News and views

Soil biodiversity and DNA barcodes: opportunities and challenges

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1. Introduction

Soil biodiversity represents a huge underground world containing a wide range of organisms, from archaea, bacteria and fungi to nematodes, insects and earthworms. These organisms interact with each other and affect the functioning of the soil ecosystem (Wagg et al., 2014). The study of soil biodiversity is continuously gaining importance in the environmental sciences due to its significant interlinkages with many other areas, such as agriculture and climate change (Wall et al., 2012). Indeed, soil biodiversity and its functioning deliver many ecosystem services that impact, both directly and indirectly, human wellbeing (Van der Putten et al., 2013). However, despite the value of soil biodiversity, this diversity remains to be better explored also in relation to the major threats that it is subjected to; so much so that international initiatives, such as the EU project EcoFINDE

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metabarcoding surveys on soil biodiversity conducted in a wide range of environments (e.g. grasslands, agricultural fields and forests, but also deserts and the Arctic and Antarctic) (Mardis, 2008; Nielsen and Wall, 2013) from small to large scale, including national surveys (Griffiths et al., 2011; Ranjard et al., 2013). The resulting large data sets yield invaluable reference data allowing a more general specification of biodiversity variation in relation to different factors such as soil type, climate and land use.

Applications of these molecular methodologies allow for either characterization of Operational Taxonomic Units (OTUs) by targeting specific fragments of the genome (Roesh et al., 2007; Bates et al., 2011; Orgiazzi et al., 2013) or for extensive sequencing in an untargeted way aiming at in depth screening for functional genes, community structure and phylogenetic diversity (Vogel et al., 2009; Fierer et al., 2012). The former approach is referred to as metabarcoding (Taberlet et al. 2012a,b) and the latter as metagenomics (Simon and Daniel, 2011) (Fig. 1). Metabarcoding is a molecular approach based on the assumption that each OTU can be unequivocally identified through a specific sequence of DNA (barcode). The general strategy consists of (i) extracting DNA from soil or organisms, (ii) amplifying a specific DNA sequence chosen for its taxonomic value, (iii) sequencing the corresponding DNA amplicons, (iv) analysing the sequences using proper pipelines, and finally (v) assessing the taxonomic diversity of the analysed soil or identifying the organism from which DNA has been extracted (Taberlet et al., 2012b). However, this common procedure is rapidly evolving towards new and innovative approaches. An example is the current tendency to move towards methods that bypass PCR amplification of a single DNA fragment by applying shotgun sequencing of e.g. the entire mitochondrial genome (Zhou et al., 2013), thereby introducing genomic approaches into the classic metabarcoding framework.

Although the strategy described above seems easy to apply, its diffusion in the study of soil biodiversity, as well as in other areas, has awakened hidden issues while simultaneously creating new ones. The increased use of this kind of approach reveals the urgent need to establish reference points in the methodology and management of this field of research. However, the optimal use of the unprecedented opportunities described above requires certain preconditions. Metabarcoding-based surveys on soil biodiversity are only now reaching a significant number, thus the time is ripe to (i) give an overview of the offered opportunities and (ii) to present and address the challenges that must be faced. Starting from what has been published so far in terms of soil metabarcoding studies (Fig. 1), we first present the opportunities presented by the application of metabarcoding to surveys on soil biodiversity and, secondly, we describe challenges that we consider relevant for the study of soil biodiversity through metabarcoding and propose possible solutions in order to obtain more accurate and comparable results and, consequently, valuable discussions. In particular, we identified two categories of challenges; the first refers to what is needed in order to obtain a reliable assessment of soil biodiversity and what is required for the application of the corresponding research. The second category refers to the strategies to obtain and manage data in such a way that they can be properly processed, compared and eventually used to develop a better management of land and soils.

2. Scientific and operational opportunities

Soil metabarcoding is increasingly applied in scientific studies with a progressing number of published papers following this approach (Fig. 1). This is most certainly due to the relative ease with which this technique can be applied together with the continuous reduction of time and costs involved in using NGS platforms and the development of new bioinformatics pipelines to analyse the data (Schmidt et al., 2013; Yang et al., 2013). Researchers are increasingly able to adapt the application of metabarcoding in order to shed light on several unanswered questions. Any soil biodiversity study can have a dual objective: the first refers to more basic and scientific research, the aim of which is to obtain in depth knowledge of the structure and the functions, i.e. the ecological roles, of soil biodiversity. The second purpose is more operational and directed toward decision makers, and aims to assess the level and possible fluctuations of soil biodiversity in different environmental conditions in order to obtain a diagnosis and establish

![Fig. 1. (a) Number of articles (published and in press) in peer-reviewed journals with the keywords and/or the article title and/or the abstract containing the words “metabarcoding and soil” (blue) and “metagenomics and soil” (red), respectively. The values refer to an online search conducted in the Scopus Database (values recorded on September 15th, 2014; search parameters: all standard except). The growing trend of “metagenomics and soil” studies will likely be confirmed at the end of 2014. (b) Distribution of authentic metagenomics-based articles (positive — orange) and metabarcoding-based approach articles (false positive — green). The false positives are calculated as the articles with the keywords “metagenomics and soil”, where they were, in fact, based on a metabarcoding strategy. The year 2012 has been chosen as a time threshold as it is the year when the term “metabarcoding” was proposed for the first time.](image-url)
measures that can preserve and protect it. Metabarcoding offers unprecedented opportunities toward reaching both objectives. For example, it offers unique opportunities related to the spatial distribution of soil biodiversity. Soil biodiversity remains to be explored at different embedded scales: from the soil aggregate, which is relevant to the size of the organisms studied (Briones, 2014), to landscapes and whole territories, which is relevant for land users and managers (Ettema and Wardle, 2002; Nunan et al., 2003) with several ecological questions that still need to be addressed (Griffiths et al., 2011; Ranjard et al., 2013; Serna-Chavez et al., 2013).

The ability to store soil DNA for prolonged periods of time (Lauber et al., 2010) also offers unprecedented chances to collect and assess the evolution of soil biodiversity over time in relation to global change (e.g. climatic changes) (Dumbrell et al., 2011). More generally, application of metabarcoding in soil surveys will provide us with a baseline measurement of biodiversity, which can be referred to, for example, in the case of an environmental disaster caused by human activity. The appropriate use of new tools, e.g. NGS, and the huge amount of data from soil metabarcoding enable the conversion of all these opportunities into new knowledge, thus allowing concrete progress towards better comprehension of soil biology and ecology. Studies of soil biodiversity through metabarcoding will also bring insights relevant for more applicative purposes. This kind of study would be particularly helpful for decision makers developing measures to preserve soil biodiversity as has already been done for aboveground biodiversity. This means the ability to screen local populations and prevent harm being done to them (Thomsen et al., 2012). In other words, the operational opportunities allow an actual diagnosis of soil biological properties and, consequently, development of concrete measures to preserve these features. This requires the development of Standard Operating Procedures (SOPs) for soil analyses and referential methods for the interpretation of the corresponding analyses depending on the soil type, climate, and land use as already done for soil physical—chemical properties.

So far the attention and available technologies have mainly been focused on the production of snapshots showing the community structure and diversity of only a portion of soil biota, such as archaea, bacteria, fungi or earthworms, present at a given time and in a specific area of interest as a result of specific features, such as the land-use type or vegetation. However, we need to move towards a wider perspective. The available DNA-based tools allow us to think about large-scale and long-term projects aiming at global and chronological description of soil biodiversity. This is increasingly becoming a key issue because of several environmental variables (e.g. soil types, climatic conditions, and land use) that can affect soil biodiversity. Indeed, fluctuations of soil biodiversity are common and are not always cause or result of soil degradation thanks to the resilience of the system allowing balancing of potential losses (Shade et al., 2012; Pereira e Silva et al., 2013). Therefore, possible variations in soil biodiversity must be interpreted in relation to all the considered variables in order to assess whether changes of below-ground biodiversity are occurring or not. The spatio-temporal assessment of soil biodiversity through the creation of specific soil biodiversity maps could represent a major step forward with regard to soil protection and restoration. Maps also allow more transparent identification of soil threats, areas requiring risk mitigation, and areas of good soil quality that require a lower level of intervention.

3. Challenges

The potential of new molecular tools together with the increased awareness of the importance of soil biodiversity is leading to an increasing number of metabarcoding studies on soil biodiversity. This increasing interest must be taken into account and will entail facing challenges including: (i) moving towards a complete study of soil organisms (ii) standardization of operating procedures and continuing methodological developments, (iii) creation and sharing databases and referential procedures, and (iv) preventing the loss of data derived from metabarcoding. In order to overcome these challenges, we present here a possible workflow aiming at creating a virtuous circle to ensure a valuable assessment of soil biodiversity (Fig. 2). Thanks to the collaboration among the interested scientists it would be possible to develop standard procedures and a common database, as demonstrated by the Barcode of Life consortium. This would allow establishment of a real spatial and temporal monitoring effort of soil biodiversity, with additional sampling areas and new data added to the list of hot points and database year by year. The obtained assessment of soil biodiversity at large scales may be used not only for scientific purposes, but also to develop appropriate measures to preserve soil biota.

Most studies on soil biodiversity address a mere fraction of this biodiversity: most studies only deal with microbial biodiversity, the majority of which only consider the bacterial and archaea biodiversity (Rob et al., 2010). Only few studies so far have encompassed a wide range of organisms with the aim of better understanding soil functioning and biotic interactions (Pimm et al., 1991; De Vries et al., 2012a, b). A major challenge is, therefore, to develop studies that encompass all biodiversity, from micro to macro scale, and not only a fragment of it. Developments are underway to apply the same type of metabarcoding techniques to the protozoa and multicellular eukaryotes (i.e. soil fauna; Andersen et al., 2012; Hamilton et al., 2009). Morphological assessments are time consuming and require a high level of taxonomic expertise. Therefore, there is an urgent need to develop metabarcoding approaches for these organisms and to calibrate them with the classic phenotypic trait-based identification. Targeted approaches have been published for some well-studied groups (e.g. nematodes; Floyd et al., 2002; Griffiths et al., 2006) but are lacking for others (e.g. soil mites or enchytraeids). Metabarcoding could also be applied in order to characterize plant root distributions (Jørgensen et al., 2012) or plant diaspore banks (e.g. weed seeds) which form components of the soil biodiversity with major impact on soil quality. The Barcode of Life and its database, which aim to develop a public reference library of species DNA barcodes, represent the way forward. This will allow the application of metabarcoding to all soil-living organisms and, therefore, to adopt a more systematic approach in order to, firstly, describe soil biodiversity as a whole and, consequently, to strengthen our understanding of it. Another issue is knowledge of sequences with taxonomic values that could be applied to allow a broader description of soil biodiversity. The development of metabarcoding calibration is currently applied to faunal organisms isolated from soils. This raises a major issue regarding the complexity and variety of this extraction step according to the type of organisms analysed. Nevertheless, this wider consideration of soil biodiversity will allow us to better disentangle the ecological relationship between below- and above-ground communities and how these relationships are regulated.

A solution to the major operational challenge of the metabarcoding studies is to follow Standard Operating Procedures (SOPs) in order to allow comparisons and to value the outputs of the surveys. There is a high variability in all steps of the analysis: from the sampling strategy to the DNA extraction procedure (Rob et al., 2003), sequencing method (i.e. different NGS platforms) and tools and parameters for data analysis (e.g. choice of bioinformatics software and genetic distance threshold to distinguish different species) (Collins and Cruickshank, 2013). One way to move forward
would be to develop general rules as, for example, has been done for sampling soils for physical-chemical analyses. For microbial DNA, significant progress toward standardization has been made in national surveys (Rutgers et al., 2009) and a unique ongoing study along a European transect. ISO standardized operating procedures are available for the extraction of DNA from soil (ISO 11063) and the extraction of live specimens of various groups of soil fauna (ISO 23611 part 1–6), and may be applied when adopting an indirect extraction approach. The next step would be to obtain a broader view of soil biodiversity, i.e. encompassing microorganisms and fauna by targeting a series of barcode fragments using DNA directly extracted from soil. Of course, this is an ambitious undertaking since the number and size of the soil samples must be adapted to the larger organisms and to fauna with regard to their ability to move within the soil, in the same way that the minimal size of samples for DNA extraction for bacterial and fungal diversity characterization was previously defined (Ranjard et al., 2003). Furthermore, methodology will have to be developed in order to optimize the procedure and the cost of DNA extraction for these larger soil samples (see e.g. Taberlet et al., 2012b). An additional methodological challenge is how to reduce bias related to DNA extraction. Extraction efficacy may vary according to the soil type, especially when considering differences in soil texture and organic matter content (Delmont et al., 2011). However, despite this bias, an ISO standard has been developed for the extraction of microbial DNA and compared across a network of laboratories, allowing data comparison (Philippot et al., 2012). For meiofaunal fauna, Fonseca et al. (2011) clearly showed the range of variation that may result from using different extraction kits on identical samples, but no standardization attempts have been published so far. Moreover, standardization of methods should be applied at each step of the analysis, from DNA extraction to the bioinformatics pipeline in order to properly analyse the DNA sequences. The uncertainty in the bioinformatics pipeline is mainly related to (i) the difficulty in obtaining a clear taxonomic affiliation of all the DNA reads because of the possible biases in the sequencing process (Balzer et al., 2011); (ii) the difficulty in identifying a fixed threshold to discriminate different species or OTUs; (iii) the large number of available software to analyse DNA sequences and the difficulty in choosing the most appropriate software and parameter settings. In practice, a clear trade-off exists between the need to eliminate low-quality readings without losing valuable information by accidentally deleting divergent but valid sequences belonging to rare species.

Furthermore, the current methodological discrepancy can affect the interpretation of the data and often impairs comparisons of soil biodiversity among studies. Even if the number of studies are continuously increasing (Fig. 1), metabarcoding applied to soil is still a young discipline and immediate actions can be taken in order to avoid future issues. Common SOPs could be more beneficial if adopted within a short period of time, as this would facilitate future meta-analysis and comparison of DNA sequences and OTUs. A reliable meta-analysis would be possible using the consensus sequences of the OTUs per dataset as input for a new OTU clustering. A prerequisite for this is to obtain OTUs through a common bioinformatics pipeline; otherwise the harmonisation process would be extremely time-consuming. Current knowledge and the related scientific literature (Plassart et al., 2012; Köljalg et al., 2013) allow a combined effort to gather and synthesize the present methodological DNA-based approaches in order to assess soil biodiversity and identify ways to improve the methods. A similar approach has been followed in other disciplines, such as genomics, and could be used as a model. One representative example is the 1000 Fungal Genomes Project and the Fungal Genomics Program with their own web portals of protocols and datasets available to the public. The distribution of standard procedures and large soil metabarcoding datasets, which will most likely be developed in the coming years, will allow both large-scale and in-depth comparisons of total soil diversity across soil types, climates and land use types (Rousk et al., 2013). Overcoming the methodological challenges is an essential step toward creating a referential point in the study of soil biodiversity. A reference not only in terms of knowledge of both the level and distribution of soil biodiversity across ecosystems, but also as a reference in monitoring and protection of soil biota across time and space.
The next challenge is the management, storage and further exploitation of the data obtained through metabarcoding of soil DNA. Prior to publication, all studies are required to deposit the obtained DNA sequences with some additional information (e.g. collection date and location, possible identity and PCR primers) in one of the most well-known databases (i.e. Barcode of Life Database —BOLD— National Center for Biotechnology Information —NCBI— database or European Bioinformatics Institute —EBI— database). These are mainly focused on visualizing sequence variation among taxa, rather than linking sequence variation to environmental, spatial or temporal variations. A unique database of metabarcoding data does not exist. In order to develop and set up future metabarcoding-based surveys on soil biodiversity, we propose a step-by-step pipeline to reach this goal (Fig. 2). The soil (e.g. physical and chemical parameters) and environmental features (e.g. climate and land use parameters) would be used as a first criterion for selecting sampling areas in order to obtain a spectrum of representative environments. Once the data have been collected and analysed through commonly accepted methods, they should be quality checked (using standardised data queries) and inserted into a database for further comparison and integration. Besides the diversity and relative abundance of different organisms, a complete dataset should also include the collected metadata. This is the common approach used for other soil properties (e.g. physical and chemical properties) with updated databases monitoring changes over time. Metadata have proven to be crucial for best interpretation of the data in past collections of other soil properties, such as soil organic carbon and soil erosion (Panagos et al., 2013). A proposed list of requested metadata includes the following from sampling areas: location, geographical coordinates, land management, land use changes, sampling strategy, laboratory method analysis, survey date, climate, and chemical and physical properties. The scientific community is asked to clearly report all this information before publishing data. Scientific journals may also help with this concerted effort by avoiding the publication of papers lacking this information. Furthermore, obtaining a comprehensive database is a crucial step since it would allow mapping and modelling soil biodiversity together with other environmental parameters. Several databases of both environmental DNA sequences (e.g. the Metagenomics RAST database and EBI metagenomics) and biodiversity information (e.g. Global Biodiversity Information Facility) are now available. Of course they represent a valuable resource and could be used to (i) assess the state of soil biodiversity and DNA metabarcoding information available so far, and (ii) develop a preliminary assessment of the soil biodiversity distribution by means, for example, of meta-analysis of the available data. Nevertheless, these huge databases include a lot of information and may be discursive. Therefore, establishing a specific database dedicated to soil biodiversity is essential. This will allow us to specify the range of variations of soil biodiversity for a given soil type, climate, land use and, therefore, to interpret the results of analyses of soil biological properties as has been done for many years with soil physical—chemical properties. This approach is required to ultimately deliver to soil managers and end users a diagnosis of soil quality in order to define actions to be taken. The development of a common database represents one of the major issues, difficult to be addressed by a limited group of people. Indeed, it requires a collaboration among the involved scientists as well as the appropriate tools and expertise to develop it. Therefore, any opportunity, such as conferences and workshops that allow us to discuss this issue would be a good way to disentangle this matter.

A further challenge related to the development of a database is the need to avoid losing data of potential interest. As stated above, many research projects are currently focused on the metabarcoding analysis of only one component of soil biota (e.g. soil microbial communities) and, therefore, do not consider any other kind of retrieved data (e.g. all the sequences identified as non-microbial or those not identifiable at all), meaning that many sequences are not kept. This is due to both the large amount and diversity of DNA information present in soil, as well as the low specificity of the available detecting tools (i.e. low specificity of primer sets for DNA amplifications) (Sipos et al., 2007). However, those eliminated data are informative since they originate from organisms living in the analysed soil. One may, therefore, consider storing them to be identified and used in the future by other research groups. This would mean the insertion the “wasted/unknown” data into a specific section of the database, which would be implemented for this purpose. The development of a common database for all types of sequences may be a major step toward a reliable assessment of soil biodiversity.

4. Conclusions

The importance of soil biodiversity for providing ecosystem services is well known (Dominati et al., 2010). DNA metabarcoding represents an unprecedented opportunity for the study and monitoring of biodiversity in a wide array of environmental conditions over time. National (e.g. CreBeo Soil Biodiversity Project in Ireland and Biome of Australia Soil Environments -BASE-in Australia) and international projects (e.g. European project EcoFINDERS, Global Soil Biodiversity Initiative) have been established in order to increase our knowledge and understanding of spatial and temporal distribution of soil biodiversity by means of DNA barcoding. This number is likely to increase in the coming years together with the amount of data collected. The knowledge and technological context is suitable for metabarcoding of soil biodiversity at large scales. Therefore, it is time to discuss and promote a systematic and coordinated effort toward common guidelines allowing the comparison of data and development of global and regional studies and assessments of soil biota. Nevertheless, there are obstacles that must be overcome soon in order to reach this goal. Fortunately, there are many interested scientists and it is necessary to reach a general consensus on certain issues: from standard methods to the creation of specific database. We are aware that such an ambitious process will require time, dialogue, and a combined effort, but its achievement is essential and inevitable. Therefore, apart from our ideas, we are also proposing a call for any other opinions and suggestions that could help in reaching this goal. Soil metabarcoding is an opportunity to be seized. If actions are not taken, we risk losing the great opportunity of obtaining a truly comprehensive analysis of soil biodiversity.

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References


for the sequencing of a soil metagenome. Nature Reviews Microbiology 7, 252.


