomonitoring.

4. COMBINING NGS WITH ENA: OPPORTUNITIES AND CHALLENGES

4.1 Using NGS to Construct Ecological Networks

Currently, ecological networks constructed using DNA-based approaches are not used to regularly monitor ecosystems. This may be partially due to the historical reliance on classic field survey methods in network ecology, which rely on observation, specimen sampling, laboratory rearing and morphological identification to construct bipartite networks. Recent work has demonstrated that NGS can be rapid, universal and relatively cheap, in comparison to conventional (i.e. 'traditional' taxonomy based) approaches to assess biodiversity (Beng et al., 2016; Ji et al., 2013; Liu et al., 2013). Beyond the characterization of biodiversity, NGS can also be used to efficiently build ecological networks (see Evans et al., 2016; Toju et al., 2014; Vacher et al., 2016). First, NGS has the potential to directly establish species interactions (Evans et al., 2016; Kitson et al., 2016; Toju et al., 2014). Second, with metabarcoding and eDNA approaches, NGS can also generate millions of DNA sequences which then can be processed and used in statistical models to construct ecological networks (Vacher et al., 2016).

However, molecular approaches and NGS in particular are yet to be widely used to build ecological networks. Non-NGS molecular approaches such as diagnostic PCRs (using taxon-specific primers to detect targeted species in samples) and Sanger sequencing approaches (see Table 2)

Table 2 Comparison of the Main Sequencing Technologies

Sequencing Platform	Sequencing Generation	Amplification Method	Sequencing Method	Read Length (bp)	Error Rate (%)	Error Type	Number of Reads Per Run	Time Per Run (Hours)	Cost Per Million Bases (USD)
Sanger ABI 3730xl	1	PCR	Dideoxy chain termination	600–1000	0.001	Indel–Substitution	96	0.5–3	500
Ion Torrent	2	PCR	Polymerase synthesis	200	1	Indel	8.2×10^7	2–4	0.10
454 Roche GS FLX+	2	PCR	Pyrosequencing	700	1	Indel	1×10^6	23	8.57
Illumina HiSeq 2500; high output	2	PCR	Synthesis	2×125	0.1	Substitution	8×10^9 (paired)	7–60	0.03
Illumina HiSeq 2500; rapid run	2	PCR	Synthesis	2×250	0.1	Substitution	1.2×10^9 (paired)	24–144	0.04
Illumina MiSeq v3	2	PCR	Synthesis	2×300	0.1	Substitution	3×10^8	27	0.15
SOLiD 5500xl	2	PCR	Ligation	2×60	5	Substitution	8×10^8	144	0.11
PacBio RS II: P6-C4	3	Real-time single-molecule template	Synthesis	~10,000–15,000	13	Indel	$3.5–7.5 \times 10^4$	0.5–4	0.40–0.80
Oxford Nanopore MinION	3	None	Nanopore	~2000–5000	38	Indel–Substitution	$1.1–4.7 \times 10^4$	50	6.44–17.90

Based on Schendure, J., Ji, H., 2008. Next-generation DNA sequencing. Nat. Biotechnol. 26, 1135–1445; Glenn, T.C., 2011. Field guide to next-generation DNA sequencers. Mol. Ecol. Resour. 11, 759–769; Niedringhaus, T.P., Milanova, D., Kerby, M.B., Snyder, M.P., Barron, A.E., 2011. Landscape of next-generation sequencing technologies. Anal Chem. 83, 4327–4341; Liu, L., Li, Y., Li, S., Hu, N., He, Y., Pong, R., Lin, D., Lu, L., Law, M., 2012. Comparison of next-generation sequencing systems. J. Biomed. Biotechnol. 2012, 251364; Escobar-Zepeda, A., Vera-Ponce de León, A., Sanchez-Flores, A., 2015. The road to metagenomics: from microbiology to DNA sequencing technologies and bioinformatics. Front. Genet. 6, 348; Rhoads, A., Au, K.F., 2015. PacBio sequencing and its applications. Genomics Proteomics Bioinformatics 13, 278–289; Weirather, J.L., de Cesare, M., Wang, Y., Piazza, P., Sebastiano, V., Wang, X.-J., Buck, D., Au, K.F., 2017. Comprehensive comparison of Pacific Biosciences and Oxford Nanopore Technologies and their applications to transcriptome analysis. F1000Res. 6, 100.

remain more intuitive and easier for network ecologists to understand than NGS (Derocles et al., 2014a, 2015; Traugott et al., 2008; Wirta et al., 2014). These two explanations focus on why network ecologists have yet to fully embrace NGS approaches over more traditional methods. The flip side to this argument is that molecular ecologists using NGS for metabarcoding studies have yet to fully realize the potential of the data they generate. The vast majority of NGS studies quantifying the diversity of ecological communities have heavily relied on descriptive statistics based on classical measures of community diversity, and/or changes in species composition between samples. However, data are often collected in such a way that (ecological) networks could be constructed, but are not, and the vast potential of the NGS data thus remains unrealized.

Conventional approaches to ecological network construction have some major drawbacks that could make them inefficient in the biomonitoring of ecosystems. Visual species identification (with or without microscopy) can sometimes be slow and labour-intensive at best, or unreliable at worst. Diagnostic PCRs need very good prior knowledge of the species composition of the ecosystem monitored, because they require the design of multiple PCR primers to detect the full range of species. For Sanger sequencing, costs increase linearly with experiment size and quickly become too expensive for large-scale biomonitoring.

In contrast, NGS metabarcoding may scale more efficiently to large samples compared with microscopy, diagnostic PCRs and Sanger sequencing, providing opportunities for much more intensive sampling of species interaction networks than has previously been possible. The investment in time and materials goes 'per plate' (96, 384 or 1536 samples) rather than 'per sample', although for large numbers of samples, additional sequencing runs may be required, which increases the cost. However, the number of samples that can be processed with a single sequencing run varies widely depending on a range of factors, including sequencing technology, chemistry techniques, and the quality of DNA extraction and amplification procedures (see Table 2). While in principle highly useful, it is these technical, and some of the theoretical issues linked to the use of NGS to quantify interactions that may have limited their adoption by researchers. We discuss ways of overcoming these limitations below and foresee that the construction of ecological networks using NGS will soon become commonplace and be integrated into biomonitoring schemes.

4.2 PCR Bias and Abundance Estimation in NGS Community Analyses

Understanding the limitations of molecular approaches in detecting species interactions is fundamentally important when correctly designing a sequencing approach and interpreting the produced network. Primer and amplification biases are well-known phenomena in the PCR. Mismatches between primer and binding site sequences or structural and compositional variation in the DNA strand can lead to variation in PCR efficiency (Polz and Cavanaugh, 1998), causing two distinct issues, respectively: (1) the inability to detect species present within the sample as the primer mismatches exclude their detection, and (2) the preferential amplification of DNA from some species at the expense of amplifying DNA from others (a cloning step is added to separate mixed DNA sample; e.g. Dunshea, 2009). However, for metabarcoding where the aim is to parallel sequence an entire community (or to identify two parties in an ecological interaction), this becomes more critical as differing PCR efficiencies among species can result in a final PCR product composition that is not representative of the input DNA composition (although some authors have reported broad correlations e.g. Elbrecht and Leese, 2015; Leray and Knowlton, 2017; Razgour et al., 2011). In practical terms, biases can lead to false negatives and read depths that are of no use for determining quantitative or even relative community composition (Elbrecht and Leese, 2015; Leray and Knowlton, 2017; Piñol et al., 2014). The most common approach to dealing with this is to develop PCR primers that are as general and unbiased as possible (Elbrecht and Leese, 2015; Leray et al., 2013), but even these are prone to the above-mentioned biases and a certain degree of PCR-induced bias is now commonly acknowledged in PCR-based metabarcoding studies (Leray and Knowlton, 2017). That said, in some instances by using carefully designed primers and targeting genes which vary in base-pair composition but not structural properties among species, elimination of PCR amplification biases is entirely possible (Cotton et al., 2014), and researchers should continue to pursue development and validation of these unbiased approaches.

Some authors have attempted to circumvent this by using a metagenomic approach (e.g. Tang et al., 2015) where they sequence all DNA present in their extraction and then filter the resulting sequences to only retain the data of use for identifying species. In theory, with no PCR step, there is no amplification bias, so read depths are more representative of the input DNA composition. In practice, the relationship between community

composition and metagenomic read depth is not so simple. The availability of mitochondrial DNA (mtDNA) for extraction varies significantly with tissue mass and metabolic activity (e.g. there is a significant nonlinear increase in mitochondrial count in developing oocytes; Cotterill et al., 2013), and this relationship can be further modified by tissue type and age (Barazzoni et al., 2000). This bias concerns mtDNA (mainly used to identify animals), but similar issues surround plastid DNA (used for plant identification), and the overall metagenome is often swamped with 16S ribosomal DNA gene reads (used for microbe identification), which may mask the presence of rarer higher taxa. Even if it were possible to know how read counts vary with tissue mass/type/age for all the organisms in our community (e.g. the contribution of multicellular organisms to eDNA has been modelled by this chapter; Sommeria-Klein et al., 2016), relative read counts can be further skewed by differences in ease of DNA extraction across taxa (Schiebelhut et al., 2017) and the extraction method used to obtain the DNA (Deiner et al., 2015; Vesty et al., 2017). Taken together we are forced to conclude that, as currently performed, metabarcoding is not generally suitable for estimating tissue biomass from sequence data (Clare, 2014) and thus any such metabarcoding-based estimations would have to be idiosyncratically calibrated using conventional abundance surveys (as in Tang et al., 2015). Estimation of abundances is also problematic except for single-celled organisms for which they can be assessed accurately by targeting genes with minimum amplification biases (see Fischer et al., 2017). For higher taxa, however, only relative abundances can be recovered from NGS data. Relative abundances may nevertheless have a limited use in a conservative biology perspective and thus in biomonitoring as well (Clare, 2014).

4.3 NGS Without a Prior PCR Step

A significant drawback of the metagenomic approach is cost (see Table 2). By sequencing all DNA in an extraction, researchers greatly limit the sample size per sequencing run and thereby either increase the sequencing costs for the study or, in fixed cost studies, reduce the statistical power of the study dramatically. They also discard much of the available read depth when they filter reads to only those of direct interest. One general approach to solve this is to enrich the DNA to be sequenced for a specific genomic region without PCR, avoiding PCR bias. This can be achieved in several ways, but the most common is to use an hybridization approach that employs sets of degenerate probes that can bind to target DNA and then themselves be bound to

magnetic beads (Gnirke et al., 2009) or a solid substrate (Albert et al., 2007) with nontarget DNA simply being washed away. The enriched DNA is biased towards useful genomic regions so a smaller proportion of the sequencing reads are discarded and more samples can be included in a sequencing run (see 'sequencing coverage' in the glossary). Variations of this approach exist, which range from very simple centrifugation-based approaches (Macher et al., 2017) to much more complex methodologies using isothermal DNA replication (Dapprich et al., 2016) that can allow researchers to enrich for extremely long genomic regions suitable for the latest sequencing technologies such as Pacific Bioscience (PacBio) Single Molecule, Real-Time (SMRT) sequencing and Oxford Nanopore MinION (see Table 2). Depending on the laboratory, these approaches can be highly scalable, but their utility for community assessment is yet to be proven.

4.4 Detection of Species Interactions Using Molecular Tools

As an alternative to attempting to infer relative abundances via read depths of bulk extracted communities, it is possible to simply analyse individual organisms separately and link the metadata for each sample (i.e. individual). In this situation, the number of samples for each species is a proxy measure of relative abundance. If the sample contains multiple DNA templates arising from a species interaction (e.g. predator gut contents, host/parasite systems or plant–pollinator systems), then it is possible to use molecular tools to detect these species interactions in a quantitative or semiquantitative manner. Prior to the advent of parallel sequencing technologies, this would have been achieved by one of the following two broad approaches. First, PCR diagnostic approaches use sets of primer pairs that each produce species-specific bands of different lengths (or with different attached fluorophores as in microsatellite genotyping) that can then be separated by gel or capillary electrophoresis (e.g. aphid/parasitoid interactions Traugott et al., 2008; predatory beetle gut contents King et al., 2011). Second, PCR amplification of all DNA in a sample using general primers, separating PCR products via cloning (e.g. Dunshea, 2009) or gel electrophoresis, followed by Sanger sequencing (e.g. Kitson et al., 2013). Third, the design of primers specific to a taxonomic group (e.g. a parasitoid family or a genus of prey) amplifying a short but variable region (such as a fragment of COI) is another approach to resolve species interactions (Derocles et al., 2012b; Fayle et al., 2015). This method allows identification of an interaction that is occurring by relying first on a PCR diagnostic (e.g. a parasitoid within a host, a prey with a

gut content) and then to identify the nature of the interaction by sequencing the organisms detected (Derocles et al., 2012b; Rougerie et al., 2011). Thus, this approach was successfully applied to build ecological networks in a farmland system (Derocles et al., 2014a) and an arctic system (Wirta et al., 2014). However, because of the linear cost of the Sanger sequencing and/or the time to process samples with these molecular tools, these approaches allow to examine a relative limited number of organisms (e.g. a low number of hosts).

The advent of NGS technologies allows researchers to parallelize this process and work more effectively on a larger scale. The use of PCR primers tagged with known sequences to track samples is well established in NGS (Binladen et al., 2007), and this has been shown to be effective not only for community metabarcoding (Yu et al., 2012) but can also be used to build webs. Toju et al. (2013, 2014) were one of the first to use NGS (454 pyrosequencing) to resolve species interactions between trees and arbuscular mycorrhizal fungi and then build ecological networks from that data. One step further, Shokralla et al. (2015) and Cruaud et al. (2017) showed that a 'nested tagging' approach for amplicons involving two rounds of PCR permits (see nested PCR in the glossary) extensive multiplexing to increase throughput of barcoding programs, and Evans et al. (2016) have proposed this as an approach to building larger, replicated networks in ecological studies. In the future, it is likely that these sorts of nested tagging approaches will be combined with PCR-free approaches to sequencing to allow quantified networks to be produced while reducing concerns over PCR bias and missing interactions caused by false negatives.

All the molecular approaches described earlier represent tools able to rapidly characterize the biodiversity of ecosystems or describe species interactions. There is no doubt that this area of research is expending very rapidly as that new advances must be expected, pushing the limits of the description of biodiversity and the understanding of ecosystems. In the future, we believe that NGS will be fully integrated by ecologists to build networks and will be a usual approach of biomonitoring programs.

4.5 How to Deal With Interactions Not Directly Resolved by NGS: Are Species Association Networks Species Interaction Networks? The Case of Microorganisms

For several decades, ecological networks have been constructed from the observations of both the species and their interactions (Ings et al., 2009; Poisot et al., 2016b). Databases of observation-based ecological networks,

such as plant–pollinator and predator–prey interaction networks, have been compiled (e.g. Interaction web database IWDB; https://www.nceas.ucsb.edu/interactionweb/) and used to describe the architecture of biodiversity (Bascompte and Jordano, 2007; Bascompte et al., 2003; Jordano et al., 2003; Lewinsohn et al., 2006; Olesen et al., 2007), to understand how species have assembled through time (Peralta, 2016; Rezende et al., 2007; Vacher et al., 2008), to elucidate the network properties that sustain species coexistence (Allesina and Tang, 2012; Tang et al., 2014; Thébault and Fontaine, 2010) and to predict the resistance and resilience of ecosystem functions to environmental change (Memmott et al., 2004; Schleuning et al., 2016; Vanbergen et al., 2017). These observation-based networks, however, often remain incomplete (Chacoff et al., 2012; Jordano, 2016), despite intense efforts to make them as realistic as possible by merging interaction types (Fontaine et al., 2011; Genrich et al., 2017; Kéfi et al., 2012, 2016; Melián et al., 2009; Pocock et al., 2012), by linking networks occurring at different times and in different sites ('multilayer networks' or 'metawebs'; Pilosof et al., 2017; Poisot et al., 2012) and by linking them to the functioning of human societies (The QUINTESSENCE Consortium, 2016). Sampling effort has been shown to significantly influence some whole-network properties (Blüthgen et al., 2008; Costa et al., 2016), while the integration of new sets of species, such as parasites, can completely disrupt the architecture of ecological networks (Hudson et al., 2006; Lafferty et al., 2008). This lack of integration of small organisms (e.g. bacteria, fungi) is a shortcoming of the ecological network literature, since these small organisms represent a major part of the Earth biodiversity in terms of species number and biomass (Dobson et al., 2008; Hawksworth, 2001; van der Heijden et al., 2008; Whitman et al., 1998).

The emergence of metabarcoding and NGS in the past decade has given us a chance to fill this gap but raised new issues (Bálint et al., 2016). Metabarcoding approaches revolutionized the field of microbial ecology because they gave us access to the composition of whole microbial communities, including noncultivable microorganisms (Hibbett et al., 2009; Peay et al., 2008). However, metabarcoding approaches only detect molecular species named operational taxonomic units (OTUs). They do not detect ecological interactions. A current challenge is therefore to reconstruct species interactions networks from species (relative) abundance data (Fig. 2), such as those obtained from metabarcoding techniques (Abreu and Taga, 2016; Biswas et al., 2016; Faust and Raes, 2012; Layeghifard et al., 2017; Vacher et al., 2016). New theoretical frameworks have been developed by community ecologists to tackle this issue (Cazelles et al., 2016;

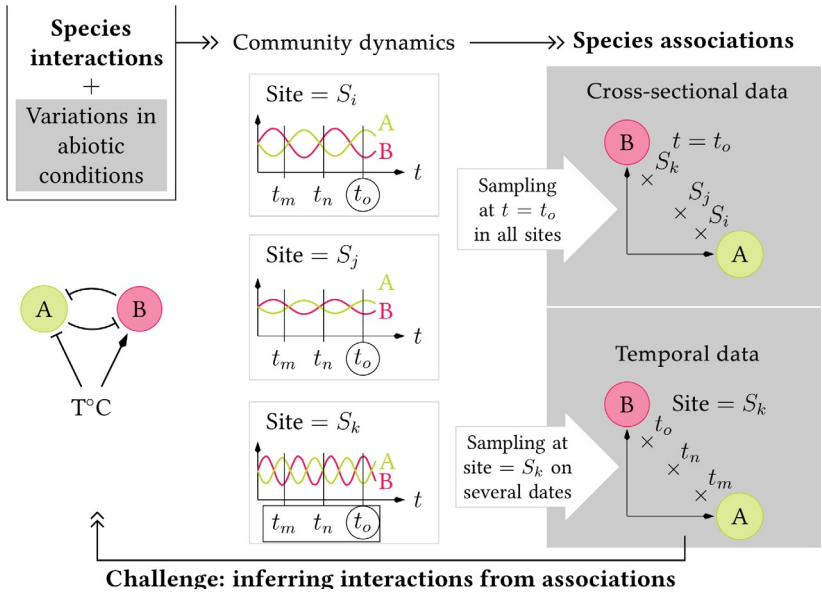

Fig. 2 Challenges to overcome in order to reconstruct species interaction networks from species abundance patterns. The structure of species interaction networks and the variations in the abiotic environment trigger spatial and temporal variations in species abundances. These variations can be captured by sampling several geographical sites at one sampling date (*circled*), by sampling one site at multiple dates (*framed*) or by combining both sampling designs (not shown). The challenge is to reconstruct the species interaction network (often unknown) from the variables and relationships that can be measured (*in grey*), including species association statistics and abiotic environmental variables. Knowledge only of the species associations is not sufficient, as illustrated in Fig. 3.

Ovaskainen et al., 2016, 2017a,b), and software adapted to metabarcoding data has been developed concomitantly (Bucci et al., 2016; Deng et al., 2012; Faust et al., 2015a; Friedman and Alm, 2012; Kurtz et al., 2015; Li et al., 2016; Shang et al., 2017; Weiss et al., 2016). These methods all produce species association networks, where a link between two species represents a significant statistical association between their abundances. This is a critical issue, because species association networks differ from species interaction networks (Fig. 3). Species association networks, which are now very popular in the field of microbial ecology, usually have two type of links, positive and negative (Agler et al., 2016; Faust et al., 2015b; Jakuschkin et al., 2016). Realistic species interaction networks have multiple types of links, including mutualism, commensalism, parasitism, predation, amensalism and competition (Faust and Raes, 2012).

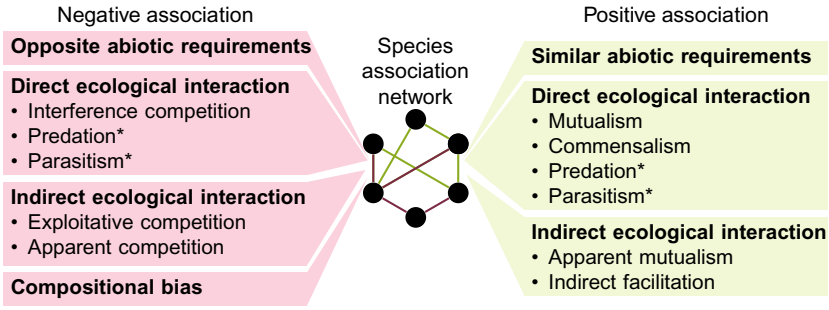

Fig. 3 Why species association networks are not species interaction networks. Significant associations between species abundances can be triggered by ecological interactions, but also by species abiotic requirements and methodological biases. Moreover, linking statistical association sign with ecological interaction type is not straightforward (*the cases of predation and parasitism are discussed in the text). These issues must be overcome to reconstruct species interaction networks from species abundance data, such as those obtained from metabarcoding approaches.

The relationship between statistical associations and ecological interactions is not straightforward (Fig. 3). For instance, parasitism is rarely captured as a significant link in microbial association networks and when it is, it is retrieved as a positive link despite the detrimental effect of the parasite on its host (Weiss et al., 2016). This might be because the copresence of the host species and the parasite species is necessary for the interaction to occur: analysing together samples where neither the host nor the parasite are present, and samples where they both interact, tends to create a positive relationship between the abundances of the host and the parasite species in cross-sectional datasets. The same reasoning holds for predator–prey interactions (Fig. 3). Predation and parasitism are also expected to trigger various types of association in the case of time series. Indeed, in resource–consumer interactions, species evolution can alter the dynamics of the interacting populations (Bengfort et al., 2017; Hiltunen et al., 2014). It can therefore modulate the statistical association between species abundances, especially in the case of fast-evolving species such as microorganisms. Eco-evolutionary models suggest that when the resource (i.e. the prey or the host) evolves faster than its interacting partner, the two-species dynamics exhibit antiphase cycles (Hiltunen et al., 2014). Such dynamics are expected to yield a negative association between species abundances in time series. In contrast, no association is expected if none of the species evolve or if both evolve at the same rate, because their dynamics would then be lagged by a quarter-period (Hiltunen et al., 2014). In real systems, both species dynamics and evolution

are actually shaped by the complex network of trophic and nontrophic interactions. Current methods generally fail at retrieving robust interaction signals from these complex dynamic systems (Sander et al., 2017; Weiss et al., 2016). New frameworks seem to be necessary to detect the causes of real ecosystems dynamics (Sugihara et al., 2012; Ye and Sugihara, 2016). The meaning of a link in a molecular-based ecological network reconstructed using current methods is thus different from that of an observation-based network: links of molecular-based ecological networks represent statistical associations (that may be triggered by biotic interactions), but they do not represent true species interactions. Because of this conceptual difference, the knowledge derived from observation-based interaction networks hardly applies to molecular-based association networks. A new line of research, using molecular-based association networks as indicators of plant, animal and ecosystem health, has therefore emerged (Bohan et al., 2017; Karimi et al., 2017; Poudel et al., 2016). To get a more complete view of ecosystem structure and function, future research should strengthen the synergies between observation-based interaction networks (involving macroorganisms) and molecular-based association networks (involving microorganisms).

Finally, even though molecular-based association networks concern predominantly microorganisms, advances in this area of research go well beyond purely microbial networks. In a biomonitoring scheme building networks based on eDNA, the issues related to the link between species association networks and species interaction networks are valid for any kind of network constructed. Consequently, recovering accurate species interaction networks from species association networks represents a major issue today in order to rapidly construct networks from monitored environments.

5. MACHINE LEARNING AS A WAY TO RAPIDLY BUILD MOLECULAR ECOLOGICAL NETWORKS IN A RAPID AND RELIABLE WAY?

5.1 Learning Ecological Networks From Data

A basic premise of ecology is that the variation in the observed data, through sampling ecosystems, contains information about past and current ecological interactions between species in the ecosystem. Thus, the abundance and variation of any one species are in part a consequence of past interactions between the individuals of previous generations, such as sexual reproduction, and of the current generation, such as competition, cannibalism or

migration. Across a community the observed abundance, phenotypic variation and diversity of species are further determined by ecological processes and interactions between species, including trophic and competition interactions. In analysing variation in sample data, ecologists aim to recover some information about these interactions, often using data sampled at particular times or in manipulative experiments that target specific interactions.

More recently, these ideas have been extended to simultaneously building whole ecological networks of interactions from data using statistical or logic-based machine learning. The idea behind these machine learning methods is simply that already used by ecologists that embedded in a dataset is the imprint of the recent processes and interactions that created the data and this information can be recovered to reconstruct networks. The underlying hypothesis of machine learning for network reconstruction is therefore that ecological interactions produce correlations and relational patterns in the abundance of species that can be recovered. In statistical machine learning, the variation in the sample is treated statistically, typically using Bayesian approaches (Jakuschkin et al., 2016; Vacher et al., 2016). Significant correlations between any given pair of species within the data are then considered as potential network edges. Logic-based machine learning treats relational patterns rather like the structure of grammar in a language (Muggleton, 1991; Tamaddoni-Nezhad et al., 2006).

In both statistical and logic-based machine learning, the trick is to sort, from the list of edges (i.e. interactions) hypothesized by the learning algorithms, those links that are ecologically meaningful from those that are artefacts. This selection approach is done differently in the two approaches. In logic-based machine learning, the grammar for an interaction can be coded as background information. In the agroecological network learnt by Bohan et al. (2011a) and Tamaddoni-Nezhad et al. (2013), trophic interactions were selected by background information that was a set of grammar rules (a model) for a trophic interaction whereby the predator and prey species must cooccur in the same samples and predators should be larger than their prey (in this case big things eat small things, but that is not always the case in nature). A trophic interaction between two species was only identified if this grammar rule was realized. In statistical approaches, links are selected using environmental factors or species functional trait covariates integrated into the modelling (Cazelles et al., 2016; Jakuschkin et al., 2016; Ovaskainen et al., 2016; Vacher et al., 2016).

Learning networks is currently limited by our background information rules. Mechanistic rules for trophic interactions, based upon body size or

gape size, allow the reconstruction of food webs. Where ecological networks are structured by processes for which we have no general mechanistic explanation, there is no background information that can be employed, and machine learning is of little value for reconstructing networks. However, recent developments in logical machine learning are now allowing background information rules to be discovered from data. Tamaddoni-Nezhad et al. (2015) showed using simple subnetworks that the trophic interaction rule 'big things eat small things' can be recovered. Developments of this work are now extending this possibility of rule learning to larger and noisier data sets (Dai et al., 2015).

5.2 Exploiting eDNA-Derived Information as a Source for Network Data

While machine learning could greatly speed up the process of constructing ecological networks, the abundance data used for the reconstruction are currently something of a bottleneck. Highly replicated and taxonomically resolved data sets, such as the farm-scale evaluations data used by Bohan et al. (2011a) and Tamaddoni-Nezhad et al. (2013) to reconstruct an agro-ecological network, are few and costly to create. One solution will be to move towards assessing the presence of species, and potentially their relative abundance and variation, using highly replicated samples of DNA taken from the environment. These eDNA samples, which in principle could be sampled (relatively) easily and cheaply, could then be used to identify the taxa present at a sampling point using NGS approaches. NGS describes a number of similar technologies for generating large numbers of nucleic acid sequences for the identification of species (OTUs) and functions. The great beauty of these methods is that the nucleic acids with which they work are common to all life forms and ubiquitous. The fact that NGS might be applied to the identification of OTUs and functions in environmental samples from any biome, habitat and environment and any source material with minimal change in protocol has driven interest in eDNA as a generic source of data (Barnes and Turner, 2015; Evans et al., 2016; Thomsen and Willerslev, 2015). Coupling machine learning and NGS data could greatly speed up the reconstruction of networks in all ecosystems.

The raw OTU information that would be produced by eDNA sampling would contain all the interactions that structure the data. Treating this complexity of information has been highly problematic and difficult to date, and thus network researchers have tended to use DNA data in which many of the

interactions are filtered out. Probably the best example is gut content data in which the sample is effectively an individual predator, and the data are the OTUs contained in the predator's gut (e.g. Fayle et al., 2015). Adopting this predator-derived data effectively selects for realized links, without a process of learning, and allows trophic ecological networks to be constructed directly. While learning approaches might be applied to these data to learn something about the processes underscoring trophic interactions, such as the traits that make some species predators of particular prey via background information, these gut content DNA samples are essentially limited to describing food webs which alone cannot explain community-level species cooccurrence and ecosystem structure (Bohan et al., 2013; Pocock et al., 2012). DNA analysis of gut content samples (or other type of DNA samples allowing a direct highlight of interactions such as host parasitized or faeces) is nevertheless mandatory as it allows to confirm the predictions from machine learning. In biomonitoring using NGS networks established with learning approach (or statistical models), it would be necessary that new interactions discovered by machine learning are systematically tested (with molecular tools or with experiments) for an accurate understanding of ecosystems.

The challenge for the future is to use appropriate machine learning and background information to tackle the problem of the many interactions that in combination have created the eDNA data we observe. While the best machine learning approaches have yet to be determined (Bohan et al., 2017), it is clear that background information will play a pivotal role here. The background information 'model' proposed by Bohan et al. (2011a) and Tamaddoni-Nezhad et al. (2013) for their agroecological network was essentially a model for a hypothetical trophic interaction and consequently selected interactions that conformed to this—trophic interactions. Subsequent work showed that a logic-based machine learning approach, called metainterpretative learning (MIL) (Muggleton et al., 2014, 2015), could discover background information directly from data. MIL was demonstrated discovering the rule used by Bohan et al. (2011a) and Tamaddoni-Nezhad et al. (2013) that 'big things eat small things' directly from a simulated, synthetic food web (Tamaddoni-Nezhad et al., 2015). This holds out the exciting possibility that reconstruction of an ever greater number of ecological networks, from eDNA, will drive a rapid improvement in our understanding of ecosystem structure and function because it will require the discovery of the background information models—the generic rules—that describe/determine the ecological interactions that structure all ecosystems.

6. NGS NETWORK DATA SHARING

With NGS approaches, network ecologists will be able to rapidly generate a large amount of data, including both DNA sequences and putative species interactions (interactions derived from machine learning must be validated through observation and experiment, see Lima-Mendez et al., 2015). This means that data curation and sharing between researchers must be done systematically for both DNA sequences and species interactions. Currently, sequences data are available publicly to researchers through databases such as Genbank, BOLD (Ratnasingham and Hebert, 2007), European Nucleotide Archive (for raw NGS data, see Leinonen et al., 2011; Nilsson et al., 2011). In network construction, reliable species identifications are directly dependent on the quality of these databases (Sonet et al., 2013; Wells and Stevens, 2008). First, databases of DNA sequences must ideally be representative and comprehensive to cover the identification of all the species entangled in the ecosystem studied. This is particularly important as relying only on OTUs would certainly allow to build networks and to describe the architecture of the ecosystems, but would also miss the important ecological functions of the organisms found interacting. For example, this was the case in the plant–fungi network built by Toju et al. (2014). In this very impressive network gathering 33 plant species and 387 fungal OTUs, only 26.4% of the OTUs were identified to the genus level and the ecological function remained to be determined for an important part of the fungi detected (Toju et al., 2013, 2014). Although, as more complete genomes become described and deposited in databases, it may be possible to infer ecological functions from OTU data. While still in its infancy, this approach has been realized via the PICRUSt algorithm, which infers complete genome data from 16S rRNA gene sequences used to define bacterial OTUs, linking the functional data held within genomes to their corresponding 16S sequences (Langille et al., 2013). Other approaches, which use trait-based databases to map taxonomic identities inferred from OTU data to that taxon's function, also exist (e.g. FUNGuild; Nguyen et al., 2016), and these will become increasingly important as the quality and quantity held within expands. We emphasize that the completeness of databases will improve over time through the increasing use of sequencing technologies and will therefore enhance the reliability of species identifications and their function. Second, the validity of NGS networks also depends on the frequency of misidentifications and sequencing errors in databases (Wells and Sperling, 2000) because

misidentifications from public libraries can spread in network construction. Taxonomic databases such as BOLD (Ratnasingham and Hebert, 2007) nevertheless limit these risks since DNA sequences (i.e. DNA barcodes in BOLD) are associated with specimen vouchers. This practice allows reexamination of specimens after DNA sequencing and also gives information such as sampling location, photography, sequencing laboratory or specimen depository. Despite their imperfections, public libraries are improving over time and there is a general consensus to share DNA-sequencing data which improve DNA-based research in a wide range of areas.

In ecological network research, data sharing is, however, not a systematic practice. Ecologists generate together massive high-value data through individual projects. However, data curation and sharing are still an important issue to overcome to enable ecologists to take full advantage of existing data as well as future generated data (see Hampton et al., 2013; Poisot et al., 2016a,b). IWDB is an example of data repository where nearly a hundred of webs are available today, half of them being plant–pollinator networks. Mangal (Poisot et al., 2016a,b) has been designed (with an R package [rmangal]) to access, curate and deposit data on ecological interactions. The Global Biotic Interactions (GloBI) database (Poelen et al., 2014) is an open structure to share and analyse species interactions in a structured data repository. Ecological data, networks included, can also be found on non-specific repositories such as Dryad or Figshare. One important challenge for these data is to conform to some common presentation standards, despite attempts at providing a common format for open access ecological network data (Poisot et al., 2016a,b).

With the rapid increase of studies using NGS to construct ecological networks, need of a common way for molecular network ecologists to store and share their highly valuable data, which includes both DNA sequences and species interactions (or at least species cooccurrence), has never been of greater importance.

6.1 The Importance of a Dedicated NGS Network Database: Linking DNA Sequences and Ecological Interactions to Limit Species Identification Errors

While it is still possible to rely on existing public repositories for DNA sequences (e.g. Genbank) and ecological data (e.g. Dryad or IWDB) to store and share NGS networks, a common way to register NGS networks would be considerably beneficial for network ecologists. Indeed, there is a need to be able to recover directly, easily and reliably the links existing between

DNA sequences and species interactions (or at least cooccurrence) within NGS networks. To date, there is no single environment providing these features. The field could build upon the standard file format (e.g. BIOM) to efficiently store OTU table associated with metadata concerning samples (e.g. treatment) or species (e.g. taxonomy). Public data-sharing initiatives have sprouted both from microbial ecology (e.g. QIITA platform; qiita.microbio.me.) and ecology (e.g. mangal; Poisot et al., 2016a,b), but NGS networks would still need to connect both fields. Taking inspiration from current databases, a systematic storage of DNA sequences and ecological interactions together would allow network ecologists to rapidly recreate the NGS networks published, compare them with their own ecological networks and make further analysis possible.

In particular, this would allow the reexamination of existing networks. Because accurate identification of species and interactions directly affect the network structure (e.g. Wirta et al., 2014), it seems important in the future to be able to reassign the species or at least the OTUs in the NGS networks created. Because species delimitation methods are improving (Kekkonen and Hebert, 2014; Puillandre et al., 2012; Zhang et al., 2013; Zorita et al., 2015) as well as public databases with the increasing number of sequencing programs, species identification or OTUs delimitation will certainly follow this trend. Consequently, the ability to reexamine existing NGS networks and reassigning species and OTUs thanks to a systematic practice of data storage will improve the precision of the networks described which may be today biased by current database completeness and species delimitation methods.

6.2 Reconstructing Ecological Networks With Different Predicting Methods of Species Interactions

As already discussed, in some systems in which interactions cannot be directly determined, such as networks of microbes (see Vacher et al., 2016), only species cooccurrence and/or abundance patterns are available, and thus statistical methods (Kurtz et al., 2015) or machine learning (Bohan et al., 2011a,b) are needed to predict species interaction. Despite their ability to rapidly build networks from NGS data, these methods are still in their infancy (Bohan et al., 2017). It is clear that this research area is still young, and future developments are expected, which will affect the way ecological networks are created and how accurate they are. As a NGS network database would allow the reassignment of species and OTUs paired with the improvement of public databases and identification methods, it

would also allow the reconstruction of preexisting networks based on cooccurrence patterns by benefiting from the arrival of new statistical inference methods and machine learning approaches. Moreover, since machine learning is very reliant on available data and information on the biological traits of species (e.g. body size), a centralized way of data storage for NGS networks and a systematic data-sharing practice between network ecologists would considerably improve the efficiency of network construction with learning approaches.

This means that not only DNA sequences and species interactions or cooccurrence must be stored and shared, but also the bioinformatics pipeline that created the networks (e.g. the mathematical and bioinformatics framework 'molecular ecological networks' to construct ecological association networks developed by Deng et al., 2012). Reproducibility of bioinformatic pipelines is an important topic in molecular phylogeny research where new phylogenetic trees are constantly built (Szitenberg et al., 2015). Network ecology should follow this example, and a NGS network database could be a first step in that direction.

6.3 Do Only Sequences and Species Interactions/ Cooccurrences Matter in a NGS Network Database?

As demonstrated previously, being able to track DNA sequences together with species interactions or abundance patterns is important. However, there is much more to ecological data than simply interactions and cooccurrences. In particular, temporal and spatial data are also highly valuable. Indeed, it is tempting to use databases to merge data from different studies in order to build large-scale ecological networks and to picture a broader view of ecosystem functioning. For instance, this has been done by Derocles et al. (2014a) for an aphid–parasitoid food web by pooling all interactions described in Europe between these organisms. However, merging data without caution would also create unrealistic ecological networks. Ecological networks cannot be created from all kinds of data: covariates (i.e. metadata) are needed. Indeed, the structure of ecological communities revealed by eDNA does not only depend on biotic interactions but also on abiotic filtering, dispersal limitation, ecological drift and historical contingency (Ovaskainen et al., 2017a,b). Temporal and spatial metadata would be the minimum information required to prevent building ecological networks with interactions or species not present in the same location (e.g. species A is eating species B in America and species C in Europe, can a network be built with the three species?)

or during the same period of the year (e.g. species A is eating species B in the winter, but not in summer). Here, BOLD is a very good example to follow since the deposited specimens (i.e. voucher) are systematically assigned with collection data (sampling date, sampling location, name of the collector).

6.4 An Example Output From a NGS Network Database: Phylogenetically Structured Networks

In this chapter, the use of DNA sequences has been considered in species identification or OTU delimitation. However, DNA sequences are too valuable to limit them only to the creation of nodes in ecological networks. Recently, DNA sequences have also been used for the phylogenetic signal they can provide in a network context (Elias et al., 2013; Hadfield et al., 2014; Rafferty and Ives, 2013). The interactions detected, and hence the network structure of ecosystems, reflect both the ecological processes and the evolutionary history of the species (e.g. Pilosof et al., 2014). Consequently, the observed network structures are necessarily constrained by the coevolutionary processes between species, as evidenced by recent studies predicting invasive species interactions from native species phylogenies (Charlery de la Masselière et al., 2017; Pearse and Altermatt, 2013). It seems very important therefore to start to account for phylogenetic signals within networks (Ives and Godfray, 2006), as pioneered by host–parasite cophylogeny studies (e.g. Banks and Paterson, 2005) and now advocated in the holobiont literature (Brooks et al., 2016; Martinson et al., 2017). Hopefully, NGS networks have the ability to easily explore this area of research through the extensive DNA sequences and species interactions they generate. A NGS database would constitute a turnkey solution to build phylogenetically structured ecological networks (see Evans et al., 2016).

As an example here, aphid–parasitoid interactions found in an agroecosystem from Western France by Derocles et al. (2014a,b) together with cytochrome *C* oxidase I (COI) mined from Genbank was used to build a phylogenetically structured network (Fig. 4). With this approach, the phylogenetic conservatism hypothesis that closely related parasitoid wasps parasitize closely related aphid species was tested (Cavender-Bares et al., 2009; Webb et al., 2002). This hypothesis seems true in some cases: for example, the genus *Lysiphlebus* attacks the genus *Aphis*. In the same way, the genus *Trioxys* exclusively parasitizes the genus *Myszocallis*.

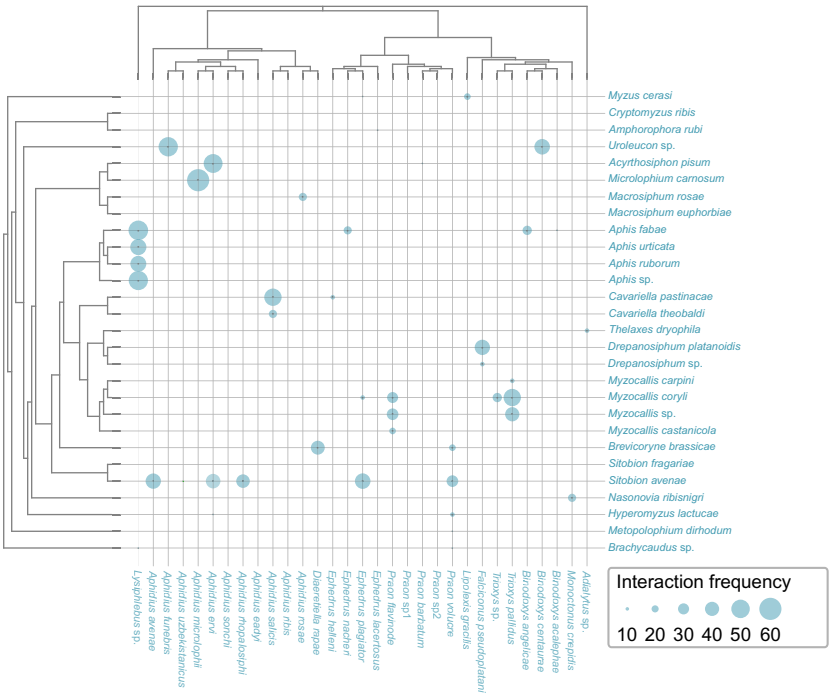

Fig. 4 A phylogenetically structured aphid–parasitoid network created using the networks from Derocles et al. (2014a,b). Cytochrome oxidase subunit I (COI) sequences for the detected species were extracted from Genbank and the Barcode of Life Database version 3 (BOLD v3). Trees were created using the default settings in RAxML version 8.2.10 (Stamatakis, 2014), and the figure was produced in R (R Development Core Team, 2017) using the library dualingTrees as used by Chaudhary et al. (2016). *Circles* represent interactions found in an agroecosystem (Derocles et al., 2014a,b). The radius of the *circle* represents the strength of the interaction. The aphids are in *rows*, the parasitoids in *columns*.

A particular case contradicts this theory: *Sitobion avenae* is parasitized by parasitoid species phylogenetically distant (*Aphidius* spp., *Ephedrus plagiator* or *Praon volucre*).

However, because the network in Derocles et al. (2014a) was based on morphological identifications for aphids and 16S sequence identifications for parasitoids, Fig. 4 must be considered with caution: COI sequences used to build the phylogenetic trees in Fig. 4 did not come from the specimens sampled by Derocles et al. (2014a). Moreover, for some species not identified to the species level (e.g. *Lysiphlebus* sp. or *Aphis* sp.), COI sequences mined from Genbank from a closely related species were used (e.g. COI of *Lysiphlebus fabarum*). Finally, the interactions used here are

certainly not completely representative of the studied agroecosystem due to the limited spatial and temporal scales considered in Derocles et al. (2014a), which may have consequences both on the phylogenetic trees and the structure of interactions. A database such as those described earlier would prevent these issues.

6.5 Improving Network Ecology Research With a NGS Network Database

A database as presented here would facilitate comparison between networks, thus allowing the detection of errors introduced during the sequencing process, species identification or network construction by pointing out unusual species or interactions. In the same way as molecular phylogenetic studies are improving over time, thanks to DNA sequence curation and sharing, ecological network research would also benefit from such practices. From a theoretical point of view, a NGS network database would open ways for large-scale comparisons of ecological networks from various ecosystems, and thus improve our understanding of how ecosystems vary in space and time and how they respond to environmental changes (Bohan et al., 2017). By definition, this is the fundamental goal of biomonitoring programs.

Nevertheless, ecologists still need to be cautious when using preexisting data to build or compare ecological networks (including phylogenetically structured networks) and particularly when making ecological interpretations. Patterns derived from associations between community ecology and phylogeny can be interpreted in very different ways, and phylogenetic patterns do not necessary reflect community assembly mechanisms (Gerhold et al., 2015; Mayfield and Levine, 2010). As demonstrated earlier, interpreting coabundance pattern-based networks is not straightforward as complex oscillatory dynamics, indirect interactions or trophic cascades may alter the structure of cooccurrence networks. Consequently, the efficient use of databases to build and compare networks must be considered as a complementary approach. Experimental evidences based on field or laboratory experiments are still mandatory to confirm the ecological patterns highlighted and the networks constructed. To sum up, if used with appropriate caution, a NGS-based network database would constitute a way to start a virtuous circle that would improve the methodology of network construction, the fundamental research in ecology and finally the biomonitoring of ecosystems.

7. CONCLUSION: TOWARDS THE CONSTRUCTION OF MULTILAYER NETWORKS IN ECOLOGY USING NGS

7.1 Towards Larger, Highly Resolved Networks

The recent conceptual revolution in ecology brought forth by the development of metacommunity and metacosystem theory (Holyoak et al., 2005; Leibold et al., 2004; Loreau et al., 2003) has stressed the importance of spatial processes for the functioning, diversity, complexity and dynamics of ecological systems (Massol et al., 2011). Recent theoretical findings have highlighted the role of the spatial structure and scales of communities, food webs and ecosystems to understand properties such as ecosystem productivity and nutrient fluxes (Gravel et al., 2010; Loreau and Holt, 2004), ecosystem stability (Gravel et al., 2016; Mougi and Kondoh, 2016; Wang et al., 2017), species coexistence (Amarasekare et al., 2004; Haegeman and Loreau, 2015; Mouquet and Loreau, 2002), food-chain length (Calcagno et al., 2011; Holt, 1997), food web complexity (Bolchoun et al., 2017; Pillai et al., 2011), alternate steady states of ecosystems (Gounand et al., 2014), nutrient colimitation (Marleau et al., 2015; Mouquet et al., 2006) and the existence of 'keystone' ecosystems (Mouquet et al., 2013). From a practical, applied viewpoint, these models have also fostered change in the understanding of human-managed ecosystems such as agroecosystems (Bohan et al., 2013; Gaba et al., 2010; Massol and Petit, 2013) or marine protected areas (Andrello et al., 2013; Parravicini et al., 2014). However, our knowledge of 'networks of networks', (e.g. multiple animal groups interacting with shared plants; Pocock et al., 2012), is still scant and poorly defined. In part, this is due to the difficulty of obtaining necessary data using traditional empirical tools (i.e. direct observation, gut contents, stable isotopes, etc.). As discussed earlier, even if it is more efficient and reliable than other approaches, NGS is not the key to every lock as it might not see some species (PCR issues for instance), misidentify them (incomplete databanks) or miscalculate the links. However, merging NGS with ENA will provide the ecological research community with the tools it needs to create multilayer networks with increased ease in the coming decades, thus improving the power to detect many kinds of spatial food web effects. Increasing network coverage (i.e. more species identified, more links identified) through networks of ecological networks is a step forward for different reasons:

(1) It provides more precise metrics on network properties (connectance, modularity, degree distribution, etc.; see Ma et al., 2017);

(2) It better connects species demographics with community and food web dynamics through decoupling the population dynamics and interaction effects of cryptic species;
(3) It improves precision regarding indirect measures of species interaction strengths, which is crucial to better understand ecosystem stability theory and test its predictions (see Jacquet et al., 2016).

7.2 NGS Networks to Link Above- and Belowground Ecosystems, as Well as Eukaryotes and Prokaryotes

Currently, ecological network research is mainly split between studies focusing either on aboveground or belowground interactions, with very few linking the two (Mulder et al., 2013; Rodríguez-Echeverría and Traveset, 2015). However, the structure of ecological networks is inevitably affected by the range of organisms considered (Hudson et al., 2006; Lafferty et al., 2008). NGS is a promising way to start to link all organisms, from above- and belowground, prokaryotes and eukaryotes, in the same network. This approach has been attempted recently with a combination of observations and DNA-based identifications to infer a multiorganism network including spiders, earthworms, Enchytraeids, nematodes, plants, protists and other microorganisms (Morriën et al., 2017). By combining existing NGS techniques, it is now possible to take this approach even further and rapidly construct networks much more complex than those currently established. With a nest-tagging NGS tool (Cruaud et al., 2017; Evans et al., 2016; Shokralla et al., 2015), it would be possible to rapidly construct both aboveground (e.g. flower visitation based on pollen sequencing carried by pollinating insects and animal DNA found in plant nectaries) and belowground networks (with arbuscular mycorrhizal fungi, as previously done by Toju et al., 2014) where all organisms are linked by the shared plants they interact with. In parallel, metabarcoding data from microbial communities and machine learning inference would enable the inclusion of microorganisms in a network of networks, and understand how they affect the network structure and how they constrain interactions.

7.3 Biomonitoring of Ecosystems With Multilayer Phylogenetically Structured Networks

NGS methods provide an unprecedented opportunity to rapidly build complex multilayer ecological networks. A combination of sequencing technologies and machine learning approaches would open new opportunities for describing networks at various spatial and temporal scales

(Bohan et al., 2017). Such 'next-generation biomonitoring' through the construction of networks of networks paves the way for an exciting new area of research and a comprehensive understanding of the response of ecosystems to environmental changes. From our point of view, we now have access to the tools needed to respond to a number of contemporary global challenges.

However, more complex networks in large-scale biomonitoring programs require new analytical tools to disentangle actual patterns of change in network structure from noise. Indeed, the construction of multilayer networks is only a step (albeit a major one) when assessing the impacts of environmental changes on ecosystems. The identification of consistent patterns within networks is another crucial step that cannot be neglected. Most of the networks analysed to date are still relatively simple as they do not integrate a wide range of organisms or different kinds of interactions. In more complex networks, such as the network of ecological networks established by Pocock et al. (2012), all species are linked by the plants. While giving a more complete view of the architecture of interactions, this approach still neglects some links such as intraguild predation, which play a role in the stability of ecosystems (see Nakazawa and Yamamura, 2006). For example, integrating the full range of interactions mediated by the ground beetles (Coleoptera: Carabidae) in the type of network of networks built by Pocock et al. (2012) would be currently problematic. Indeed, these organisms can interact with plants, herbivorous species (e.g. aphids), as well as natural enemies of herbivorous species (e.g. parasitoids). Describing the structure and the robustness of very complex ecological networks, integrating a wide range of interactions as well as prokaryotes and eukaryotes, therefore requires new statistical methods.

Combining phylogenetic information with ecological networks is also a new area of research that remains to be more systematically explored (Evans et al., 2016). NGS is generating an increasing amount of new data which give the opportunity to investigate how the structure of species interactions and the phylogenetic signals are linked. This approach would certainly be an added value to the current biomonitoring programs as ecosystem condition and coevolutionary processes could be monitored together. More importantly, in a changing world, phylogenetic signals may be key to predicting ecological interactions (Elias et al., 2013; Ives and Godfray, 2006; Rafferty and Ives, 2013; Rezende et al., 2007).

The future of network ecology is exciting, with a great opportunity to biomonitoring and to improve our understanding of the ecosystem functioning and services as well as finding ways to mitigate the impact of environmental changes or to restore ecosystems.

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GLOSSARY

Molecular Biology

Amplicon a DNA fragment amplified by primers during the polymerase chain reaction (PCR).

Diagnostic PCR specific primer pairs are designed for each targeted species or higher taxonomic group to produce amplicons of different sizes. These primers are then used in a multiplex and/or several singleplex PCRs for taxonomic identification. Identification of taxonomic groups in samples is then based on the presence/absence of bands on an electrophoresis gel (taxonomic group is present or not) as well as the position of each band (which taxonomic group is present). Quantitative PCR (qPCR) or digital droplet PCR (ddPCR) could then be used to get a precise estimate of the abundance of a species/group. This approach does not require DNA sequencing but requires a prior knowledge of the studied organisms in order to design specific primers.

DNA barcoding in a broad sense, a taxonomic method to identify organisms (ideally to the species level) using DNA sequences.

Environmental DNA (eDNA) remnant DNA in the environment; cells or tissues left behind by organisms (e.g. faeces, hair, epithelial cells).

Gel electrophoresis method used to visualize DNA fragments according to their size usually on an agarose gel.

Metabarcoding approach where several millions of DNA sequences of a specific genomic region are generated (e.g. eDNA sample) to characterize the taxonomic diversity present in a sample.

Metagenomics often confused with metabarcoding, metagenomics sequencing does not target specific genomic regions. Instead, it provides insights into entire genomes from multiple organisms in a particular ecosystem.

Molecular network/molecular ecological networks in the context of this chapter, molecular networks refer to ecological networks constructed with DNA-based techniques (Sanger sequencing, PCR diagnostics, NGS).

Multiplex PCR PCR where more than one primer pair is employed to simultaneously amplify several PCR fragments within a single reaction.

Nested PCR PCR comprising two successive steps. In the second step, the PCR product obtained in the first step is amplified. Nested PCR can improve the amplification of recalcitrant target gene regions. In NGS metabarcoding protocols, a nested PCR allows the addition of a second step for tagging ('nested tagging method', see Evans et al., 2016; Kitson et al., 2016).

Next-generation sequencing (NGS) or high-throughput sequencing sequencing technologies designed to simultaneously generate up to several millions of sequences (see Table 2 for more details).

Operational taxonomic units/OTUs specimens/samples assignment to a taxonomic group sharing similar sequences, but without naming actual species.

Primer short strand of DNA (or RNA) between generally 15 and 25 base pairs. A primer is the starting point used by the DNA polymerase to synthetize a complementary DNA brand.

Sanger sequencing 'first-generation' sequencing method where the output is a single DNA sequence per sample and PCR (see Table 2 for more details).

Sequencing coverage the depth of coverage refers to the number of times a region has been sequenced by independent reads. The breadth of coverage refers to the percentage of bases of a region covered with a given depth (e.g. 90% of the targeted region was covered three times). The number of samples in a NGS run depends mainly on the sequencing coverage. The number of samples in a sequencing run does not depend on labwear units (i.e. 96- or 384-well plate).

Sequence reads DNA sequences produced by NGS.

Sequencing run step during which the DNA is sequenced (see Table 2 for more details).

Singleplex PCR where one pair of primers is used to amplify one specific PCR fragment.

Specimen voucher a sample of an organism deposited and stored in a facility. A voucher is generally associated with a scientific species name, the collector's name, the expert who identified the specimen, the date and location (with GPS coordinates) of collection. Researchers may request the permission to examine a voucher for further studies (see Culley, 2013).

Tag unique short sequence (usually 8–12 bp) added to the 5′ end of a primer. Samples with unique tags can be pooled and sequenced in the same sequencing run. Sequences are later assigned to samples using bioinformatic pipelines.

Ecology and Ecological Network Analysis

Bipartite networks a bipartite network can be divided into two disjoint sets. The nodes (i.e. species) of these two sets are connected with links (i.e. interactions). Plant–pollinator networks and prey–predator networks are two examples of bipartite networks.

Compartmentalization the degree to which an ecological network is divided into weakly connected subwebs.

Connectance the proportion of observed links over all possible links. A simple measure of connectance is given by the ratio number of link/number of species^2. More complex measures of connectance account for the frequency of interactions (see Bersier et al., 2002).

Conventional approach/method in the context of this chapter, this refers to 'traditional' taxonomy-based approaches to identify species. These methods do not rely on DNA to identify species.

Ecosystem functions/ecosystem functioning ecological processes that control the fluxes of energy and matter between trophic levels and between the organisms and the environment.

Ecosystem services humankind benefits from ecosystem functioning. Ecosystem services are often grouped into four categories: (1) provisioning: e.g., food and water production; (2) regulation: e.g., control of climate, pests and diseases; (3) supporting: e.g., nutrient cycles, pollinations; (4) cultural: e.g., historical, recreational, educational and therapeutic.

Generality mean number of consumers per prey. In a trophic network, a simple measure of generality is the mean number of prey species per predator species. More complex measures of generality account for the frequency of consumer–prey interactions.

Indicators in biomonitoring, indicators are used to assess risks to human health and environment. These indicators include pollution indicators (pesticides, elements, metabolites) and ecological indicators (species, population, community, behaviour). Indicators are used for communication to the general public and policy makers.

Modularity a measure of compartmentalization within a network. In ecological networks, it refers to a group of species interacting more frequently with themselves than with other species. A wide range of methods have been developed to assess the modularity of a network (see Poisot, 2013).

Multilayer networks set of ecological networks built at different times and/or in different sites and or/with different interaction types (see Pilosof et al., 2017). For example, the 'network of ecological networks' built by Pocock et al. (2012) is a multilayer network gathering different interaction types (mainly mutualist and trophic).

Nestedness tendency of nodes with few connections to be linked to a subset of nodes interacting with more connected nodes. In nested ecological networks, a core group of generalist species interact with each other and with specialist species, while specialist species interact only with the core group of generalist species. A wide range of methods have been developed to assess the nestedness of a network (see Almeida-Neto et al., 2008).

Node in ecological networks, nodes represent taxonomic units (generally species, but it can be other taxonomic levels or OTUs) connected by links (i.e. interactions).

Robustness a measure of the tolerance of ecological networks to species extinctions (Dunne et al., 2002b; Memmott et al., 2004).

Specimen rearing specimens sampled in the field are reared in the laboratory after collection under controlled conditions (e.g. temperature, humidity and photoperiod). Specimen rearing is typically used when the development stage of specimens collected in the field does not allow a reliable taxonomic identification. Specimen rearing is also commonly used for parasitoid identification. In this case, parasitoid hosts are collected in the field and reared in the laboratory until the emergence of adult parasitoids.

Vulnerability mean number of prey per consumer. In a trophic network, a simple measure of vulnerability is the mean number of predator species per prey species. As for the generality, more complex measures of vulnerability account for the frequency of prey–consumers interactions.

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