# The search for the meaning of life in soil

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# The search for the meaning of life in soil: an opinion

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**Summary**

The introduction of impressive technologies in the search for life's diversity and activity in soil has led to impressive new techniques and knowledge concerning the soil microbial community; with some important links to function found.  However, we attest that the general lack of causality found between the many diversity/numbers metrics of soil microbes and function is due, at least in part, to the lack of understanding of the microbial populations/dynamics links to their physical habitat and attendant moisture conditions.  In this opinion paper we explore the importance of this interplay between organism and habitat.  Further, as an example of this interplay, we introduce the potential importance of nematode movement and gene transfer in bacterial populations.

**Highlights**

- The importance of the physical habitat is highlighted in soil microbiology studies

- The interplay between the soil-root-habitat is emphasized.

- Seeking a functional understanding of biodiversity rather than a 'biology of numbers and differences' approach is highlighted.

- The movement of nematodes with respect to Horizontal Gene Transfer is discussed.

**Context**

The factors affecting the survival of a bacterium or fungi in soil are surprisingly similar to those affecting our own survival on Earth.  We all rely on sufficient flow of gas into and from our bodies for adequate respiration.  We need refuge to shelter from adverse environments and from biological intruders.  Access to sufficient resources, water and food is key, as is the ability to move when required.  Proximity to similar biological organisms is desirable for protection and gene transfer.  Connecting such general statements to specific operational functions in the soil environment, however, is a difficult task.

Much effort and research money have been invested in describing and measuring the soil microbial population and in particular aspects of microbial biodiversity.  Within soil science, the greatest scientific investment has gone into these biodiversity areas in the past few decades, and

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by a substantial margin. The arguments that justify such research often appeal to 'common sense' (perhaps, teleo)logic: soil contains the world's greatest diversity of micro-organisms and this diversity itself must be related to important soil functions, such as carbon sequestration and decomposition, and plant production. Further links are often inferred to aspects of soil health and sustainability.

The search for 'meaning' in the many soil–omics technologies when applied to soil microbial communities continues unabated: for instance, how can we connect what we measure to what we can control to the benefit of plant production, in the case of agriculture, or species diversity, in the case of ecological concerns. Nannipieri *et al*., (2003) list and comment on 16 methods to measure microbial activity and in their recent reflections on their paper (Nannipieri *et al.*, 2017) they comment that even with such an array of accessible methods there must be a mix of methodologies that quantify microbial activity and composition. However, it is our contention that, despite the significant improvement in many of these technologies, such approaches will always be doomed to failure when we seek to link the many metrics of biodiversity and or activity to function. For without the link to the physical habitat that imposes heterogeneity on all life in soil, no diversity–function link will be possible. Below, we develop our rationale that seeks to encourage a more multidisciplinary approach to seeking the 'meaning' of life in soil, both from an experimental and theoretical point of view, with a focus on soil physics.

**Background**

Research on soil microorganisms is long established. Ackert (2006) summarizes the work of Sergei Vinogradskii in soil microbiology from the 1890s through to 1910. A review of Google

Scholar (February 16, 2017) returns on the phrase "soil microorganisms" emphasizes such early interest with 10 noted publications up to 1900, rising to just above 500 in 1950. Bhaumik's PhD thesis on the subject of "*Soil moisture tension and microbiological activity*" (Bhaumik, 1947) cites 40 references, including research that links bacteria to soil water and one related to a very early paper on soil bacteria and evaporation (Hoffman, 1912, cited by Bhaumik, 1947). In the 1980s and 1990s, investment in the general area of soil microbiology increased greatly. Interestingly, "soil biodiversity" appears in Google Scholar for the first time in 1926 with one publication; the number of publications rose substantially between 2010 and 2017.

This focus of activity in this area of soil science has had important successes: a deeper understanding of the diversity of microbial communities and how they change over time and in response to environmental perturbations, and the development of impressive new technologies to observe and measure (at least in a semi-quantifiable manner) related metrics. For example, in ground-breaking research Fierer & Jackson (2006) related phylotype richness and diversity (Shannon index), at large spatial scales, to a single variable, soil pH on 98 soil samples from a wide range of ecosystem types. Treves *et al*. (2003) measured for the first time the role of spatial isolation in microbial diversity in unsaturated sand systems.

Turbe *et al.* (2010), however, in perhaps the most exhaustive analysis and review of research on soil biodiversity gave a more sobering analysis of the large body of research; they observed no consistent links between soil species diversity and function. Aside from research on the many soil pathogens, this statement holds true today. Although, Ritz (2014) states that soil microbes "undoubtedly play a principal role in crop productivity", it seems less obvious that this holds in

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intensive cropping systems where pharma-agriculture, a reliance on synthetic chemical inputs, prevails. Furthermore, serious doubts remain about the various mechanisms that control biodiversity, how such biodiversity links with soil function and importantly how such discoveries can be used in agriculture and ecological management. The statement by Turbe *et al*. (2010) emphasizes what has become a 'biology of numbers and differences approach' in soil biodiversity research: add a chemical, put in a perturbation, look at changes in land use, plant use and see if we can find a correlation with some aspect of the microbial community.

Ranjard *et al*. (2010) in a review of the French National Initiative on biodiversity of soil microbial communities state that "soil diversity could be linked to soil functioning to improve management and protection…of soil." One would imagine that is one of *the* main reasons for research into soil diversity, rather than simply a statement of possibility. Nannipieri *et al*. (2003) state that "The central problem posed by the link between microbial diversity and soil function is to understand the relations between genetic diversity and community structure and between community structure and function." They state that this lack of understanding appears to be solvable, at least partly, by better assays and techniques that can determine inactive and active microbial cells in soil. Prosser *et al*. (2003), however, are correct in stating that "..advances in microbial ecology are limited by a lack of conceptual and theoretical approaches" and that the over reliance on new technologies alone might lead to expensive and functionally redundant research that leads nowhere, and where even the journey rather than simply the final destination is worthless. It is possible that the advance in ecological theory in aboveground systems results from the intimate, observable, link between biological activity and the physical habitat. In soil, this link is far harder to observe and measure. Although there have been some 'form to function'

successes in biodiversity research, they are but a very small percentage of the total given the large concentration of scientific and financial resources invested.

Soil biodiversity research, however, is not alone in having this problem. Philip (1991), in relation to the increasing use of modelling over experimentation in soil science, states that soil scientists increasingly "presented themselves as computer bound, incurious about real-world phenomena and innocent of laboratory and field skills…" and bemoans the lack of real advances in understanding of water flow in soils.

**Finding a home for the microbial diversity**

Soil biodiversity science seems to be evolving into 'soil microbiome' research; there were 1260 publications between 2010 and 2017 and seven publications between 1990–2000 (Google Scholar, February 16, 2017). Here the term microbiome is defined as "... a characteristic microbial community occupying a reasonably well defined habitat which has distinct physio-chemical properties. The term thus not only refers to the microorganisms involved but also encompasses their theatre of activity." (Whipps *et al*., 1988). To achieve substantial advances, this new approach must represent more than mere textual camouflage for future plans to avoid doing much the same type of research. Unfortunately, evidence suggests that although there are strong research publications the desire to link the soil microbiome causally to soil function appears as problematic as linking function to biodiversity, and partial correlations or trends seem

to be a typical result. Perhaps, however, hope is warranted.  The term 'biome' potentially promises a better connection to microhabitats which, in soil, are linked implicitly to microbial activity, yet this link is often forgotten (Young & Crawford, 2004).  Where habitat is mentioned in past publications it is generally in the context of artificial 'aggregates' that are prevalent in laboratory microcosms, but are rare in most field systems. We use such systems to help us smooth out any variation that would 'mask' the differences in responses by plants and or microbes to a wide variety of internal and external perturbations.  The reality is that the attempt to link the presence and activity of microbial communities functionally to a wide range of functions that translate to field conditions has largely failed. Clearly, fundamental research in many areas does not translate easily to new products or indeed new knowledge.  Therefore, biodiversity research is not alone in that respect, because we are dealing with the most complex biomaterial on the planet, soil.

It is clear that the research community has been very good at quantifying and exploring biodiversity in soil microbial communities.  Now our lens into the life of microbial populations is much more focused due to the development of new hardware and molecular technologies. Nevertheless, our understanding of how such microbial diversity links to function remains poor. The paucity of such formal links of diversity with function might arise because the examination of microbial populations is isolated from their physical environment. The interconnections of microbial activity, habitat and water might be the key to gaining a far better, fuller and causal understanding of how important microbial populations and their actions are for the terrestrial biosphere.

There is a growing body of evidence that emphasizes the importance of linking habitat micro-habitat with biodiversity.  Lennon *et al.* (2012), in an interesting mapping exercise of niche space of soil microbes, examined optimal matric potentials for soil microbes in terms of respiration. Indeed, Terves *et al.* (2013) linked hydraulic connectivity (i.e aquatically connected or disconnected) of pores through to spatial isolation and the generation of microbial diversity in sand.  Ramette *et al*. (2009) in a mini-review discussed microbial habitats in relation to microbial diversity and abundance from a statistical viewpoint. Papers from Dani Or's group in ETH Zürich focus on the fundamental mechanistic relations between habitat, water and bacteria (e.g. Tecon & Or, 2016; Ebrahimi & Or, 2015), both from an experimental and modelling perspective. Manzoni *et al*. (2012) present compelling evidence, through meta-analysis, that links the changes in matric potential and reduced microbial activity to water potential thresholds where physical constraints (diffusion) have the defining effect on microbial activity. This research has a direct link to that of Linn & Doran (1984) on the effect of water-filled pore space (WFPS) on the production of $CO_2$ and $N_2O$ in tilled and non-tilled soil in which they postulated threshold limits of microbial activity and function on WFPS.

It is clear that the habitat and attendant water films play crucial roles in the activity of microbes and are vital to take account of in terms of the creation and sustainability of biodiversity, and how such 'diverse' metrics can be linked to function.  However, what is the actual microbial habitat and why does it matter in respect to microbial activity and function?

**The microhabitat of microbes**

Soil, at any scale is complex: opaque, composed of a myriad of organo-minerals, roots, large and small organisms, and exhibits truly impressive gradients in its biology, chemistry and physics over large and small spatial ranges. It represents the most complex 3-D architecture known, which, because of clay minerals, can 'shape-shift' its geometry and thus its function, simply by the addition or subtraction of water and solutes.

Microbes, by definition, live and organize themselves at small scales. Fungi, on the other hand, are indeterminate organisms that can cast their bodies far and wide, but like bacteria are still constrained by the habitat they live in (Toyota *et al.,* 1996). It also appears that whilst this complex physical architecture imposes constraints on microbiological activity, the architecture is markedly affected by the activity of the microbes (Young & Crawford, 2004), and the soil–plant–microbe complex can be defined as a self-organized system where in some cases fungi dominate in terms of habitat design (Feeney *et al.,* 2003). In combination, water and architecture have prime positions? as factors in controlling the location of microbes, the flow and activity of individual and populations of microbes, and the diffusion of gas (Young & Ritz ,1998). We have few technologies that can see microbes directly in soil. Impressive biological thin sections and nano-sims techniques (e.g. Nunan *et al.,* 2001; Herrmann *et al.,* 2007) have been developed that have revealed fascinating data on the spatial distribution of bacteria and fungi. However, these are limited to 2-D, which will always leave us wondering what is 'going on round the corner' in 3-D. Microbes are too small to be seen by any magnetic resonance imaging (MRI) or micro-computed aided tomography (μCT) systems, and so we have no technology that can reveal how they behave in real-time at the micro-habitat scale. Fungi, which are larger in size, suffer from much the same limitations. Added to these problems, it is clear that apart from hotspots (e.g. rhizosphere, organic debris) the soil is devoid of microbial life and even searching for them is

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problematic unless we provide the system with food resources and wait. In soil, physics dominates the spatial distribution of microbes with between only 0.01 and 0.00001% of the surface area covered by any microbial form (Young & Crawford, 2004). Such figures are usually not discussed. and the focus is typically on the absolute number of things and types, for eaxmple prokaryotic abundance, largely bacterial (Torsvik *et al.,* 2002) can be as large as $4.8 \times 10^9$ to $2.1 \times 10^{10}$ cells cm$^{-3}$ with, in some cases, up to 8800 different species genomes.

Where is the water?

The soil moisture characteristic (SMC) reveals the interaction between water and the soil's physical architecture by providing a unique dynamic fingerprint for any soil ecosystem that relies on the equilibration of soil at specific matric potentials. This characteristic not only provides information about the volume of water, but also its distribution within the 3-D soil and how, whether in an adsorption or desorption phase, that volume and distribution changes with time. It defines the hydraulic and gaseous connectivity of the soil and thus the ease with which gases and water may flow. Connectivity is the key phase here because although resources are unevenly spread in soil, even close to a root, the potential connectivity of microbial communities to other communities and resources, and potential spread of fungal hyphae are intimately connected to the SMC, which also defines the thickness of the water films. Tecon & Or (2016) highlighted the importance of the thickness of water films on rough surfaces in relation to bacterial flagellar motility. They found that dispersal of bacteria and their mean velocity decreased rapidly between saturated (very connected) and unsaturated (–2kPa; and less connected) matric potentials on rough surfaces as the 2-D habitat became less hydraulically connected. Of course, the SMC can also exhibit considerable hysteresis.

Given that bacteria, protozoa and archaea are water-borne organisms, constricted in activity and movement to water-films or biofilms, the activity of such microbes is even less in terms of 'active' micro-surface area. The soil may be teeming with life (Haq *et al*., 2014), but only around a series of spatially fragmented oases (Nunan *et al*., 2003). Communities in such cases exist in splendid isolation from others; perhaps they rarely mix or sense each other's presence. Such communities are linked spatially by water and solutes, but these fluid connections often fluctuate rapidly. Bacteria find it very difficult to move through a soil profile by mass flow alone, unless there are some large pores and a large volume of water is involved. Water at these micro-scales exhibits some fascinating properties. In an essay, Purcell (1977) discusses "Life at Low Reynolds number" and introduces Berg's brilliant research on bacterial motility (eg. Berg, 1988, Blair & Berg, 1989, Wolfe & Berg, 1989). Although Purcell's essay is somewhat known in the soil physics literature, it is virtually unrecognized in molecular ecology.

Water at small-scales has unusual properties that have a direct effect on the fecundity and activity of microorganisms. Microbial life at the small scale takes place in liquids at low Reynolds number. At these scales, because of the small Reynolds number, viscosity dominates the flow of moving objects in pore water (Hatton & Choset; 2013). Therefore, rather than an image of a motile bacterium as a human swimming in water, imagine yourself wading through treacle to obtain a better idea of the fluid drag forces acting on microbes as they try to move. This small-scale environment where viscosity dominates means that it is very difficult for a superficially motile microbe to escape its local environment. From a bacterium's viewpoint, sitting still might be an evolutionary advantage if sufficient local resources diffuse to you: an exploitation strategy. However, where resources are spatially isolated and not locally available, then moving to 'greener pastures' conveys an important evolutionary advantage that a motile

organism can take advantage of: a combined exploitation and exploration strategy. Therefore, a pore-water environment with a small Reynolds number might allow the existence of both motile and non-motile bacteria. This is not new knowledge in the sense that we already know that different strategies for movement are expressed in bacteria and other microbes. What is interesting is that we can relate the evolution of those strategies to the physics of the growing medium (Purcell, 1977), and perhaps now also approach the question of what can we do to manipulate this local environment to improve specific functionality? The interaction between soil microbes and both roots and soil fauna exert a major effect on the soil environment at small scales.

**Add the exploring root**

One area in soil microbiology and biodiversity that is still relatively unexplored is the effect of the plant root on the engineering of the (micro-)habitat space as new soil is explored and a new rhizosphere is generated and colonized (Watt *et al.,* 2006). With a new focus on rhizosheaths, this area is expanding to take into account the role of both root hairs and mucilage. Once a root has initially penetrated a new region of the soil matrix or biopore, everything that enters or leaves the root must do so through the root–soil interface, making its properties of great importance. The biophysical nature of the interface does not remain static however, but changes continually with the prevailing soil water regime, the age and development stage of the root tissue, and with microbial activity that is powered substantially by the release of carbon from the root itself. A conceptual illustration of the microhabitat of soil before, during and after a root elongates into a soil volume is provided in Figure 1. The root–mycorrhizal fungal conceptual

model, partly derived from Jones *et al.* (2004), shows that the root exerts pressure on the surrounding soil and creates a markedly different habitat for microbes to colonize and spread. The key aspect of this model is recognition of the temporal dynamics and how the root may leave a legacy in terms of newly formed habitats with very different characteristics, including different microbial loadings.

We know from a large portfolio of research papers the 'before and after' of rhizosphere and bulk soil in terms of types and numbers of microbes before and after perturbations. Less clear is the functionality of these two different soil volumes, which is only now becoming clearer in terms of water regime and physical architecture.

The root itself exerts substantial control over its local environment by modifying chemical and water-retention properties through the release of polysaccharide gel, phospholipids, sugars and amino acids. Substantial progress has been made recently in understanding the complex relations between exudation and water, following surprising observations that soil water content of rhizosphere soil may be wetter than bulk soil (Young, 1995) and that root exudates may change the water retained in the rhizosphere at a given suction (e.g. Read *et al.*, 2003). The combined 'cocktail' of exudates released by roots amplifies the hysteresis-like behaviour of the rhizosphere soil; polysaccharide mucilages probably make the soil around the root slower to dry (i.e. decrease in water content), whereas it is more difficult to rewet once it has dried especially adjacent to older roots (Carminati, 2013). Undoubtedly, this has consequences for water uptake by crop root systems in the field and for soil microbial activity. Whether it also represents a rhizosphere trait that could be mapped genetically and manipulated to increase crop productivity

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remains uncertain. The presence of mucilage also changes the way in which soil structure develops around roots during successive wetting and drying cycles, the way that a root tip shears and deforms the soil as it penetrates and the adherence of soil particles to root hairs.

Roots increase the area of intimate contact between root tissue and the soil particles by growing numerous root hair projections, typically around 10 μm in diameter, but potentially extending several millimetres into the rhizosphere. These root hairs increase the effective radius of the root and facilitate the uptake of nutrients and water (Nye & Tinker, 2000). Interestingly, although there is a great deal of knowledge about the growth of root hairs and their function in idealized experimental conditions (e.g. *Arabidopsis thaliana* on gel), relatively little is known about the growth of root hairs and their function for field crops in soil environments. Understanding of the dynamics of root colonization remains poor, although Watt *et al*. (2006) clearly demonstrated that this is likely to be strongly affected by the properties and expansion of the root surface, including the exuding sloughing root cap with the zone of extension and root hair zone behind.

As roots penetrate within soil structural pores, root hairs serve a much-neglected physical function in anchoring the root tip to pore walls, giving the root tip a reaction force to push against as cells in the elongation zone expand (Bengough *et al.,* 2016). This can enable root tips to grow from loose soil regions into strong soil layers more successfully (Haling *et al.,* 2013). The anchorage of soil particles to the root is apparent when the rhizosheath of soil adheres tightly to root hairs, despite physical shaking (George *et al.,* 2014). There is potential to use rhizosheath mass per length of root to provide a rapid measure of root hair growth and interaction with the soil around the root, and there are quantitative trait loci (QTLs) associated with rhizosheath production in both barley and wheat (George *et al.,* 2014; Delhaize *et al.,* 2015). This makes root

hair and root exudate traits a possible target for plant breeders, whereas direct screening of the growth of root hairs is currently too tedious to screen many lines in large genetic populations.

**The biased uber nematode taxi (BUNT) hypothesis and gene transfer in bacteria**

The research of Wallace (1958) on the movement of *Caenorhabditis elegans* provides a good overview of the importance of the physics of soil, specifically water films for soil biology and potentially has an important link to microbial dispersion, activity and horizontal gene transfer. Wallace (1958) in a classical set of experiments on agar, saturated soil and monolayers of soil particles emphasized the importance of the thickness of water films to define the spread of nematodes, and also the role of soil structure in nematode movement and dispersal. Wallace showed that nematode forward movement is maximal when the water film clings tightly and covers the body of the nematode, minimizing sideways movement: the agents that the nematodes sense force them to move in certain directions. The 'taxis' of nematode movement is an interplay between that hydraulic connectivity and water-film thickness together with the diffusion of volatile compounds through the unsaturated pore space. Once the nematode senses the volatile substances, it may react by changing direction towards the largest concentration of volatiles, which in this scenario would be bacteria in the rhizosphere.

With a parallel biophysical theoretical and experimental approach, Anderson *et al.* (1998a,b) showed through 2-D simulations and experiments that the diffusion gradient within a complex heterogeneous structure is highly variable, as is the hydraulic connectivity. Consider the actions

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of a nematode. Driven to find food reserves, it forages in an exploration phase, searching for clues to the location and type of food available in a 3-D unsaturated maze. As a root grows into the soil (see Figure 1), it exudes a range of possible food sources for the nematode population at some distance from the root–soil interface. At this point the nematode's movement is restricted solely by the connectedness and thickness of water films, and it moves in a random exploratory manner. A diffusion gradient developed from the root–soil interface spreads out from the rhizosphere volume, depending on the gaseous diffusion of attractants in the volatile phase. The matric potential of soil water controls the degree of diffusion by determining the air-filled conduits through which the volatiles spread: gaseous diffusion changes by orders of magnitude when a wet soil drains to become dry. At some point, above a certain threshold concentration, the nematode will sense the gradient and attempt to move in the direction of the source using biased random-walk strategies. However, it has to use a quasi-random strategy because of the intricacies of how the soil architecture and attendant moisture interact to affect the chemotaxis process. Nematodes move in a sinusoidal fashion, snake-like within water films—they have to, to overcome a fluid that is characterized by a low Reynolds Number. Unlike snakes, however, nematodes can reverse, which is useful in their complex world where dead-end narrow pore necks are the norm. This is where nematodes have to shift from a directed movement in response to volatile compounds that pour through the soil architecture at scales smaller than the dimensions of the nematode. If the nematode could not switch to a random motion and find a route around the blocked maze, it would be stuck behind small inaccessible pores: much like the 'dead man's handshake' experienced by unfortunate divers stranded in submerged caves. Thus, the reversing of a nematode shows neatly how the complexity of any architecture affects gas diffusion and the activity of organisms in the soil. An interesting question is: how does a nematode manage such a coordinated set of movements when it is assumed that the movement is

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linked to a taxis process governed by the diffusion of volatiles through the soil? What makes the nematode so positionally aware? Of course we know that nematodes are not the only biological actors involved in transporting bacteria in soil. However, the key characteristic of nematodes is that they are excellent integrators of the soil architecture, water and its combined function; they sense at the molecular scale, feed at the microbial scale and move at the metre scale. Thus they are perhaps a unique biological integrator of soil biophysical systems across so many scales.

It is reasonably well understood how soil architecture and attendant moisture feature in the probability of horizontal gene transfer (HGT). The dissimilarity between bacterial species increases where water films are discontinuous or where soil structural attributes deny the movement of bacterial species within the soil. However, another factor might help explain HGT within complex soil systems. The chemotaxis process of nematodes, explained above, relies on the detection of a diffusion gradient from a bacterial source to the spatial position of the nematode. Consider a nematode moving towards a colony of bacterial species, *A;* the nematode will consume the bacteria on contact and, depending on bacterial density, might have a considerable amount of bacteria adhering to its own exterior. At this point, the volatile diffusion gradient will not be present as it will be locally equilibrated around the biochemical receptors of the nematode. Within a short period, the nematode will then revert to random movement, seeking additional food resources some distance from bacterial species *A*, but unable to identify additional populations of the species *A* that emits the cocktail of volatiles that it used to detect them. Rodgers *et al*. (1998) observed such a trait in *C.elegans* populations after they were fed *Escherichia .coli*. They termed the observations 'substrate legacy' because the initial substrate of

the nematodes had a functional effect on the ability of that population to recognize and source a range of bacterial populations.

In terms of the probability of HGT in soil, the substrate legacy hypothesis, which has been observed in other higher order organisms, presents interesting insights. The separation of large portions of bacteria in soil suggest small probabilities of HGT. Indeed, given that active bacterial populations are often spatially isolated from one another, it is clear that an alternative HGT catalyst must be present to ensure HGT occurs. In this case, the nematodes may be likened essentially to symbiotic biased Uber taxis in soil. The bacteria, emitting volatiles, 'call' the nematodes to their location. The diffusion gradient (akin to the the taxi's GPS navigation system) signposts pathways towards the bacterial resource. On contact, the nematodes feed on the bacteria. Then, disregarding calls from similar bacteria, they recognize incoming calls only from a different bacterial population. The initial population is transported distances within soil that they would never be able to move to either on their own or by mass flow to other bacterial populations. The nematode not only ingests the next bacterial population, but mixes different bacterial species in the same location. The probability of gene transfer between populations is potentially increased as is the functional engineering of bacterial populations. The question arises as to what happens to bacterial diversity and functioning in soil where nematode taxis are sparse. We term this the Biased Uber Nematode taxi (BUNT). Does the Uber nematode decrease or increase diversity? Does it matter functionally at the management scale? Indeed, what happens genetically to nematodes who carry 'passenger' microbial genes across the soil's interior landscape? If true, the proposed BUNT process is potentially a powerful mechanism by which we can encourage microbial engineering of HGT *in situ* at the micro-habitat scale with potentially large effects on microbial function in soil.

**Epilogue**

Decades ago, the soil science and ecological community embraced the various -omics techniques used in soil science. An avalanche of publications has followed and our knowledge of the microbial population in soil has increased rapidly and massively. What has followed, however, can be best described as incremental improvements in our understanding of the functionality of the soil's microbial communities. Molecular technologies are vitally important, but, used in isolation from a deep understanding of the micro-ecology of communities, their use is limited. Integrating our new knowledge of the physical world of microbes offers us an unprecedented opportunity to close this knowledge gap by looking at the soil microbial community in the context of the complex system that it lives in.

As Wardle & Giller (1996) observed, pre-2000 we were bereft of any serious, sustained, development of theory related to soil molecular ecology and indeed related to the general soil–plant–microbe system. More recently Prosser *et al*. (2007) eloquently repeated and added to that statement.

We contend that the success of developments of ecological theory in aboveground terrestrial ecology has resulted substantially from the readily observed and intimate link between organism and habitat space. With more connections established between soil microbial diversity and micro-habitats, this should facilitate advances in theory.

Habitat and water are key ingredients to achieve a fuller understanding of how soil biodiversity links with soil functioning. We must resist the temptation to be too technology driven and seek to understand better the interactions between physics, biology and indeed chemistry of the soil. all within a dynamic theoretical framework. Soil microbes operate in the most complex environment on the planet and our studies must somehow reflect and take account of this complexity. What we do not need is a continuation of the biology of numbers and differences approach, across any aspect of soil science, but in particular soil microbiology. Sufficient examples of before and after papers already exist to provide adequate illustration for many different environments.

**Acknowledgements**

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**Figure caption**

Figure 1. Conceptualized single root exploration in heterogeneous soil, adapted from concepts in Jones *et al*., (2004; Figure 3). (a) Bulk soil with microbial loading of fungi (brown filaments -

arrow) and bacteria (blue mats - arrow), (b) root tip covered in mucilage enters bulk soil volume reassembling soil at the root–soil interface, (c) elongation of root with different microbial loadings and proliferation of both fungal hyphae and microbial colonies (arrows) that extend axially and radially through the soil volume. Root hairs evident on root (arrow), (d) root exiting soil volume, e root senescence on-going, leaves gaps around root (arrow), (f) root leaves a legacy of its presence with a physical biopore, (g) structure around the legacy rhizosphere changes and partially collapses (arrow) because of wetting and drying cycles and biophysical perturbation and (h) a new root entering the soil volume.  The cycle repeats.

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