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REVIEW

Meeting the challenge of scaling up processes in the plant–soil–microbe system

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Abstract The scaling up of processes in the plant–soil–microbe system represents one of the greatest challenges facing environmental scientists and yet is essential for sustainable land management worldwide. The latter encompasses, for example, the mitigation of and adaptation to anthropogenic climate change, the bioremediation of industrially contaminated sites, catchment management of human pathogens such as *Escherichia coli* O157 and integrated crop management on the farm. Scaling up is also essential for the regional and global biogeochemical modelling that will inform policy-makers of the critical environmental factors driving climate change. Despite increasing understanding of the links between gene expression and process on a microscale, there is still much progress to be made when relating this to processes at the macroscale. In this paper, we explore the challenges this poses and examine key case studies of successful up-scaling.

Keywords Scaling · Bioremediation · Rhizosphere · Microbial diversity · Ecosystem

Introduction

The issue of scaling up microbial process and function is rapidly becoming the primary challenge of microbial ecology, not least because a more holistic approach to the role of soil microbial ecology is required. To understand what happens to a particular process or function at the landscape-scale, for example, we need to understand the connections, linkages and feedbacks and, most importantly, the scale-related relevance of its constituent parts. Many of these relationships are non-linearly connected in self-organised, non-equilibrium states. The fundamental 'harmonic' of any soil-based system is an understanding of the principles that control soil structure formation, with respect to the role of microbial biota to soil pore space and stability (Feeny et al. 2006). The soil structure determines water availability to plants and diffusion rates of gases and other compounds into and out of the soil. Importantly, these rates are highly sensitive to small changes in soil structure (Young and Crawford 2004). However, even with an understanding of the soil physical micro-environment, it is a non-trivial task to characterise microbe–microbe interactions within that environment. These may be either directly competitive (i.e. for limiting nutrients) or metabolic, in that metabolic by-products may be a substrate/nutrient source, or inhibitory to growth, or trigger gene expression of neighbouring populations through production of signal molecules (Newman and Banfield 2002). With the mainstream use of powerful molecular techniques, it has been possible to begin the description of microbial diversity and to link it with ecosystem function (Dubey et al. 2006). However, to begin to fully understand the complex interplay of biogeochemical interactions, we must continue to develop computational modelling tools. Such modelling will, optimally, follow two interconnected development trajectories. For the first trajectory, we need highly detailed deterministic models able to simulate the complex interaction of physical and biological processes of a particular experiment. This is the classical mode for model development, and most current models have been developed using this approach. The second trajectory

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leads to the development of models driven by integration and prioritisation from the process descriptions in experiment-specific deterministic models: *Integration* is the process that assimilates information about a particular scale to find an aggregate relevant to the next higher scale. Examples are decomposition rates of different soil organic matter pools. There is an abstraction of very complex microbial processes integrated over time and space. *Prioritisation* means the implementation of processes in models based on their scale-dependent importance. One example would be the transport of dissolved organic matter (DOM) in surface and sub-surface runoff that might be of minor importance at a small scale but is very significant at the catchment or regional scale. This second trajectory is so important because the role of modelling has changed: Modelling no longer solely provides research tools designed to understand complex systems but now provides tools for decision support. A prominent example of models being used as predictive instruments across scales can be seen in the Intergovernmental Panel on Climate Change (IPCC) Fourth Assessment Report. The policy decisions, however, are made regional, national or even global scales, a fundamentally different scale from that at which most experiments are conducted to understand underlying processes. Our main challenge is to define the uncertainties that arise from using model results that cross these scales.

An exemplar of this style of approach is provided by Paustian et al. (1995) who present a general approach for integrating long-term, spatially explicit data from regional-level databases with process-oriented simulation models. The model framework calls for the synthesis of process data derived from microcosm, growth chamber and field experiments with geographic information (soil maps, land use data, remote sensing) to form a predictive, regional-scale model. However, the aggregation of process functions, describing site-scale conditions, to make regional-scale estimates is problematic (Paustian et al. 1997), and there remains a need for multi-scale models that fulfil the criteria of being both strategic and tactical. Paustian (2001) succinctly summarises this tension in modeling–analytical philosophy by suggesting that researchers should be 'measuring the modellable' and 'modelling the measureable'. The emerging C market provides a powerful driver for such model development. The Global Environment Facility Soil Organic Carbon (GEFSOC) Modelling System has been successfully developed in response to this (Milne et al. 2007). GEFSOC estimates stocks of soil organic carbon (SOC) under varying and diverse conditions.

Anthropogenic disturbances to the environment have helped focus research and development efforts into specific scenarios. Pollution and pathogen monitoring and bioremediation, for example, have proved to be strong drivers for research into specific microbial gene function, elucidated at a cellular level, validated at mesocosm scale and implemented at a landscape level (Fig. 1). The issue of climate change represents the ultimate challenge for understanding processes across scale and presents the probability of global redistribution of resources. Understanding the consequences of anthropogenic activity on production of greenhouse gases is vital to our ability to mitigate against climate change.

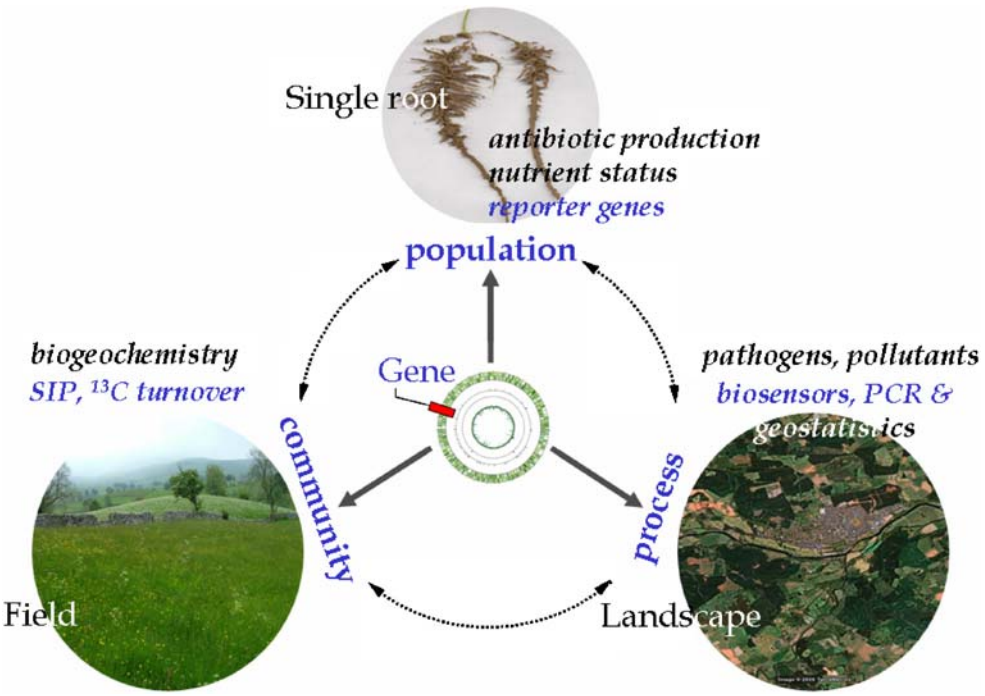

Fig. 1 Schematic illustrating the transition from gene through organism to population and community. The *blue text* shows the scale and technique appropriate to that scale, whereas the *black italic* text shows the target

Typically, an experiment with soil micro-fauna will use an experimentally convenient mass of between 0.1 and 1 g of soil. A huge amount of information can be gained from this mass, but it still only represents an infinitesimally small fraction of the microbial biomass that drives ecosystem biogeochemical processes. Technical constraints often prevent us from up-scaling our primary measurements (cf eddy fluxes and satellite imagery); thus, we must look to our results for general trends that can be applied over larger scales.

With drivers such as climate change, soil pollution, pathogen transmissibility, etc., it is policy makers, regulators, legislators, industry and other end users that also require these data to enable appropriate land-use choices on field, catchments, landscape, national and global scales. Thus, the need for up-scaling facilitates cross-disciplinary solutions (e.g. global carbon and nitrogen models). As has been pointed out by Balser et al. (2006), one of the key limitations to multi-scalar understanding of ecosystems is rather prosaic: It is the schism between two schools of science, micro- and macro-ecology. Both schools use differing experimental designs and methodological processes and, importantly, 'have different comfort levels with uncertainty' (Balser et al. 2006). However, although this is the general case, there is increasing awareness of common ground.

In this paper, we illustrate successful examples of scale-up in the study of the plant–soil–microbe system, focussing on combining the use of microbiological tools [reporter gene systems, polymerase chain reaction (PCR), stable isotope probing (SIP)] with modelling (geostatistics/GIS, individual based models) approaches to scale-up in the following case study areas:

- Contamination and bioremediation
- Human pathogens and catchment management
- C, N, P and rhizosphere dynamic modelling
- Relating rhizosphere microbial diversity to ecosystem processes

It is worth briefly considering here the interrelationship between scale and functional redundancy as applied to soil microbial biodiversity. Functional redundancy can be regarded as a metric of the number of different species within various functional groups or guilds (Yin et al. 2000). For the bioremediation of anthropogenic contaminants, the functional diversity may range from very low, where only one or two species possess the appropriate degradative pathway to break down a particular PCB or dioxin congener, to high for the degradation of simple hydrocarbons (Young and Cerniglia 1995). Functional redundancy tends to increase with a shift towards natural systems; for example, the diversity of the antibiotic synthesis gene *phlD* has a worldwide distribution amongst pseudomonads (De La Fuente et al. 2006; MacSppden Gardener 2007), and the selective removal of some pseudomonad species would not affect the overall process. Among such widespread soil processes as denitrification or plant litter decomposition, functional redundancy may be so large that the relationship between below-ground biodiversity and ecosystem functioning loses relevance at scales above the micro.

Finally, we briefly consider how technical considerations affect the incorporation of data from soil denitrification and nitrous oxide emission studies, with respect to global warming models.

Case studies

Biosensors for pollutant assessment and bioremediation

Whole-cell microbial biosensors are now routinely used for screening of contaminated soil (Killham and Paton 2003) as a complementary tool to standard, chemical analysis. *Lux*-marked bacterial biosensors expressing bioluminescence reporter genes either under the control of a strong, general, constitutive cell promoter (in the case of toxicity biosensors) or under the control of a contaminant catabolic or resistance promoter (in the case of contaminant specific biosensors) have proved particularly useful in this regard. This is because their inherent flexibility, in terms of host selection and construction, offers a high degree of environmental relevance and both the ability to screen for overall toxicity (because of contaminants or some other soil factor) or the presence of specific, bioavailable contaminants (Paton et al. 1995). It is the capacity of these types of biosensor to address contaminant bioavailability that so effectively complements chemical analysis, as well as to screen (using toxicity biosensors) all toxic contaminants and to identify otherwise unknown problems that the highly specified, analytical approach may miss (Killham and Paton 2003). The use of biosensors to assess contaminant bioavailability involves integration of the many physical, chemical and biological factors that influence this availability (Fig. 2).

In systems such as acid forest soils, where the scarcity of prokaryotes may restrict the relevance of bacterial sensors, the use of micro-organisms such as filamentous fungi may provide a useful alternative (Weitz et al. 2002; Horswell et al. 2006).

Scaling up biosensor-based assessment of contaminated sites from the small sample scale (based on extraction of a few g of soil) associated with most biosensor toxicity-screening procedures is currently, effectively achieved by using geostatistical tools such as block krieging that facilitates interpolation of spatial data, assuming the sampling coverage/density of the area is adequate. Killham and Staddon (2002) highlight the strengths of geostatistics

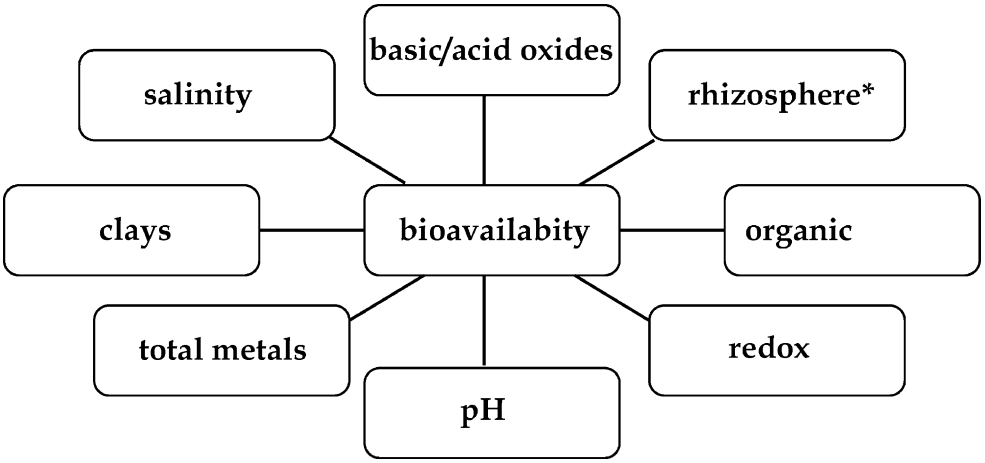

Fig. 2 Illustration of various factors affecting the bioavailability of any target pollutant

for quantifying soil health in this way. Figure 3 shows an example of this type of plot, using the "Surfer" software package (Golden Software Inc., Colorado, USA) in which the contours on the figure join points of equal soil toxicity.

The use of a simple colour scale that grades from non-toxic through to highly toxic (in terms of biosensor response) is qualitative but enables such maps to be readily interpreted and form a valuable tool in screening sites for toxic pollutants and identifying/quantifying hot-spots where there are likely to be serious toxicity constraints to intrinsic bioremediation of normally degradable, organic pollutants (Killham and Paton 2003).

Human pathogens and catchment management (PCR, reporters, GIS)

The availability of a raft of detection techniques (culture based, immunological, molecular) for human pathogens in environmental matrices (Campbell et al. 2001; Ritchie et al. 2003; Shelton et al. 2006) has greatly enhanced our ability

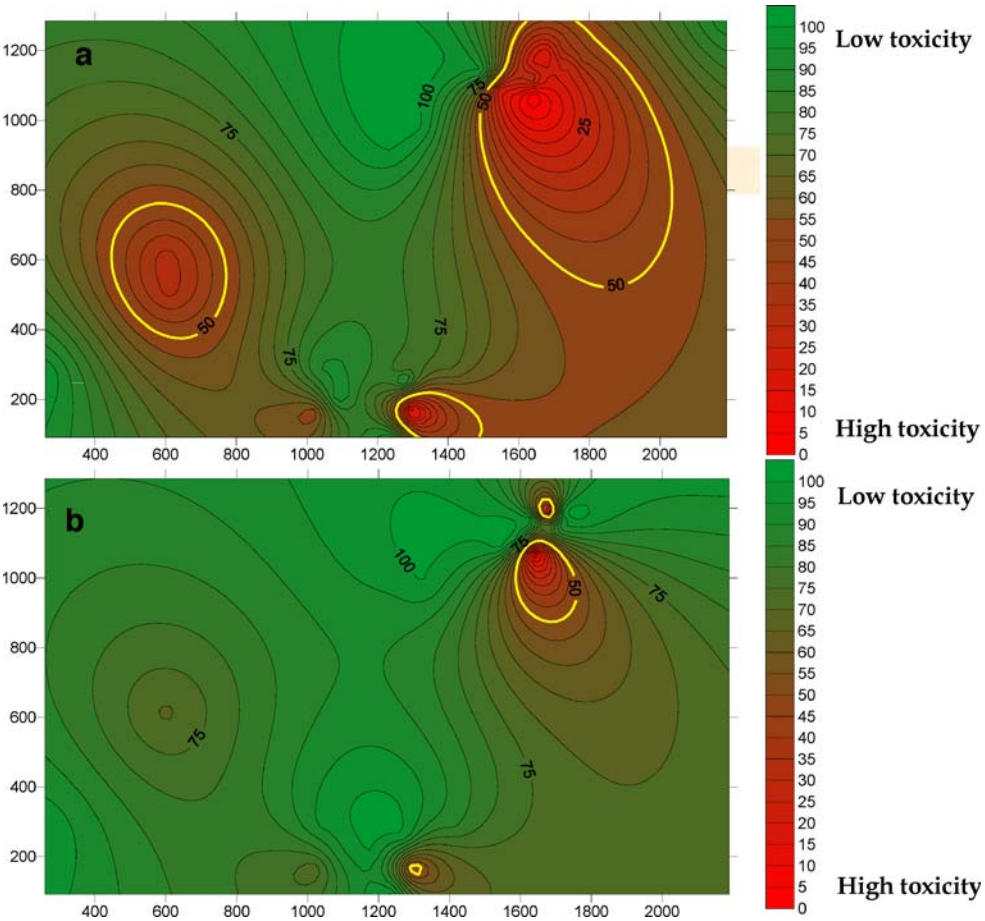

Fig. 3 An aerial photograph and corresponding "tox map" showing the spatial distribution of contaminant toxicity on a field scale

to develop a predictive understanding of the infection risks associated with human contact with pathogens in the environment. This can be well illustrated for *Escherichia coli* O157 where infection, such as from drinking contaminated well water draining from soils receiving animal wastes, can cause human health impacts ranging up to serious kidney damage and even death (Artz and Killham 2002). Because of the very low infective dose associated with this pathogen (10–100 cells), detection techniques need to achieve a commensurate level of sensitivity, and this has been achieved with molecular approaches [generally based on quantitative PCR (qPCR) targeting of key genes associated with this particular pathogen], as well as the use of reporter-gene-marked non-pathogenic surrogate strains. Figure 4 shows how effective such detection techniques are for screening soil and water samples and for assessing how these pathogens move through the soil environment and enter drainage waters (and potentially contaminating drinking waters).

However, our capacity to track pathogens such as *E. coli* O157 in environmental samples introduces the challenge of scaling up to facilitate the development of risk management strategies.

For human pathogens such as *E. coli* O157 where surface water is a major vector, risk management is relevant at the catchment scale. Scaling up for catchment management can be achieved by studying the leaching/dispersal properties through individual soils that dominate catchment hydrology. This is readily done using laboratory-based mesocosms. Data from such experiments can be combined with digitised, soil spatial distribution data from sources such as the Scottish Soils Database (Macaulay Institute, Aberdeen, UK) and together facilitates a GIS-led approach to scale-up and catchment management. GIS is a particularly valuable tool here, as the availability of other digitised data-sets such as cattle density on pastures and proximity to private water supplies can be simply overlaid to further facilitate this scale of risk management approach (Fig. 5).

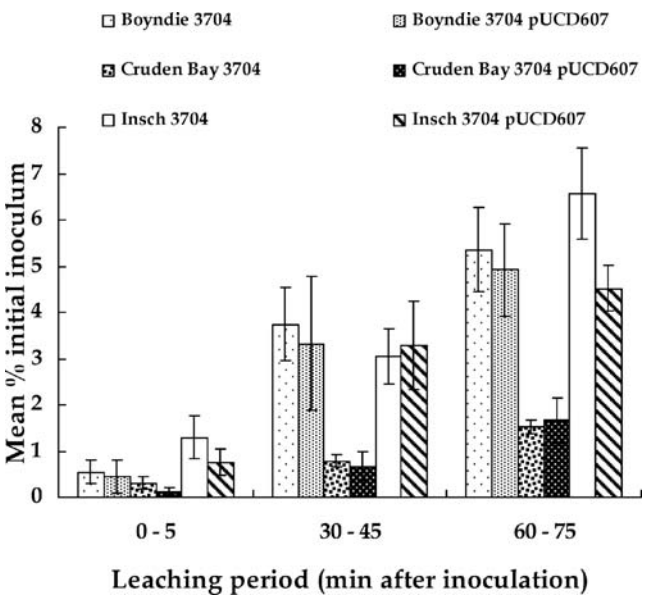

Fig. 4 Soil leaching studies involving a lux-marked construct of *E. coli* O157:H7 (adapted from Artz et al. 2005)

Rhizobacterial reporters and dynamic rhizosphere modelling

Bacterial reporter gene systems offer the opportunity for interrogating rhizosphere C-flow in situ without uncoupling plant–microbial interactions. This is vital in maintaining the integrity of the C flow, both quantitatively and qualitatively. When the metabolic activity of individual cells is in direct proportion to *lux* output, then the single-cell metabolic approach lends itself greatly to investigations of plant-root carbon flow. Plant roots exude carbon into the zone of soil immediately surrounding their roots. This carbon forms a selective substrate for growth of specific groups of root-associated bacteria (rhizobacteria). Rhizobacteria form a living 'screen' around the root, across which all soil nutrients and water must pass. The zone of soil affected by this plant-originated carbon is termed the 'rhizosphere' and is the subject of intensive research. Understanding rhizosphere processes at a bacterial population level, therefore, holds the key for the possibility of engineering the rhizosphere for improved crop management at field scales. As rhizosphere processes are underpinned by carbon flow, it is logical to investigate the relationship between bacterial activity and carbon quality. Yeomans et al. (1999) demonstrated that the Michaelis–Menten kinetics of the *lux* response of *Pseudomonas fluorescens* 10586 to different carbon fractions (reducing sugars, organic acids, amino acids) could be used to determine the composition of rhizosphere carbon flow. Analysis of these kinetic profiles showed that carbon flow from young wheat roots was primarily composed of low-molecular weight sugars. Chemical analysis of wheat root exudates confirmed this. In this way, the known response of a bacterial population to different C fractions was used to interrogate processes occurring at scales 10,000 to 100,000 times greater.

A potential disadvantage of using general metabolic reporters is that a different but limiting factor may modulate their response to carbon. Kragelund et al. (1995) used Tn5 mutagenesis to insert *luxAB* cassettes into rhizosphere fluorescent pseudomonads. They identified several strains that responded to N or P starvation. In this way, they developed specific starvation reporters for N and P, thus, enabling the potential to explore questions about macronutrient limitation within the rhizosphere. Standing et al. (2003) furthered this by developing a protocol using a tripartite of reporters to explore the question of what was

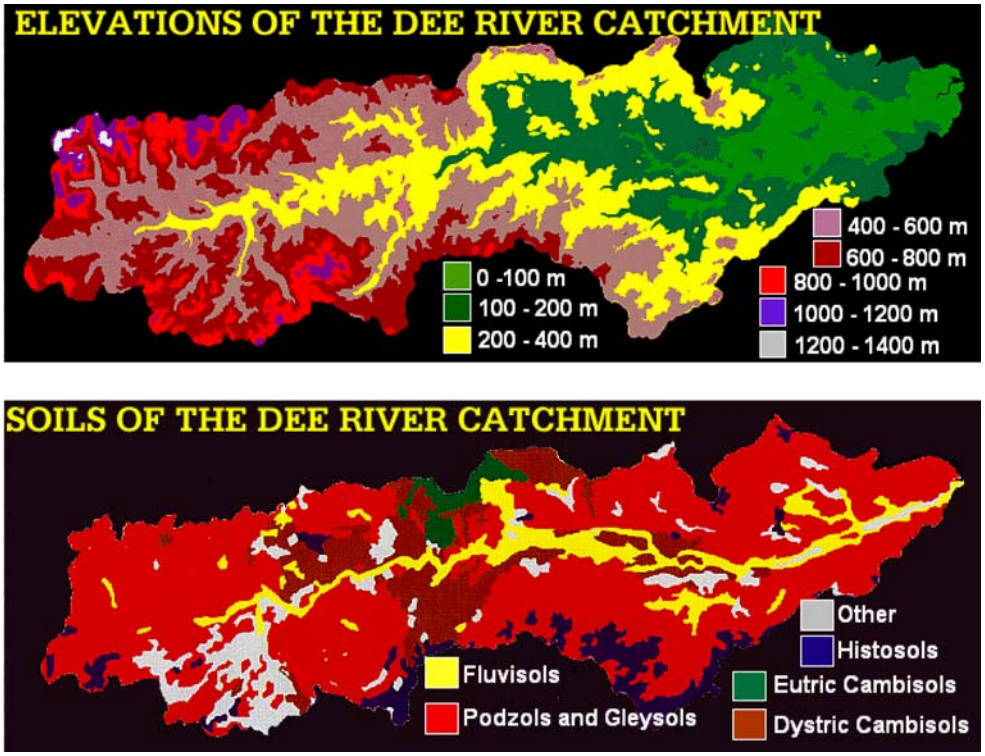

Fig. 5 River Dee catchment elevations and soil map (Fitzpatrick 1999)

the most limiting nutrient within the rhizosphere. Knowledge of such limitations is critical in the design and parameterisation of simulation modelling approaches particularly considering that C, N and P together gives useful information on potential nutrient limitation and causes. For example, Parton et al. (1988) developed a C, N, P, S model to investigate the dynamics of those macronutrients both cultivated and uncultivated grassland soils. The model simulates a number of temporal phenomena—impact of cultivation, dynamics of soil organic matter nutrient mineralisation and soil formation—and correctly predicted that N and P are primary limiting nutrients for plant growth. Parton et al. (1994) have extended this model to the Century model (NREL, Colorado State University, USA), and detailed data on rhizosphere C, N, P dynamics would be most useful in initialising values for the active soil microbial fraction.

Studying rhizosphere function is notoriously difficult because of the need to quantify microbial and substrate concentrations in a dynamic system over minute distances. This difficulty is further compounded by having to incorporate bi-directional fluxes of C in and from plant roots (Darrah and Roose 2007). Whilst there have been a number of experimental developments in recent years, it remains troublesome to quantify all of the relevant components of the system at the same time. However, combined experimental modelling approaches allow us to study various aspects of rhizosphere dynamics in intact systems at appropriate scales (Fig. 6).

As previously stated, the primary driver of rhizosphere microbial community development is the release of plant-derived labile carbon into the soil. In addition to reducing sugars such as glucose (Yeomans et al. 1999), amino- and organic-acid compounds are released, although in lesser amounts (Farrar et al. 2003; Jones 1998). These compounds are largely released from root cells because of passive diffusion as a result of the large electrochemical potential gradient that exists between the root cytoplasm and the soil solution (Farrar et al. 2003). Roots can also recapture C lost into the soil using active H^+-ATPase-driven proton co-transporters. Consequently, plants have the potential to control C accumulation in the rhizosphere by either regulating C efflux (exudation) or influx (recapture). The bi-directional flow of C at the soil–root interface, together with microbial uptake and abiotic removal of C, through sorption onto the physico-chemical matrix, from the soil solution, makes C dynamics in the rhizosphere extremely complex at both temporal and spatial levels (Fig. 7).

However, considering the fundamental role that the rhizosphere is believed to play in processes such as root nutrient uptake, plant–microbe signalling, bioremediation and soil C sequestration, it is important that we understand at a mechanistic level the dynamics of C in the rhizosphere. Modelling provides a useful test-bed with which to interpret data gained from bench or field experiments. In particular, multi-dimensional simulation modelling techniques such as cellular automata are useful, as these do not require large amounts of computer power. The use of 3D cellular

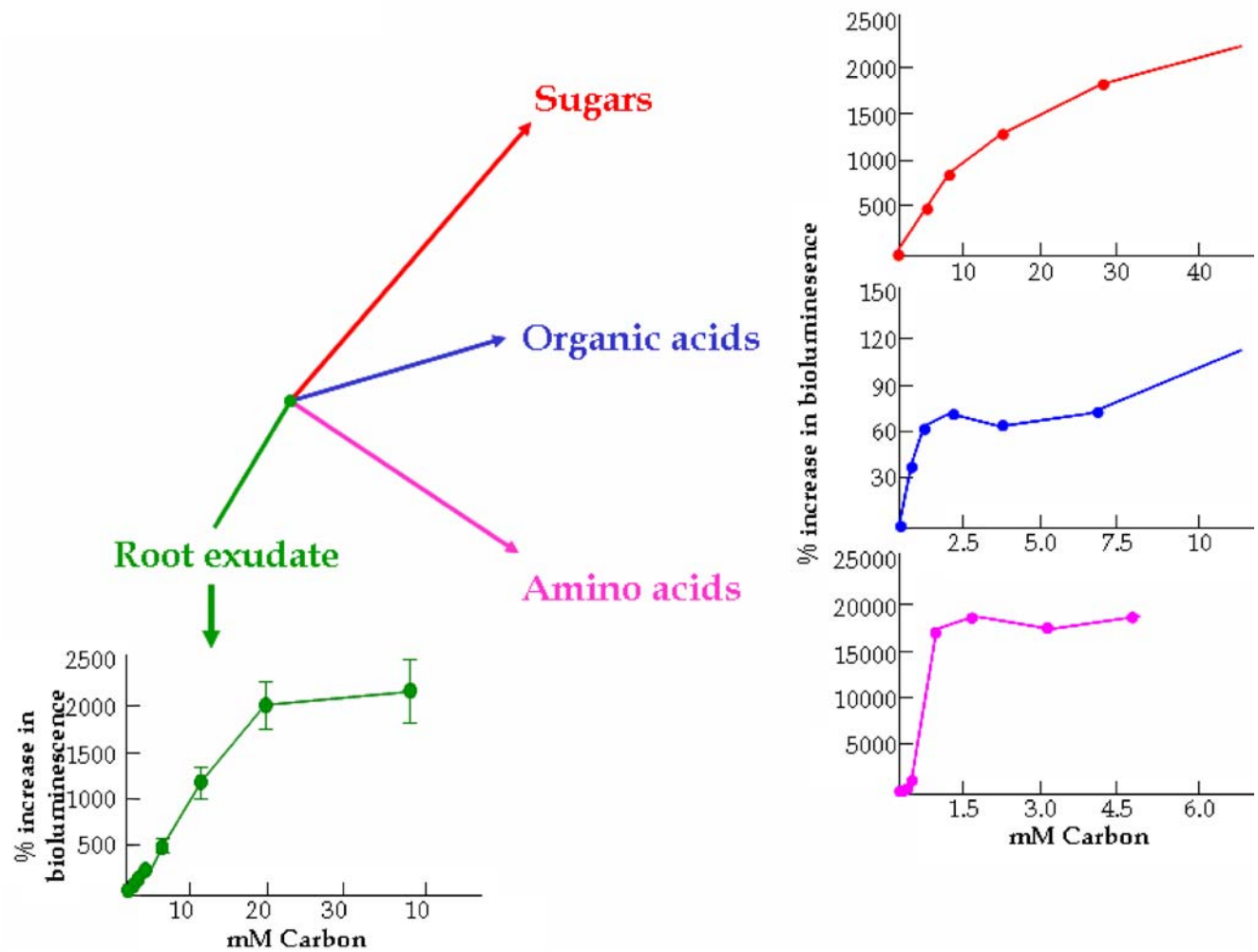

Fig. 6 Comparative kinetic responses of rhizobacterial biosensors enables identification and quantification of rhizosphere carbon flow fractions (adapted from Killham and Yeomans 2001)

automata is of particular value as spatial aspects are explicitly considered and cell size, within any individual model, can be altered to explore scale-dependent issues (Fig. 8). System non-linearity is accommodated, and state variables such as diffusion coefficients, bacterial population size and growth rates can be easily changed to incorporate experimental data.

Relating rhizosphere microbial diversity to ecosystem processes—antibiotic production and rhizosphere engineering

As previously mentioned, the necessity for understanding scaling has many applied drivers, particularly bioremediation and pathogen control. Although simple to state, it is not a trivial matter to scale up from plant-originated C through microbial uptake, utilization and protein expression to crop management. Foremost, it requires a fuller understanding of the links between C and microbial gene expression. Photo-assimilate is primary growth substrate for rhizobacterial, and it is, therefore, possible to link this plant-originated C to active members of the soil microbial community. Understanding how exudate quality can affect microbial gene expression is of particular interest, as it allows for the potential to engineer specific functions within the plant-root–microbe system (Fig. 9).

Many *Pseudomonas* strains found in the rhizosphere produce secondary metabolites, some of which are effective antibiotics. As such, they have great potential as biocontrol agents. In this paper, we present an example of this using a recognised pseudomonad biocontrol strain (*Pseudomonas fluorescens* CHA0) and production of an antibiotic, phenolic polyketide, 2,4-diacteylphloroglucinol (DAPG). DAPG has been shown to be effective against the wheat root-disease fungal pathogen *Gaeumannomyces graminis* var. *tritici* (Take-all). Duffy and Defago (1999) and Shanahan et al. (1992) demonstrated in vitro the selective effects of different single-C sources and minerals on DAPG production. However, rhizosphere carbon flow is more complex, and the question of how DAPG production is

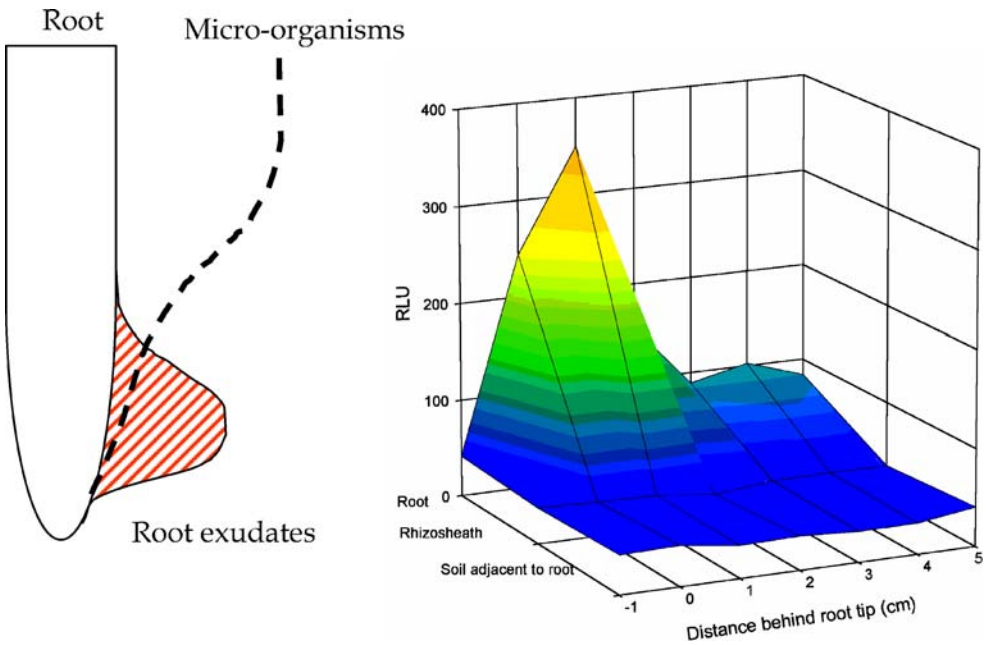

Fig. 7 Development of a rhizosphere microbial population in response to carbon availability (Standing et al. 2005)

affected by qualitatively changing complex C, i.e. admixtures of reducing sugars and acids typifying root C flow, has not received a great deal of attention.

A 96-well plate method was used to investigate the effects of different but C-balanced growth media on DAPG production. The C treatments were designed to reflect early monocot rhizosphere C flow, i.e. to be based primarily on simple reducing sugars with admixtures of organic and amino acids. *P. fluorescens* CHA0 were inoculated into the plate wells and incubated for 5 days. The plate was harvested for DAPG by drawing the cell-free contents of each well through a C-18 solid phase extraction 96-well plate (Porvair Microlute, Porvair Sciences, Norfolk) and quantifying any DAPG in the eluent with high-performance liquid chromatography (HPLC).

Analysis of the DAPG concentration within each treatment showed significantly more antibiotic when the

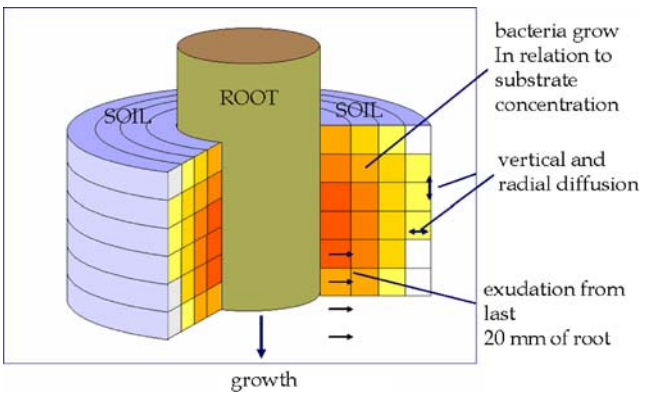

Fig. 8 Cellular automaton diffusion—root model. Substrate movement (both positive and negative) is modelled as a series of individual events, from a source ring into a neighbouring receiving ring (above, below, inside or outside; Standing and Killham 2006)

total reducing sugars comprised 90% of the total C with the remainder split between organic acids and amino acids (data not shown). This illustrates that a relatively small shift in substrate C quality may result in dramatically altered rhizobacterial bio-control activity. Plant quantitative trait loci involved in organic acid production are an obvious target for plant breeding for biocontrol.

SIP is an elegant technique allowing us the potential to investigate microbial gene expression and metabolic activity in complex environments without the need for culturing. SIP is not a new technique—in 1958, Meselson and Stahl (1958) used 'heavy' ^{15}N and 'light' ^{14}N to prove the Watson–Crick model of DNA replication but has recently emerged as a powerful technique for linking microbial diversity to function, particularly, where the driver is C (Prosser et al. 2006). Briefly, this method relies on the incorporation of a 'heavy' isotope (^{13}C, ^{15}N, ^{18}O, etc) into genetic material [commonly, biomarkers such as phospholipid fatty acids (PLFA), DNA or RNA] and subsequent separation of that labelled biomarker by isopycnic centrifugation. The isolated biomarkers can then be visualised with standard techniques such as denaturing gradient gel electrophoresis (DGGE) or gas chromatography (GC). DNA–SIP has been successful in establishing the links between the flow of plant root exudate to microbial community structure (Rangel-Castro et al. 2005; Fig. 10). However, as with all techniques, caveats exist: According to Manefield et al. (2006), a critical aspect of SIP methodology concerns the degree of labelling. This, in turn, depends on the number of organisms using the labelled substrate. If a wide diversity of organisms are consuming the substrate, then substrate dilution can occur, and the degree of labelling in any substrate-utilising taxa

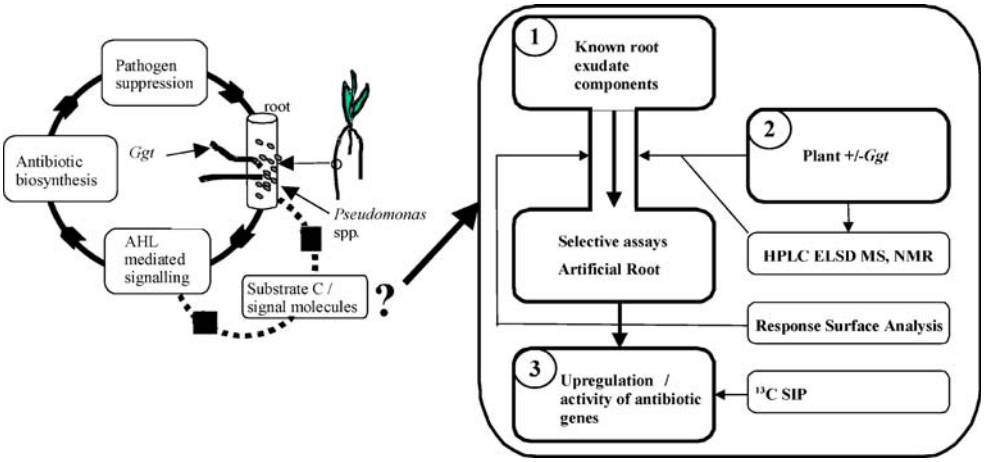

Fig. 9 Rhizosphere antibiotic production—a strategy for identifying the components of rhizosphere C flow that activate functional genes involved in antibiotic suppression of soil-borne plant pathogens. *AHL* Acyl homoserine lactone, *Ggt Gaeumannomyces graminis* var. tritici, *HPLC* high-pressure liquid chromatography, *ELSD* evaporative light-scattering device, *MS* mass spectrometry, *NMR* nuclear magnetic resonance, *SIP* stable isotope probing

will be low. Conversely, if the substrate is consumed by a small number of taxa, then label dilution is not so much of a problem, and the degree specific taxa will be high, facilitating isolation by density. The latter conditions may occur in the rhizosphere soil.

Complementary to this, PLFA–SIP has been used to highlight spatial variation in rhizosphere communities (Lu et al. 2007). Although these studies used different biomarkers, both metrics of structure give essential insights into larger scale rhizosphere processes, particularly the rapid incorporation and turnover of C into the microflora, as well as the activity of these communities both vertically and horizontally in the rhizosphere-bulk soil continuum.

Both such fine-scale analyses can be used as a basis for incorporation into nested modelling approaches. These rely on a graded series of increasingly larger model domains, with increasingly coarser resolution, that cover the area of interest, e.g. landscape, continent, global. An important determinant of the predictive ability and reliability of the model is its scale-bridging capacity, i.e. the ability of the model to reproduce observed data at any relevant scale.

Our present lack of understanding of scaling processes within soil is partly a result of the many technical difficulties associated with the study of soil structure. These difficulties are primarily driven by the inherent heterogeneity of soil structure, itself the determinant all physico-chemical (micro- and macro-variations in pore size distribution, texture, bulk density, OM, hydraulic conductivity, pH, CEC) and biological processes such as the accessibility of nutrients, water and air for the soil biota and plant roots. There has been success in interpreting soil structure in terms of fractal analysis (see Pachepsky et al.

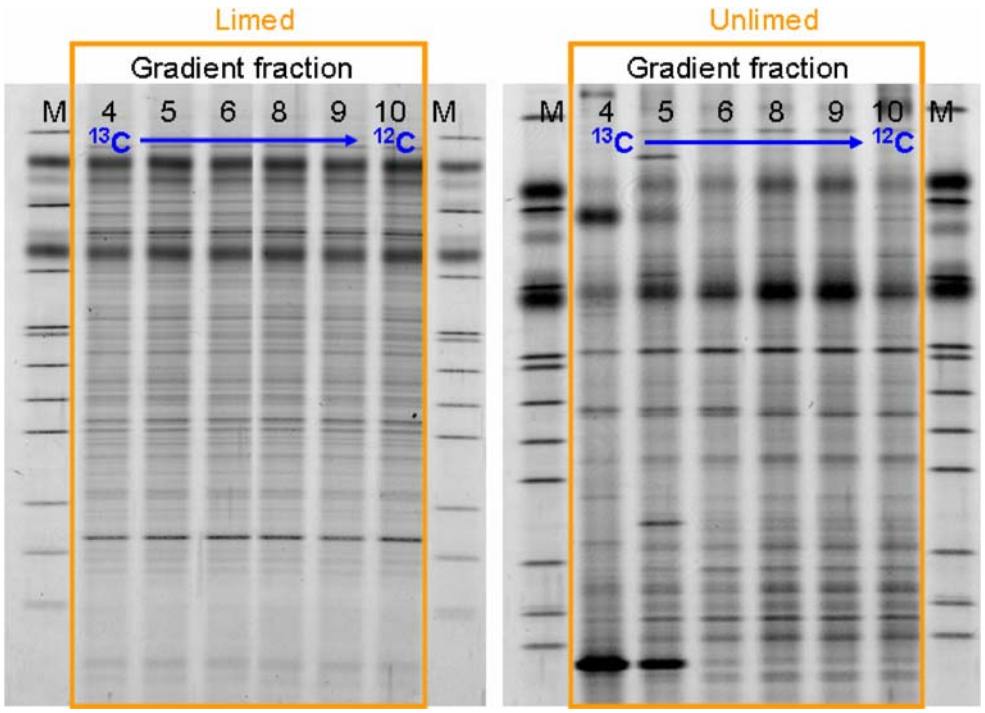

Fig. 10 16S DNA DGGE-SIP of rhizobacterial community in a upland grass pasture soil to identify changes in terms of rhizosphere carbon flow utilisation under different management regimes (Rangel-Castro et al. 2005)

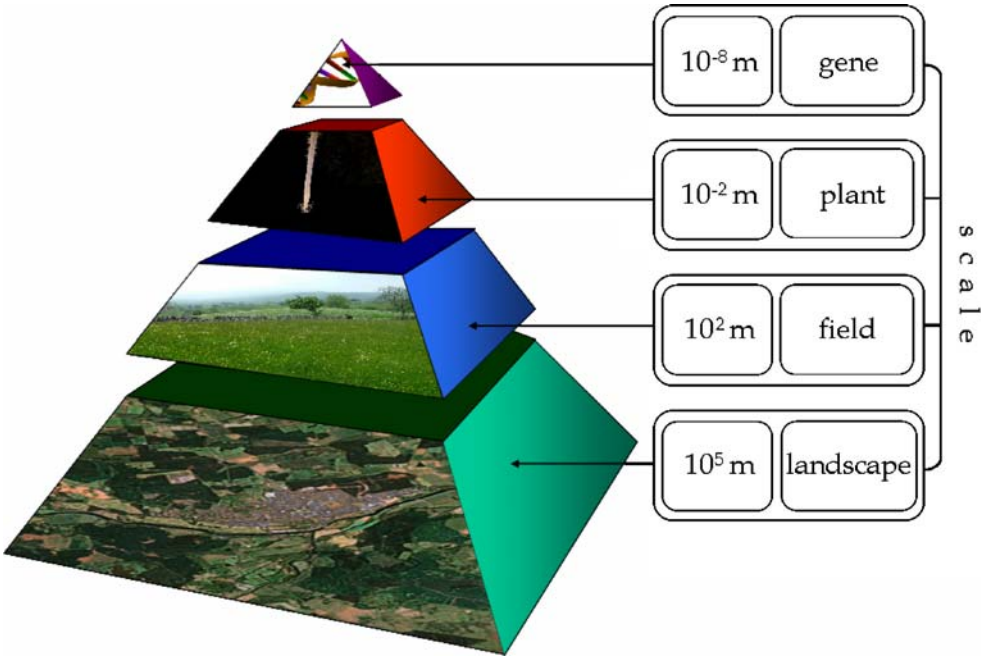

Fig. 11 The 13 orders of magnitude of scale from the molecular to the landscape

2000 and references therein), but this has not yet been possible with microbially driven processes.

Although not soil-based, a collaborative group[1] are using ^{13}C to investigate scaling relationships in aquatic systems. This group is investigating the question of whether scaling rules that describe the relationship between stream size and nutrient utilization also hold for DOM. To determine which components of the microbial community are actively assimilating DOM, they have created stocks of ^{13}C-labelled DOM originated from composted trees grown in a ^{13}C-enriched atmosphere. By following the fate of labelled organic molecules through SIP analysis of stream water samples, they will identify which microorganisms are actively incorporating the label. By relating existing scaling rules of stream growth and nutrient uptake, the group will be able to elucidate microbially driven energy networks leading to a better understanding of nutrient uptake and scaling rules in stream systems. At present, this is the only study the authors are aware of that attempts to follow the fate of ^{13}C across many scales within an ecosystem (Fig. 11).

Scale is a primary consideration when quantifying the soil's contribution to climate change. Soil is a major source of N_2O and CH_4 (IPCC 2007) but also acts as a sink for these potent greenhouse gases and offers the potential for C sequestration. With better understanding of the controls on trace gas production and reduction or oxidation, it will be possible to develop appropriate mitigation strategies. For this to be effective, we need to bridge the differences in scale considered from soil microbial sources of these gases, the active key microbial groups responsible and the fate and turnover of C within different soil fractions to vegetation type, land-use and even up to national inventories. This remains a challenge. This is in part because of the different techniques utilised to quantify processes at different scales, ranging from stable isotope, molecular and chamber techniques at the micro- to plot scale (Baggs et al. 2003; Deiglmayr et al. 2004) to micro-meteorological measurements using Fourier transform infrared spectroscopy or tuneable diode laser absorption spectroscopy at the medium scale (Smith et al. 1994) up to aircraft at the catchment or landscape scale (Pattey et al. 2006). As the scale increases, the technique utilised provides a more aggregated response that inherently loses the level of detail provided at the microscale. Reductionist and holistic approaches diverge in this respect, and we now need to find solutions and tools to unite these approaches.

Recent advances in stable isotope techniques have facilitated major advances in quantification and source attribution of greenhouse gases in relation to process rates at plot and mesocosm levels (Baggs and Blum 2004; Wrage et al. 2005), and there is now the potential with NanoSIMS to understand the spatial heterogeneity of the microbial functional groups within the rhizosphere (Herrmann et al. 2007). There are fundamental questions underlying the concepts of up-scaling from these studies that now need to be addressed, such as are the key process drivers, for example, of denitrification, the same and of the same ranked significance with differing scale? If so, can responses measured on the microscale be mathematically extrapolated to accurately give responses at the landscape

[1] The application of scaling rules to energy flow in stream ecosystems. National Science Foundation, USA.

scale? Those adopting process-based modelling approaches, e.g. DNDC (Li et al. 1992) and Daycent (Del Grosso et al. 2000) to estimate emissions of N_2O, inherently assume that this is true, and this may well be the case before the microbiology is considered. However, whilst these models can fairly accurately measure total/annual emissions from particular environmental systems, they are poor at accurately capturing temporal variability in the magnitude of fluxes (Bakken and Dörsch 2007). It is possible that the level of uncertainty in these models can be lowered by considering better integration from the microscale, as well as the nature and significance of interactions between the C and N cycles.

Studies on diversity of denitrifiers have shown dramatic shifts in community structure in response to pH change in the rhizosphere (Deiglmayr et al. 2004; Enwall et al. 2005), but when considering denitrifier-N_2O production at the field or catchment level, soil hydrology and frequency and duration of rainfall events often become the primary drivers (Sexstone et al. 1985; Dobbie and Smith 2006). This strongly suggests that the process of denitrification within the soil community is functionally redundant, and thus, the underlying microbiology no longer becomes important at the larger scale. However, if this is the case, at what scale-point do the microbiology and the measured process decouple?

Conclusions

This review has drawn on diverse case studies to illustrate the considerable progress being made and the remaining challenges to scale up processes in the soil–plant–microbe system.

Using biosensors and other techniques such as geostatistics, we can reliably scale up in a static way and exploit the diverse gene pool of soil micro-organisms for bioremediation of contaminants. On a smaller scale, we can use reporter gene systems to map the carbon and nutrient status of the rhizosphere habitat in real time, with the potential to scale up to multiple root systems for reliable rhizosphere-dynamic modelling. Using a combined approach of reporter genes and real-time PCR, we can reliably determine the survival and dispersal of human pathogens and use GIS to manage catchments.

SIP is enabling us to explore the link between biodiversity and function, demonstrating that soil physico-chemical properties determine the "key players" that underpin large-scale biogeochemical processes. Current work tracking heavy labelled organic molecules through aquatic systems is paving the way for soil systems.

The progress in scaling up exemplified by these case studies, using tools such as geostatistics, should offer real encouragement to researchers of other aspects of the soil–plant–microbe system where drivers, such as sustainable land management and climate change, require extrapolation to greater scales than those that are most readily studied at present. The challenge now is the dynamic modelling of these processes across scales. As we emphasised in the introduction, integration and prioritizing now needs to take place. Geostatistics gives us a tool to understand the spatial autocorrelation of processes. We need to do the same for temporal autocorrelation to find integrators ready to interlink the micro- to the landscape and continental scale. This should embrace the concept of genetic functional redundancy—defining the area or phase space where scale and process no longer correlate remains one of the key questions in functional soil microbial ecology.

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