SOIL AS A BIOLOGICAL SYSTEM AND OMICS APPROACHES

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Abstract

Soil as a biological system is characterized by: i) the presence of a remarkable diversity since thousands of bacterial genomes can be present in one gram of soil. In addition microbial biomass is huge; ii) only a minor proportion of the available space is occupied by microorganisms in soil (microbiological space); iii) soil colloids can adsorb important biological molecules such as proteins and nucleic acids. Nucleic acids can be adsorbed and retain their biological activity; iv). soil components show enzyme-like activities. Unfortunately there is no methods to distinguish enzyme from enzyme-like reactions but these methods are needed to quantify both contributions; v) virus are more abundant than in other systems such as aquatic ones. A book "Omics in Soil Science" (Nannipieri et al 2014) has been recently published; it presents the state-of-the-art of omics in soil science, a field that is advancing rapidly on many fronts. The various omics (mainly metagenomics, metatranscriptomics, proteomics and proteogenomics) 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.

Introduction

After the Agenda 21, prepared from the United Nations Conference on Environment and Development in Rio de Janeiro 1992, there have been many studies on the importance of biodiversity in every ecosystem. In terrestrial ecosystems, observations and the consequent theories developed for the aboveground part of these ecosystems cannot be applied to the below-ground part due to the peculiarities and complexity of soil (Nannipieri et al 2003). It is well established that soil governs plant productivity and completes biogeochemical cycles due to the activity of organisms (macrofauna, nesofauna, microfauna and microflora) inhabiting it. Among soil organisms bacteria and fungi carry out almost all known biological reactions; however, we do not know all microbial species

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inhabiting soil since only a minor percentage of these can be culturable and studied in laboratory; therefore, most of soil microbial species are unknown. The use of molecular techniques, based on the extraction of nucleic acid and their characterization, has allowed studying unculturable soil microbial species but does not permit determining microbial diversity since only dominant species are usually determined. The application of omics (metagenomic, metatranscriptomic and proteomic) techniques to soil may permit determining rare microbial species and discovering new compounds (antibiotic, enzymes, etc) from expression of genes of unknown microbial species (Myrold and Nannipieri, 2014). However, the use in soil of omics techniques, developed in environmental microbiology, should be carried out considering the peculiarities and complexity of the soil system. Therefore, in this short review I shall discuss firstly the complexity of soil as a biological system and then advantages and drawbacks of soil omics techniques.

Soil as a biological system

Soil is a heterogeneous and structured system dominated by the solid phase where microorganisms occupy only a small percentage of the available volume (Nannipieri et al 2003, 2014); usually, soil microorganisms, are located in "hot spots", for example in the rhizosphere soil, around a plant residues or fertilizer particles, etc (Nannipieri et al 2003). However, the size of soil microflora is quite high accounting to 2% of soil organic C, as an average. It has been calculated that on average soil microflora has a biomass equivalent to 100 sheeps whereas 2 sheeps are grazing on the aboveground 1 ha grassland (Brookes, personal communication). Usually soil bacteria live on the surface of aggregates whereas fungi can explore the soil through their growing ifa. Bacteria live on water films surrounding soil particles and these communities are separated each other when soil is dry; on the contrary after irrigation or rainfall, water can fill soil pores and in this case the separate bacterial communities enter in contact each with the other. (Nannipieri et al 2014). The microbial diversity, that is the presence of different species, is very high and thousand of different species can live in one gram of soil (Nannipieri et al 2003).

Living organisms of soil are component of complex trophic webs; for example bacteria can grow in the rhizosphere upon the release of root exudates, which are mainly C sources; thus bacteria should mineralize organic N to satisfy the N requirements of their growth promoted by the C sources (Clarholm 1985). Bacterial growth occurs around the part of roots releasing exudates; the increase in bacterial number promotes protozoa grazing on bacteria and since the C/N ratio of protozoa is higher than that of bacterial cells, there is the release of inorganic N upon grazing; then, the released inorganic N can be taken up by roots (Clarholm 1985). Another interesting aspect of this trophic web is that the protozoa grazing stops when bacterial abundance decreases under a certain number, probably due to some molecular messengers relative to the number of bacterial cells. Nutrients blocked in the bacterial biomass can also be released and made available to roots by other microfaunal grazers, such as nematodes (Griffiths et al. 2012)

Another distinctive property of soil is the presence of surface.-reactive particles and important biological molecules, such as enzymes and nucleic acids, can be adsorbed or entrapped maintaining their activity. For example, enzymes, released after cell death and lysis or extracellularly released by living cells, can be entrapped by humic substances or adsorbed by clay particles maintaining their activity and being thus protected against microbial degradation (Nannipieri et al 2012). DNA molecules, released after cell death, can also be adsorbed by surface-reactive particles, be protected against the degradation by extracellular nucleases and be taken up by competent bacterial cells; thus the relative genes incorporated in the genome of the host bacterial cell (Pietramellara et al 2009).
The role of the smallest soil biota, virus and prions, on microbial ecology has been neglected since virus has been generally studied for their effects as pathogens of plants, animals and insects; nowadays it is known that most of soil viruses are bacteriophages and need to infect a bacterial cell to replicate (Williamson et al. 2012). Virus adsorption to surface-reactive particles can increase their persistence in soil (Lipson and Stotzky, 1986; Vettori et al., 1999). The ratio between viral abundance to bacterial abundance increases in agricultural soils (330-470) compared to waterlogged soils (10-60) and aquatic systems (0.5-50), because viral production is much faster than viral decay in agricultural soils (Williamson et al 2012).
As mentioned above soil microorganisms carry out almost all known biological reactions but there are inorganic and organic components of soil, which carry out enzyme-like reactions; the contribution of these abiotic reactions can be important under conditions limiting microbial activity (Gianfreda and Ruggiero 2006; Huang 1995; Ruggiero et al 1996).

Omics approaches in soil

There are two different approaches to better understanding soil functionality due to microbial activity: the need of imaginative research with innovative approaches and that of the development of new methods. Of course both are important for better understanding composition and activity of communities of soil organisms. Molecular and "omics" techniques represent important advancements from a technical point of view.
Studying microbial diversity is nowadays popular because by knowing microbial diversity it is possible to manipulate soil functioning and it is believed that the capacity of a soil to resist to stresses may depend on microbial diversity (Nannipieri et al 2003). There are soil processes, such as C and N mineralization, carried out by many different species and a decrease in microbial diversity does not affect the rate of the process, whereas this may not the case for processes, such as nitrification, which are carried out by only some microbial species. Culturable techniques only determine 1-10% of the microorganisms inhabiting soil and the problem of culturable microbial species has been overcame by using molecular techniques based on DNA extraction from soil, characterization of ribosomal RNA genes, which are present in high copies in active cells and have metabolic

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relevance (Nannipieri et al 2003). The presence of conservative regions in rDNA allows determining the abundance of microbial groups, whereas the presence of variable regions allows a deeper taxonomical characterization. There are several molecular techniques based on PCR amplification of rDNA with universal or specific primers. The denaturing gradient gel electrophoresis (DGGE) is one of the most used techniques and DNA molecules are separated as different bands in the gel with the denaturating gradient, even if they only differ for a couple of basis. It is a popular technique since the diversity of two samples can be easily compared by observing the presence of different bands in the two gels. All fingerprint techniques determine the most abundant microbial species of soil. The sequencing of soil DNA, due to the increasing performances of the present sequencers, has increased the detection of less abundant microbial species in soil. Of course the detection of all genes (soil metagenomic), and thus all microbial species inhabiting soil, is a utopia due to the huge microbial diversity and bias in the present methods. Among these bias there are incomplete DNA extraction from soil and difficulties in the bioinformatic step since it is not possible to fully assemble metagenomic or also metatranscriptomic data (Kim et al 2014; Myrold and Nannipieri 2014; Van Elsas et al 2014). Indeed identification of short reads, generally obtained by the present sequencing systems, can be obtained with a strong homology with previously described genes of database. However, the soil metagenomic approach can give insights on soil functionality by detecting the presence of functional genes, such as those involved in soil processes like nitrification, denitrification, etc. In addition, it is possible to discover genes enconding important molecules, such as antibiotics and novel enzymes, by soil metagenomic (Van Elsas et al 2014). However, determination of gene expression is needed to have an actual view of soil functioning. Problems also exist for determining gene expression in soil at the level of synthesis of RNA (soil metatranscriptomics). Indeed, there is no universal RNA extraction method, methods for enriching bacterial and fungal mRNA need to be improved and only 20-40% of total reads can be assigned to known functions (Liesack et al 2014).

It is well established that the sentence “one gene one protein” is not anymore valid since several processes can occur both after gene expression with synthesis of RNA and after translation, that is after protein synthesis from mRNA. Therefore, evaluating the functioning of a system, such as soil, requires determining all synthesized proteins, that is, to carry out the proteomic approach. Proteins are extracted from soil, purified, separated, usually, by 2D electrophoresis; after staining the gel, bands corresponding to single proteins are excised, solubilized and subjected to trypsin hydrolysis; then the tryptic peptides are analyzed by mass spectrometry; also in this case the detected aminoacid sequences are compared with previously described proteins of database (Renella et al 2014). A limit is the poor development of both fungal and plant protein databases compared to bacterial and animal databases. According to Renella et al (2014) the internal standard for the soil proteomic should be a microorganism with a known proteome. Soil proteomic is also biased by the fact that the amount and quality of extracted

proteins depends on the used method (Renella et al 2014). It is not possible to extract all proteins from soil once the microbial cells are lysed since surface-reactive particles, such as montmorillonite, can adsorb proteins. In addition, the contact of proteins with surface reactive soil components, such as humic molecules, can modify the protein conformation so that the trypsin hydrolysis may not occur in some parts of the protein molecule; thus these molecular parts are not analyzed by mass spectrometer being thus undetected (Arenella et al 2014).

Conclusions

Soil is a complex biological system whose functioning mainly depends on microbial activities. Understanding soil functioning may allow a better sustainable use of the soil source for crop production and this is essential for supplying food for a growing human population. Many microbial species inhabiting soil are unknown and by knowing their properties and physiology we can not only better understand soil functioning but also discover new compounds, such as new antibiotics and new enzymes, which can have important application in several human health and activities. The use of omics techniques can give further insights on activity and composition of soil microbial communities despite the several drawbacks of these techniques. These drawbacks can be considered when interpreting the relative research data but they should not be an obstacle for the use of these techniques is soil research. Imaginative research is also required and combined with the use of powerful techniques, such as the omic ones. Inoculation of sterilized soils with the same unsterilized soil or with other unsterilized soils has shown that soil properties drive composition of microbial communities (Delmont et al 2014). This is an example of imaginative research showing that undetected species with the soil metagenomic approach can be detected when soil conditions allow microbial grow.

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