Classical Techniques Versus Omics Approaches

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Abstract

This volume presents the state-of-the-art of omics in soil science, a field that is advancing rapidly on many fronts. The various omics approaches hold much promise but also await further refinement before they are ready for widespread adaptation. One way to judge their readiness is to compare them to methods that have become standards for soil microbiology research. Methods become standards because they provide useful information quickly and inexpensively. There is no question that omics can provide useful information, some of which cannot be obtained with traditional techniques, and integration of omics methods may provide insights into ecosystem functioning. In particular, the potential for omics to provide comprehensive coverage of genes and genes products make them well-suited for the study of general soil microbiological phenomena, such as decomposition, response to water stress, etc.

Introduction

What limits our ability to advance soil microbial ecology? Some suggest that we need to think harder, or better, and focus on identifying underlying principles that are founded, at least in part, on ecological theory (Prosser *et al.*, 2007; Fierer *et al.*, 2009). Others express the long-standing opinion that progress in microbial ecology is intimately linked to the development of new methods (Jansson *et al.*, 2012). Of course, both ingredients – theory and technique – are important drivers of advances in our understanding of the structure and function of microbial communities in soil. Our purpose in this chapter touches on this interplay by contrasting insights that can be gained from classical procedures compared to the newly developed and rapidly evolving omics methods that have been highlighted in this book. We begin by highlighting two, now classic, methods that revolutionized soil microbial ecology.

Microbial biomass by chloroform fumigation

The number, or biomass, of microorganisms is a key property of soil because microbes are catalysts for processing C and nutrients, and key players of several soil functions. The microbial biomass is also an important reservoir of C and nutrients in soil. Until the development and refinement of the chloroform fumigation method (Jenkinson and Powlson, 1976; Brookes *et al.*, 1985), extrapolation of microscopic counts was the only approach available to estimate the content of the main elements (C, N, P and S) present in soil microbiota.

The chloroform fumigation method directly determines the content of the main nutrients in soil microbial biomass whereas other methods, such as those based on the components of living cells (e.g. ATP content) or on the response of soil microorganisms to the addition of an exogenous substrate (the substrate-induced respiration method, SIR), permit only the indirect determination of the nutrient content (Anderson and Domsch, 1978; Jenkinson and Ladd, 1981).

The utility of the chloroform fumigation is shown by its rapid and widespread adoption – the articles by Jenkinson and Powlson (1976) and Brookes *et al.* (1985) have each been cited about 1400 times (Web of Science, 23 May 2012). Indeed, prior to 1976 there were no papers in the literature on the topic of 'soil microbial biomass', but thereafter such papers increased exponentially, with 93, 1543 and 5145 articles in the three succeeding decades. Clearly the chloroform fumigation method was instrumental in advancing soil microbial ecology research, particularly in monitoring nutrient fluxes through the soil microbiota. For example, using the chloroform fumigation method it is possible to trace the microbial fate of an isotopically labelled nutrient by monitoring its abundance in the microbial biomass. This in turn has fostered a more holistic approach for studying complex systems, such as soil, which is based on dividing the system into different pools linked by fluxes representing physical or abiotic and/or biotic transformations (Nannipieri *et al.*, 1994). Such current models of nutrient dynamics are based on (i) microbial biomass as a source and sink of nutrients; (ii) simultaneous microbial decomposition of organic matter and microbial synthesis; and (iii) multiple pools of organic matter with different degradation kinetics.

Denitrification rates by acetylene blockage

Quantifying denitrification – the stepwise microbial reduction of NO_3^- to N_2 – has been plagued by several technical challenges, foremost among them was the difficulty of measuring relatively small fluxes of N_2 gas into the large pool of atmospheric N_2 (Groffman *et al.*, 2006). The latter difficulty could be overcome by using ^{15}N-labelled NO_3^- (Hauck *et al.*, 1958); however, this method is also not particularly sensitive and requires access to an isotope ratio mass spectrometer, which greatly limited its adoption. Indeed, it took more than 20 years for this N-isotope distribution approach to be applied (Focht and Stolzy, 1978; Siegel *et al.*, 1982).

In contrast, the discovery by Yoshinari and Knowles (1976) that acetylene can be used to specifically block the final step in the denitrification pathway – the reduction of N_2O to N_2 – was quickly adopted and has been cited about 500 times (Web of Science, 23 May 2012). The so-called acetylene-block method removed the measurement bottleneck because it was relatively quick and inexpensive to measure small changes in N_2O against its ppb background in the atmosphere, and led to an exponential increase in soil denitrification studies. Use of the acetylene-block method spawned the development of additional widely used methods (e.g. Smith and Tiedje, 1979), provided insights into the high temporal and spatial variability of soil denitrification (Parkin, 1987; Sexstone *et al.*, 1988), and generally contributed to a quantifying losses of N by denitrification from soils of diverse ecosystems (Smil, 1999; Janzen *et al.*, 2003; Sietzinger *et al.*, 2006).

The promises of omics methods

Will omics methods – metagenomics, metatranscriptomics, metaproteomics, etc. – have a similar impact on microbial ecology as previous innovations in methodology? It is not possible to predict with certainty at this point, but the rapid and widespread adoption by soil microbiologists of the first generation of molecular methods, such as DNA fingerprinting (e.g. DGGE, T-RFLP) and Q-PCR, and more recently next generation pyrotagged sequencing, suggests that there is a demand for better tools to describe soil microbial communities.

In theory, omics approaches offer two advantages over existing methods: comprehensiveness and complementarity. As their prefix 'meta' implies, omics methods strive to measure all the genes, transcripts, or proteins in a given soil. For example, if a complete catalogue of genes exists, then it may be possible to identify all potential functions of every microorganism in a soil. Included in this complete parts list will be novel genes, whose potential function is not yet known (Vogel *et al.*, 2009). Precisely because they are unknown, their discovery requires a metagenomics approach. The same principle applies for transcripts and proteins.

Because of the canonical relationship of gene to transcript to protein, the three omics methods are inherently complementary. Linking them together provides an integrated picture of the relationship between potential activity – the structure of the microbial community – and realized activity – the function of the microbial community. For example, Jaffe *et al.* (2004) proposed a proteogenomic approach to validate genes and visualize the real location of the coding domain sequences in the genome by directly mapping proteins analysed by the proteomics on genome sequences, as discussed by Bland and Armengaud in Chapter 8. By combining genome and proteome information new genes and new proteins have been detected, giving more useful information than the separate use of metagenomics and proteomics.

Omics data also offer great potential to examine linkages or interactions among organisms or their gene products. For example, network analysis approaches have been used to predict microbial community assemblages and to identify interactions among community members based on gene sequence data (Larsen *et al.*, 2012a; Zhou *et al.*, 2011). These types of analysis may ultimately provide the information needed to develop the framework for ecosystem models (Allison *et al.*, 2010; Treseder *et al.*, 2012).

The pitfalls of omics methods

Will omics methods become widely used? Any method that becomes commonly used meets most, if not all, of the following criteria: uncomplicated and quick to perform, relatively inexpensive, uses readily available instrumentation, and produces clearly interpretable and useful information. Presently, all of the omics methods fall short in several of these criteria, although that is probably the case for most new methods. With greater use, many of the challenges associated with omics methods (e.g. extraction and purification of biomolecules, data handling and analysis) will likely become more tractable.

Challenges typical of working with soils

Some challenges for applying omics methods to soils are also common to any study of the biology of soils (Lombard *et al.*, 2011). These include the collection of a representative soil

sample, and the extraction and purification of an adequate amount of the desired biomolecule from the soil matrix.

Soil is a heterogeneous system and the biological space represents a small percentage of the overall soil space, probably because only a small number of soil microenvironments have the right set of conditions (nutrients, protection against predators, growth factors, absence of toxic substances, suitable pH, suitable oxidation-reduction potential, etc.) for the microbial life (Nannipieri *et al.*, 2003). There are some 'hotspots' in soil with increased microbial activity, such as the rhizosphere soil and zones around fresh organic matter. This spatial heterogeneity has led to studies that focus on such microhabitats (Davinic *et al.*, 2012).

Studies at the microscale can help to establish predictive relationships between soil characteristics and microbial processes in soil. Observations by electronic microscopy are useful for visualizing spatial distribution of bacterial and fungi in soil but are limited with respect to their ability to describe microbial community composition or activity. One rare exception is that active acid phosphomonoesterase has been detected in microbial cell membrane fragments in sections without soil particles (Ladd *et al.*, 1996). Consequently, a microsampling strategy has been proposed by Grundmann *et al.* (2001) and used to characterize the spatial distribution of microbial microhabitat and microbial species at the microscale; for example, the spatial distribution of *Agrobacterium* spp. Bioavr 1 in 1 cm^3 of soil was investigated by collecting 865 microsamples, each 500 μm in diameter (Vogel *et al.*, 2003). This sampling strategy is limited in application for measuring the metagenome, metatranscriptome, or proteome at the microscale because the amount of extracted molecules from these microsamples is too small to be analysed by the current techniques. Consequently, larger samples that average across microhabitats are used.

In a study that examined the effects of sample size, Delmont *et al.* (2011) determined that 100 g of soil was sufficient to represent the metagenome of soil microbial community of the Park Grass soil, the reference soil for metagenomic studies (Vogel *et al.*, 2009). As part of this study, a fingerprinting technique, ribosomal intergenic spacer analysis (RISA) of DNA, was used to explore spatial variation at the field scale. That analysis showed that vertical variation in microbial diversity was higher than horizontal variation (Delmont *et al.*, 2011).

This challenge of spatial heterogeneity, which is common to all soils studies, can be further complicated by temporal variability, which is caused by the dynamic nature of the soil microbial community as it responds to changes in environmental conditions or other perturbations. For example, temperature and moisture are important determinants of microbial activity in soil, and these commonly change seasonally. Therefore markedly different results could be obtained when soils are sampled in different seasons, not only for gene expression activities but also for the metagenomic approach. For example, metagenomic approaches usually describe predominant microbial communities (Delmont *et al.*, 2011), which can be different under different soil conditions through the year. For example, and high microbial activity may mean consumption of available P, which represses the synthesis of phosphomonoesterase and thus the expression of the relative genes encoding these enzymes (Nannipieri *et al.*, 2011). The analysis of spatiotemporal data can be difficult; however, methods are being developed to tackle this challenge (Graf *et al.*, 2012; Larsen *et al.*, 2012b).

Regardless of the size of the soil sample used, whenever we extract something from soil, the architecture of the soil is destroyed. According to Kubiena (1938), soil is like a city whose architecture is destroyed after soil sampling, breakdown of soil aggregates, and extraction. Any determination involving soil extracts, such as the characterization of nucleic

acids and proteins for evaluating structure and functionality of microbial communities, does not permit the localization of the measured parameter in the soil matrix.

Extractions of nucleic acids and proteins from soil can present several problems affecting metagenomic, transcriptomic, and proteomic analysis, and the relative interpretations of results. It is not only a problem of yields but also completeness: A method should extract these molecules from all members of soil microbial communities. This is not often the case. For example, different methods of DNA extraction from soil give different results about microbial diversity (Bakken and Frostegård, 2006; Delmont *et al.*, 2011). By analogy, it is reasonable to assume that the different methods used to extract proteins from soil for proteome analysis will give different proteomic profiles, although this has not been evaluated (Nannipieri, 2006; Giagnoni *et al.*, 2010). In addition, extraction methods typically release the desired biomolecule from microbial cells but in the process also release other organic compounds present in soil and expose the biomolecule to interactions with other components of the soil, such as clays. Clays can bind the released biomolecule and extracellular enzymes, such as nucleases and proteases, can degrade the biomolecule, reducing yields. The organic 'contaminants' often interfere with downstream analysis of the biomolecule of interest. Procedures have been developed to help alleviate these problems with extraction efficiency and inhibition, but none are universally effective. This has led to the general sentiment that extraction methods should be tested and optimized for individual soils, which is not particularly practical.

Lastly, it is important to remember the challenges inherent in scaling up processes or functions at the field, regional, and landscape level, which is essential for suitable land management (Standing *et al.*, 2007). Scaling from the gene level (10^{-8} m) through plant (10^{-2} m) and field (10^{2} m) up to landscape (10^{5} m) requires an understanding of the connections, linkages, feedbacks, and the scale-related relevance of its constituent parts. In addition, scaling up is made difficult by the use of different techniques at different scales (Standing *et al.*, 2007). For example, soil can be a source and sink of two greenhouse gases, N_2O and CH_4, and the underlying mechanisms of the relative processes as well as the active soil microorganisms are studied by molecular techniques, including the omics approach at the microscale whereas their evolution can be studied with chamber techniques at the plot scale, micro-meteorological measurements at the medium scale, and use of aircraft at the catchment or landscape scale (Standing *et al.*, 2007).

Challenges unique to omics data

Assuming that it is possible to obtain biomolecules of adequate quantity and quality that are representative of the soil being studied, additional hurdles common to meta-omics studies must be overcome. Many of these are related to the quantity of data needed for complete coverage of the genes, transcripts, or proteins in a soil. Although improvements in sequencing technology have enabled ever greater amounts of DNA or RNA sequence data, it is questionable if even terabytes of data will provide complete coverage of the soil metagenome or metatranscriptome (Brown and Tiedje, 2011). Shotgun proteomics are not as readily scalable. In this case further advances are needed to detect more than the most abundant proteins (Siggins *et al.*, 2012).

Analysis of what has become known as the 'data deluge' is another significant challenge. Currently, it is not possible to fully assemble metagenomic and metatranscriptomic data, although some partial assembly is possible and information can be gained from unassembled

sequences (Luo *et al.*, 2012; Pell *et al.*, 2012). The small read length generated by the present sequencing systems contributes to this difficulty. Short reads reliably identify sequence homologues only when there is a strong homology to previously described genes (Morales and Holben, 2011). In addition, short sequences reads produce a series of non-contiguous genetic fragments and their assembly into sequences representing genetic organizational units is problematic, particularly for the unknown functions. Advances in bioinformatics algorithms and access to larger computer resources (e.g. cloud computing) will improve this situation, but an additional limitation is the depth and quality of annotated sequences that are queried for gene and protein identification (Raes *et al.*, 2007; Raes and Bork, 2008). A further stumbling block is the need for statistical and modelling tools to interpret the vast quantities of information generated with omics methods. Recent applications of different types of network analysis have shown promise in this area (Zhou *et al.*, 2011; Faust and Raes, 2012; Larsen *et al.*, 2012a).

Successful integration of omics data are still an outstanding challenge for bioinformaticians grappling with the large and complex datasets that are produced, but doing so holds great promise for understanding the connections and interactions that form the basis of microbial processes in soil. In addition to developing bioinformatics tools, it will be necessary to gain a deeper understanding about the time scales and synchronicity of the responses of metagenomes, metatranscriptomes, proteomes, and metabolome, and whether their relationships are linear or nonlinear and how much stochasticity is involved.

Collectively, the challenges that omics data present will make it difficult, and perhaps impractical, to do replicated experiments, which is the hallmark of soil microbial research (Prosser, 2010). Nevertheless, omic-driven research should go beyond being descriptive and be hypothesis-driven, with a greater focus on experimental design rather than on the technological prowess.

Research that is best suited for omics approaches

As noted previously, soils present some common challenges in terms of measuring their properties and processes, such as their variability in space and time, potential interference of methodology with the variable being measured, etc. In most cases, multiple approaches are applied with the hope that the different perspectives will provide insight into the property or processes being measured. An important consideration with omics approaches is to identify the questions that they can answer better than traditional approaches.

Processes that are directly associated with specific enzymes and their affiliated genes and transcripts, such as nitrification with ammonia monooxygenase or denitrification with nitrate, nitrite, nitric oxide, and nitrous oxide reductases, are probably more efficiently studied with a targeted approach. Since the study of Cavigelli and Robertson (1980) first suggested a link between microbial community composition and denitrification, many studies have used molecular methods based on denitrifier genes to explore relationship between denitrifier community structure and function (Wallenstein *et al.*, 2006). More recent studies have also used denitrifier gene transcripts (Pastorelli *et al.*, 2011). For example, Liu *et al.* (2010) found that induction of denitrifier genes was related to denitrification rates; however, the effect of pH on the fraction of denitrification as N_2O appeared to be due to post-translational events.

We are of the opinion that meta-omics data are best suited for obtaining a holistic

understanding of generic processes that cannot be easily addressed with a targeted approach, such as soil organic matter mineralization or the response of the entire microbial community to a perturbation, e.g. tillage or irrigation. The mineralization of organic matter involves most of the heterotrophic bacteria and fungi present in soil – making it impossible to focus in on a specific functional taxa, which metabolize a plethora of organic compounds – making it impossible to focus on specific functional genes, and produce a range of metabolites – making it impossible to follow a single end product. A meta-omics approach has the potential to capture this taxonomic and functional diversity, and explore the interactions among taxa and their metabolic processes assuming, of course, that the challenges to dealing with the large and complex data thus obtained can be overcome.

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