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Review paper

Measuring biogeochemical heterogeneity at the micro scale in soils and sediments

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ABSTRACT

Steep physiochemical gradients and diffusive limitation associated with microscale features such as cracks and pores make soil and sediments remarkably heterogeneous environments, which is reflected on many environmentally important processes. If we are to understand and attempt to control the ecology of the microorganisms which inhabit these environments we must not only characterize their inhabitants, but also the complex biogeochemical landscape they live in. This includes local concentrations of electron acceptors and donors, microbial metabolites and key physical and chemical parameters such as pH and soil structure.

To this end, an array of techniques for collecting data at the microscale has been developed, deployed and refined, ranging from microsensor probes to planar sensors. This review provides a general reference for and a critical comparison of microscale techniques available to the fields of soil and sediment microbial ecology. Techniques are evaluated based on their ability to provide spatially resolved data at the microscale, with focus on performance characteristics, potential for repeated measurements, degree of physical disruption they create, and accessibility.

Microscale studies have given us many insights, but we outline further progress needed to make the microscale toolkit more accessible and to extend the range of analytes that can be measured simultaneously, so that we may expand our knowledge of the complex environmental microscale heterogeneity and its impact on soil and sediment ecology and functioning.

1. Introduction

Soil and sediments are complex, heterogeneous environments. Some of this heterogeneity is visible to the naked eye and/or can be readily measured using samples of gram to kilogram size. However it is becoming more and more evident that heterogeneity is not just present at the macroscopic scale, but also at the microscopic scale, the scale directly relevant for the ecology and functioning of microorganisms. Here local conditions in microscale features such as cracks in sand grains (Minagawa et al., 2008) and pores in organic remains (Widerlund and Davison, 2007) impact important processes, such as the cycling of carbon, nitrogen, sulfur and metals (Widerlund and Davison, 2007; Hansel et al., 2008). In this review, 'microscale' is reserved for structures and processes measured or occurring at scales from 1 to 100 μm, 'macroscale' from 1 cm and above, and 'mesoscale' for anything in between. Similarly, the term 'microniche' here refers to 1 μm^3–100 μm^3 subdomains associated with reactive compounds where diffusion limits the exchange with the bulk of the studied system.

Diffusive limitation caused by microscale features can lead to substantial spatial heterogeneity in the biogeochemical environment, and if we want to understand the ecology of the microorganisms that inhabit these heterogeneous environments, then we must not only be able to describe the microorganisms themselves, but also characterize the spatiotemporal niches they inhabit and how, and to which extent, the microorganisms and niches interact with each other (Nunan et al., 2007; Vos et al., 2013; Raynaud et al., 2014). While the total volume occupied by a given microniche is very small compared to the bulk system, its surfaces often provide plenty of attachment opportunities for microbes which are exposed to the ambient gradients but also modify them and create new ones. Even though one gram of soil can contain 10^7–10^{12} microbial cells (Watt et al., 2006), they can be restricted to as little as 1% of the total volume (Young et al., 2009). The resulting microbial

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microcolonies and biofilms are hotspots of activity reacting to and contributing to the complex web of spatial and temporal physiochemical gradients (Nunan et al., 2007).

An early example of the importance of soil hotspots was a study of denitrification in a soil core retrieved from a corn field, where it was found that 85% of the overall denitrification took place in as little as 0.0015% of the soil core (Parkin, 1987), illustrating that analyzing the spatiotemporal dynamics of such soil hotspots may yield important insights into soil functions. Since then, the concept of soil hotspots and our understanding of their importance for soil processes have been expanded upon greatly. We know now that soil is characterized by physical and temporal heterogeneity across scales ranging from nm to km (Young and Ritz, 2000), however the impact of individual microbial cells is too low to be of note on higher scales (Kuzyakov and Blagodatskaya, 2015), so the minimal size of a soil hotspot should be that of microcolonies and biofilm, in the vicinity of a few μm (Dechesne et al., 2003; Raynaud et al., 2014). Features measured on such small scales, such as pore pathways, influence processes relevant at much larger scales such as water retention (Vogel, 2000), plant productivity (Stirzaker et al., 1996) and nitrous oxide emission (Arah and Vinten, 1995). Using the latter process as an example, nitrous oxide is a greenhouse gas of high environmental concern, with a global warming potential per unit mass 300 times higher than carbon dioxide (USEPA, 2013). Nitrous oxide emissions from soil systems are often dominated by periods as short as a few days with extremely high emissions, primarily when the soil thaws (Flessa et al., 1995; Teepe et al., 2000; Holst et al., 2008) or is wetted after a long dry period (Davidson et al., 1991, 1993; Garcia-Montiel et al., 2003). Moving on from temporal heterogeneity to spatial heterogeneity, small scale spatial redistribution and concentration of nutrients plays an important part in creating intense nitrous oxide pulses, such as it happens during freeze–thaw cycles (Groffman et al., 2009). The occurrence of microbial hotspots in soil has recently been reviewed by Kuzyakov and Blagodatskaya who examined both the microbial hotspot occurrence, size, spatial distribution, lifetime and the associated intensity of microbial activity. The study revealed links between hotspots, their consumption of labile carbon, microbial growth, competition and other processes, concluding that despite occupying only 0.2–5% of the soil volume, the hotspot niches may be responsible for most processes measured at much larger scales, such as organic matter degradation and nitrogen cycling (Kuzyakov and Blagodatskaya, 2015). This heterogeneity has not only biogeochemical consequences; microniches can also function as a reservoir for important human pathogens such as *Staphylococcus aureus*, *Pseudomonas aeruginosa* (Mohammed et al., 2012), and *Vibrio cholera* (Vezzulli et al., 2010), all responsible for infections, hospitalizations and deaths every year (Palavecino, 2004; Hota et al., 2009; Ali et al., 2012). The knowledge that many important soil processes are concentrated in both small areas and timeframes underlines the need to study them at these scales, with a resolution high enough to resolve their finer details.

We still know little about the complex interactions of microniches, physiochemical gradients and spatial organization of multispecies microbial communities, and removing them from their natural environment and taking them apart and analyzing them is likely to modify or destroy both structure and functions (Epstein, 2009). Many of the early works on heterogeneity in soil and sediments utilized the core sampling technique followed by core slicing and analysis due to its ease. Unfortunately, various retrieval artefacts mean that data from measurements on the cores might not reflect the conditions found *in situ* (Pamatmat and Fenton, 1968; Murray et al., 1980; Emerson et al., 1982; Blomqvist, 1991). When average concentrations in each slice are determined, all potential gradients within the slices are overlooked (Brendel and Luther, 1995). Additionally, the mixing of the sample may allow chemicals that were localized in separate niches to meet and react, or simply give the impression that there is an overlap of redox species which did not exist in the untreated sample (Luther et al., 1999, 1998). These effects obscure the true environmental spatial heterogeneity.

Therefore, our understanding of the microbial ecology of soils and sediments benefit from the use of microscale techniques, which should not only be sensitive, robust, durable, minimally invasive but also economically accessible. For a long time, the impact of microscale heterogeneity was not evident, and progress in our understanding of these systems has been dependent on a gradual move towards studying the microenvironments directly in minimally disturbed samples and at a higher and higher resolution. This has allowed us to piece together an increasingly detailed view of their complex spatial organization, steep physicochemical gradients and diffusion and flow patterns (Groffman et al., 2009; Blossfeld et al., 2011; Elberling et al., 2011; Kuzyakov and Blagodatskaya, 2015).

1.1. Early studies of microscale environmental heterogeneity

The hypothesis that there exist niches in some environmental systems where a high content of organic matter results in localized spots of reducing conditions was suggested long ago as an explanation for the presence of metal sulfides within oxidized marine sediments (Emery and Rittenberg, 1952) and was brought up again by others making similar suggestions (Emery et al., 1963; Hallberg, 1968). This hypothesis was expanded on by Jørgensen who developed a theoretical model for the maintenance of a reduced microniche within an oxic environment. He also suggested that the existence of such reduced microniches may be important for other metabolic and diagenetic processes (Jørgensen, 1977). Structural microscale studies of soils and its microbial inhabitants using electron microscopy gave early hints at the complex microscale heterogeneity of soils. The technique relied on extensive preparation protocols that served to both stabilize the soil physically and render it electron dense (Foster, 1988), but were not artefact-free. Early electron microscopy studies provided important clues to the functioning of soil, such as soil being composed of many small microbial colonies, each surrounded by its own immediate microenvironment (Hattori and Hattori, 1976) and that the extracellular polysaccharides of an autolyzed bacterial colony may continue to hold an aggregate together well after the demise of the colony (Foster and Martin, 1981).

For some time, the microniche hypothesis was not confirmed, in spite of the availability of the necessary tools. Electrochemical microsensors capable of providing data at high spatial resolution have been available for over six decades. However they went unnoticed by microbial ecologists for almost three decades (Revsbech and Jørgensen, 1986): intracellular capillary microsensors with tip diameters of less than 1 μm were used for studying action potentials and iron transport over biological membranes by neurophysiologists in the 1950s (Draper and Weidmann, 1951; Hagiwara and Watanabe, 1954). A few years later, ion-sensitive microsensors for analysis of H^+, Na^+ and K^+ were developed (Caldwell, 1958; Hinke, 1959), and in the 1960s the first polarographic microsensors for the measurement of oxygen were described (Bolie, 1960). At this time, both types of microsensor were used for measurements in tissues and cell cultures but it would not be until the 1970s that microbial ecologists discovered them and introduced them to their field (Revsbech and Jørgensen, 1986). They have since been utilized to confirm the presence of steep microscale chemical gradients in soil. An example of this was the discovery that what had first appeared to be oxic silt loam soil contained small soil aggregates with central