The microbial habitat in soil: Scale, heterogeneity and functional consequences

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Abstract

The microbial habitat is rarely studied in soil microbial ecology even though microbial cells are exposed and adapt to their local environmental conditions. The physical environment also constrains interactions among organisms. The nature of microbial communities and their functioning can only be fully understood if their habitat is accounted for. Here, I describe the soil microbial habitat and show how our understanding of microbial functioning has been shaped by this line of investigation.

Introduction

In contrast to higher organisms, microbial cells are highly exposed to the conditions of their immediate environment and are rather limited in their ability to alter these conditions to suit their needs (*Egli*, 1995). In order to survive and grow, microorganisms adapt their physiology to the local physical and chemical conditions to which they are subjected. Drought and rewetting in soil for example, induces wholesale changes in microbial physiology and has profound effects on the makeup of the microbial communities (*Placella et al.*, 2012; *Schimel et al.*, 2007). The physical environment also constrains the range over which organisms can interact, thus affecting the boundaries, size and composition of communities (*Konopka*, 2009). In soil, microbial communities exist in a three-dimensional physical environment that is heterogeneous in both space and time and that regulates the flows of water, energy, gases and nutrients (*Young and Ritz*, 1998). It is likely therefore, that microbial functioning and the nature of microbial communities can only be fully understood if the properties of their immediate environment and the manner in which they interact with their habitat are accounted for.

Soil microbial ecology is distinct from many other branches of ecology in that relatively little interest has been paid to the microbial habitat. A rapid analysis in Web of Knowledge™ shows that habitat is mentioned in approximately 10 % of publications in soil microbial ecology, considerably less than the approximately 30% found in many branches of macro-ecology (data not shown). This may be because the microbial habitat is not easily grasped, either from a measurement or a conceptual point of view, but there may also be a certain disregard among microbial ecologists for variations in environmental conditions and soil properties that occur at the scale of the soil aggregate or the microbial community. Nevertheless, the microscale variations in soil properties result in a myriad of micro-niches, which is believed to contribute to the high microbial diversity of soils (*Treves et al.*, 2003) from which soil biological functionality emerges (*Crawford et al.*, 2005). In the following I describe how recent research has shed light on the nature of soil microbial habitat and on the functional consequences of the intimate relationship between microbial communities and their micro-habitat. I will further show how these insights have shaped our understanding of what soil microbial communities are and of soil microbial functioning.

What is the microbial habitat in soil?

The microbial habitat in soil can be viewed at different scales. At the coarsest scale, different soils constitute different microbial habitats. Biogeographic studies have shown that the properties of soils are significant drivers of microbial composition (*King et al.*, 2010; *Ranjard et al.*, 2013). However, the majority of the variability in bacterial diversity is not explained at these scales of analysis, but occurs at finer scales (*King et al.*, 2010; *Ranjard et al.*, 2013), suggesting that microbial interactions with their habitat at finer scales play an important role in structuring communities. Soils can be partitioned into different spheres of influence, such as the rhizosphere, the detritusphere or the drilosphere. The properties of these spheres of influence differentiate them from the bulk soil and have significant impacts on microbial communities. Microbial activity is significantly higher (e.g. *Marschner et al.*, 2012) and the copiotrophic conditions result in a differentiation in microbial composition as well as a reduction in microbial diversity (*Peiffer et al.*, 2013; *Poll et al.*, 2010). The reduction in diversity may be due to the more amble supply of substrate, allowing the more competitive species to dominate. However, the physical structure of the rhizosphere is more spatially correlated (*Feeney et al.*, 2006) which, coupled with the likely increase in substrate availability throughout the rhizosphere, may also result in a certain homogenization of the rhizosphere micro-niches, thus reducing microbial diversity. The same may be true of the detritusphere or the drilosphere. In-

deed, soil is made up of a wide range of spatially distinct microbial ecological niches with different physical and chemical properties. These micro-niches, or micro-habitats, in effect, isolate microbial species from another, resulting in the non-competitive diversity patterns and the high levels of microbial diversity that are generally found in soils (*Treves et al.*, 2003).

The micro-habitat

Early observations of soil microbial communities in their natural environment suggested that they are predominantly found in pores with diameters between 0.8 and 10 *μ*m (*Kilbertus*, 1980) and are associated with humified organic matter, in the case of individual bacterial cells, or a diverse range of organic masses, including plant and cellular remnants, in the case of bacterial colonies (*Foster*, 1988). A number of technological developments (e.g. X-ray tomography, synchrotron-based spectroscopy or nanoSIMS) have allowed us to construct a more complete picture of the micro-environments in which microbial communities reside.

The structure of pore space in soil results in a huge diversity of micro-environments in which the biological component exists and is active (*Nunan et al.*, 2006; *Young and Ritz*, 1998). The diversity of micro-environments exists because the physical organisation of solid and pore space causes a complex distribution of oxygen, water films and solute gradients, spanning distances as small as a few micrometers, to develop. These micro-environments are highly dynamic in nature as microbial activity, plant root growth or alterations in the water status can all affect their physical and chemical properties (*Crawford et al.*, 2005). During aggregate formation for example, changes in the spatial organisation of the solids and voids affects the architecture and connectivity of the pore space which can, in turn, affect the distribution of water films and the diffusion of substrate and gasses (*Horn and Smucker*, 2005), the distribution of organic matter (*Mueller et al.*, 2012; *Rawlins et al.*, 2016) or the distribution of a range of elements (*Jasinska et al.*, 2006). *Lehmann et al.* (2008) also showed that organic matter at the scale of the microbial habitat is highly heterogeneous in nature. This means that microbial communities residing in different micro-habitats experience different local environmental conditions, even when the overall environment of the soil is constant.

Relationship between micro-habitat and microbial communities

The different local environmental conditions can be expected to exert different pressures on microbial communities, potentially affecting both the makeup of the communities and their activity. Indeed, the heterogeneity of the physical and chemical micro-environment in soil has been shown to have a significant structuring effect on microbial communities: microbial community structure is dependent on whether they are located within or at the surface of soil aggregates (*Jasinska et al.*, 2006; *Mummey and Stahl*, 2004). Different regions of the soil pore space also harbor significantly different communities (*Ruamps et al.*, 2011). The intimate relationship between microbial communities and their local habitat is further demonstrated by the rapidity with which community fingerprints respond to changes in aggregation (*Blaud et al.*, 2012). The phylogenetic composition of microbial communities also varies significantly among and within aggregates of the same soil (*Kravchenko et al.*, 2014). It should be noted that the differences among microbial communities from aggregates of the same soil and from different portions of the same aggregates were of the same order of magnitude as the differences measured in bulk samples of different soils (*Kravchenko et al.*, 2014), suggesting that an accurate picture of microbial communities cannot be obtained simply from bulk measurements.

Interaction distances or « calling distances » in the microbial world are extremely short. On leaf surfaces for example, interactions among bacteria have been found to occur principally in the 5 to 20 μm range (*Esser et al.*, 2015) and diffusion limitations on solid surfaces reduce competition for a common resource to such an extent that a less competitive bacterial strain can coexist in close proximity (~100μm) to a more competitive strain (*Dechesne et al.*, 2008). In soil, the physical environment potentially constrains interactions among microbial populations even further, particularly in unsaturated conditions where the aqueous zones in microbial habitats are fragmented (*Long and Or*, 2005). The few estimates of interaction distances in soil that have been made suggest that the majority of microbial interactions occur over distances no greater than a few tens of micrometers (*Gantner et al.*, 2006). Although the numbers of microbial cells in soil are large, they only occupy a small part of the total surface area (*Young and Crawford*, 2004), a consequence of which is that individual microbial neighbourhoods contain very few cells and even fewer species that are within interaction distances (*Raynaud and Nunan*, 2014). If one considers a community to be a collection of individuals that interact with one another, then soil microbial communities may, in fact, be quite small and relatively species poor, which is in stark contrast to the very large numbers often used to describe soil microbial communities measured in bulk soil samples (*Roesch et al.*, 2007).

What are microbial communities?

Communities can be defined as closed networks of interacting populations (*Sterelny*, 2006), where the interactions among members of a community regulate members' relative abundances and determine the community's assembly rules (*Jaillard et al.*, 2014). However, communities can also be « communities of a place »; in other words a collection of individuals or species that exist in a defined space, generally because they tolerate the prevalent local conditions, but do not necessarily interact or affect each other's existence (*Bryant*, 2012). In view of the large disparity of scale between the size of the samples used for measuring microbial communities on bulk soil samples (0.25-0.5g) and the distance over which microbial cells interact (100s μm), measurements of microbial community structure, composition or diversity cannot provide much reliable information on the identities of interacting species or the extent of the interactions. The measurements are more a reflection of « communities of a place », those microorganisms that can tolerate, or thrive in, at least one of the micro-habitats present in the soil. In these communities, habitat filtering regulates the composition of communities, as is often found for soil microbial communities (*Hughes Martiny et al.*, 2006), rather than interactions among community members. One should therefore exercise caution when using co-occurrence network analysis for gaining insight into biotic interactions within soil microbial communities, as these analyses are unlikely to shed much light on microbial associations other than similar tolerances of soil environmental properties.

Microbial communities and activity

In communities where there are causal interactions, the composition may affect ecosystem functioning through competition for resources, facilitation, mutualism and a better coverage of habitat heterogeneity (*Hector et al.*, 2001). Where there are no causal interactions, the composition of the communities is only likely to affect ecosystem functioning through a better coverage of habitat heterogeneity. In soil, more diverse microbial communities are more likely to be able to inhabit a greater portion of the available micro-habitats and to harbor a greater catabolic diversity. They

might therefore be expected to better decompose the chemically heterogeneous soil organic matter. However, most studies suggest that there is little or no relationship between microbial community composition or diversity and soil organic matter decomposition in mineral soils (e.g. *Wertz et al.*, 2006). This is commonly ascribed to the existence of a high functional redundancy with respect to heterotrophic respiration in soil microbial communities (*Nielsen et al.*, 2011). The functional redundancy theory requires that communities with low levels of diversity carry out the same functions across the available micro-habitats as more diverse communities. There are alternative explanations for the stability of a range of microbial functions to changes in composition or diversity of microbial communities. The first is that it reflects an averaging effect, as described by the law of large numbers or Bernouilli's theorem (*Bernoulli and Šejnin*, 2005). The law of large numbers states that the average obtained from a large number of realizations converges towards the expected value, and, the greater the number of realizations, the closer to the expected value the average will be. If each microorganism-micro-habitat pair is viewed as a realization of a particular activity, then even communities with low diversity levels (say with several tens or a few hundred taxa) would result in a large number of microorganism-micro-habitat pairs, the average activity of which would be expected to converge towards the average activity calculated from all possible combinations of microorganisms and micro-habitats for a soil. Although this explanation and the functional redundancy explanation are virtually indistinguishable from an experimental point of view, it has the merit of not requiring that different micro-organisms function in a similar manner. A second explanation is that microbial activity is constrained to such an extent by the environment in which microbial communities exist, that all communities have similar activity, regardless of their composition or diversity.

Micro-habitat constraints and microbial activity

The relationship between the availability of substrate and microbial respiration in soil is well established. Soils, or regions of a soil such as the rhizosphere, that have higher organic C contents generally have higher respiration rates (e.g. *Kuzyakov*, 2002). Interactions between the physical structure and water content of soil modulate respiration rates by regulating O_2 levels and the access that decomposing communities have to the substrate necessary for their activity (*Moyano et al.*, 2012). A number of studies have measured increases in respiration following the disruption of soil structure (e.g. *Salome et al.*, 2010). The flush of respiration after physical disruption illustrates in a simple manner how fine-scale spatial disconnections between substrate and decomposers can constrain microbial activity. However, it is not yet clear what mechanism(s) regulate the availability of organic substrate to microbial decomposers. The significance of these regulatory mechanisms relative to biological or chemical limitations of activity has not been established either. Investigations into how the local environment constrains microbial activity have yielded contradictory results: no significant relationships between respiration in individual aggregates and aggregate properties that might be expected to influence the access microbial decomposers have to substrate (total pore volume or spatial proximity of organic matter and pores in tomographic images) have been found (*Rawlins et al.*, 2016; *Sierra and Renault*, 1996), and changes in the structure of the pore network do not appear to influence C mineralization at a given matric potential under steady-state conditions (*Juarez et al.*, 2013). Nonetheless, others have found that organic matter placed in different regions of the pore network is mineralized at different rates, suggesting that the local environmental conditions affect microbial activity (*Ruamps et al.*, 2011; *Strong et al.*, 2004). The differences, however, cannot be conclusively attributed to local

environmental constraints because microbial communities also vary with the local environmental conditions (*Ruamps et al.*, 2011), meaning that possible microbial community and local environment effects are confounded. In order to distinguish the roles of microbial communities and the local environment in regulating microbial activity, Nunan et al. (2017) used a reciprocal transplant approach in which microbial communities from two soils were placed in sterilized samples of either soil, in more or less connected aqueous environments. During the period of rapid growth, when the inoculated microbial communities were recolonising the sterile environment using labile organic matter release from dead microbial cells, the respiratory rates of the different microbial communities were significantly different. As the incubation progressed however, and the microbial communities decomposed organic matter unaffected by the sterilisation process, the mineralisation rates of all the communities decreased dramatically. The mineralisation rates of the different microbial communities converged according to soil and connectivity of the micro-environment, regardless of the community of decomposers. This suggests a significant and dominant local environmental control on microbial decomposition of soil organic matter. The limitation may be such that intrinsic functional differences among communities cannot be expressed, resulting in a « habitat-induced functional redundancy ».

The nature of the limitation is not clear, but in their influential study on N mineralisation, (*Stanford and Smith*, 1972) found that there was a linear relationship between the activity and the square-root of time. They remarked that this would be expected for diffusion-controlled reactions. Were C mineralisation constrained by the diffusion rates of organic substrates, then soil respiration curves would also be linear with the square-root of time. A re-analysis of some of our data shows that this can indeed be the case (Fig. 1). Were this widespread, then a description of the pore and water structure of soil may be necessary to accurately describe C mineralisation.

The heterogeneity of the soil habitat means that it may be more appropriate to consider soil as a juxtaposition of different « biomes » with different factors limiting process rates and microbial community assembly. In « biomes » where the availability of substrate is relatively high and microbial communities can grow (i.e. the rhizosphere, the drilosphere or the detritusphere), the capacity of microbial communities to use the available substrate may dominate process rates and competitive exclusion may play a role in structuring microbial communities. However, where organic resources are less accessible, local environmental constraints, such as diffusion, may well regulate the rates at which processes occur. In these situations, interactions among micro-organisms are unlikely to structure the communities because resource availability is too low. Recent work on the soil microbial habitat has revealed a highly heterogeneous environment, from both physical and chemical perspectives, that has significant influences on both the makeup and activity of microbial communities. This has still not been fully integrated into our understanding of the biological functioning of soil. It is likely to be profitable to pursue a line of research that better identifies the microbe-habitat interactions, and the scales of these interactions operate, that structure microbial communities and regulate their activity.

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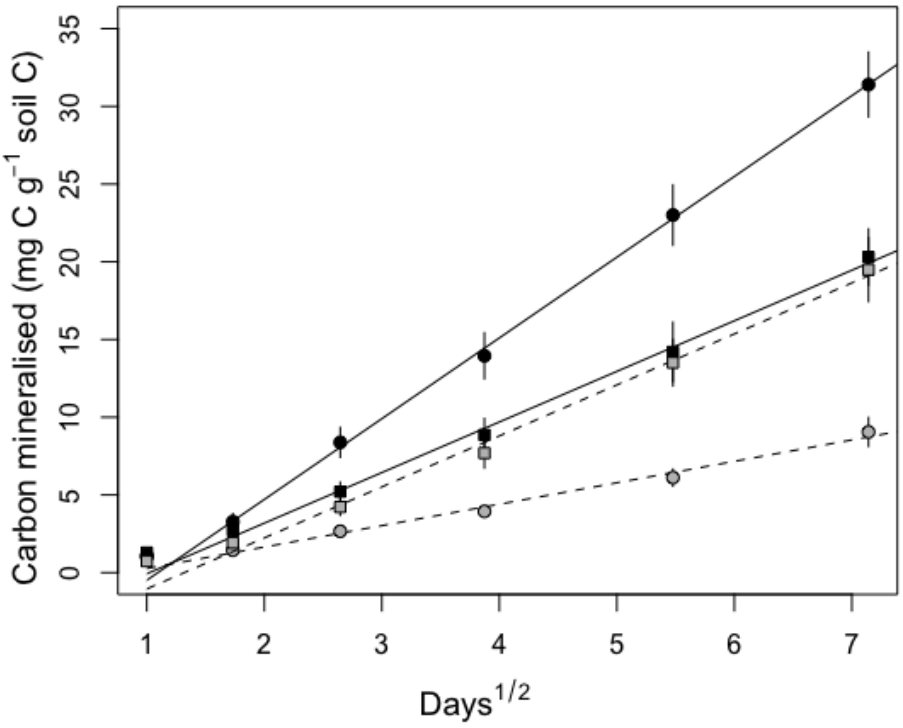

Figure 1 Linear relationship between cumulative C mineralisation and the square-root of time in topsoil (■) and subsoil (●). Grey symbols are undisturbed samples and black symbols are sieved samples. Symbols show experimental data and lines show linear fit (R2 all > 0.96). The measurements on the first day were not included in the linear fit these values may have been influenced by advection after adjustment of the soil moisture content. Bars show standard error of the mean, where error larger than size of symbol. Data from Salomé et al., (2010).

315