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The role of soil microbes in the global carbon cycle: tracking the below-ground microbial processing of plant-derived carbon for manipulating carbon dynamics in agricultural systems

Christos Gougoulias, Joanna M Clark and Liz J Shaw

Abstract

It is well known that atmospheric concentrations of carbon dioxide (CO₂) (and other greenhouse gases) have increased markedly as a result of human activity since the industrial revolution. It is perhaps less appreciated that natural and managed soils are an important source and sink for atmospheric CO₂ and that, primarily as a result of the activities of soil microorganisms, there is a soil-derived respiratory flux of CO₂ to the atmosphere that overshadows by tenfold the annual CO₂ flux from fossil fuel emissions. Therefore small changes in the soil carbon cycle could have large impacts on atmospheric CO₂ concentrations. Here we discuss the role of soil microbes in the global carbon cycle and review the main methods that have been used to identify the microorganisms responsible for the processing of plant photosynthetic carbon inputs to soil. We discuss whether application of these techniques can provide the information required to underpin the management of agro-ecosystems for carbon sequestration and increased agricultural sustainability. We conclude that, although crucial in enabling the identification of plant-derived carbon-utilising microbes, current technologies lack the high-throughput ability to quantitatively apportion carbon use by phyllogenetic groups and its use efficiency and destination within the microbial metabolome. It is this information that is required to inform rational manipulation of the plant–soil system to favour organisms or physiologies most important for promoting soil carbon storage in agricultural soil.

Keywords: carbon cycling; rhizosphere carbon flow; decomposition; soil microbial respiration; climate change; methods; carbon tracking; agro-ecosystem management

THE SOIL CARBON CYCLE AND MICROBIAL DECOMPOSERS: FUNDAMENTAL PRINCIPLES

All living organisms depend on the supply of necessary elements from the Earth. Since the Earth is a closed system with a finite supply of essential elements such as hydrogen (H), oxygen (O), carbon (C), nitrogen (N), sulfur (S) and phosphorus (P), recycling of these elements is fundamental to avoid exhaustion. Microbes are critical in the process of breaking down and transforming dead organic material into forms that can be reused by other organisms. This is why the microbial enzyme systems involved are viewed as key ‘engines’ that drive the Earth’s biogeochemical cycles.¹

The terrestrial carbon cycle is dominated by the balance between photosynthesis and respiration.² Carbon is transferred from the atmosphere to soil via ‘carbon-fixing’ autotrophic organisms, mainly photosynthesising plants and also chemoautotrophic microorganisms.³,⁴ that synthesise atmospheric carbon dioxide (CO₂) into organic material (Fig. 1). Fixed carbon is then returned to the atmosphere by a variety of different pathways that account for the respiration of both autotrophic and heterotrophic organisms⁵ (Fig. 1). The reverse route includes decomposition of organic material by ‘organic carbon-consuming’ heterotrophic microorganisms that utilise the carbon of either plant, animal or microbial origin as a substrate for metabolism, retaining some carbon in their biomass and releasing the rest as metabolites or as CO₂ back to the atmosphere (Figs 1 and 2).⁶ Globally, most soils are unsaturated and oxic, so CO₂ is the main respiration flux. In waterlogged anoxic soils such as rice

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Figure 1. The terrestrial carbon cycle with the major processes mediated by soil microorganisms (adapted from Prosser).  

Figure 2. Fate of primary production inputs to soil. Plant-derived organic carbon (after appropriate extracellular depolymerisation) is processed by soil microorganisms to CO₂, microbial biomass and extracellular substances. Microbial necromass and metabolites are the precursors for stable soil organic matter, while extracellular microbial carbon may also influence the stability of soil organic carbon (SOC). Enzymes may catalyse the depolymerisation of soil macromolecular constituents, while other extracellular substances may promote aggregation and the physical protection of SOC. SOC (red boxes) is depicted as a continuum of structures derived from the progressive decomposition of litter and exudates and includes the microbial biomass carbon. Dissolved and exposed organic carbon (A) is available for microbial cellular uptake and metabolism (catabolism + anabolism) to produce CO₂ and new biomass respectively. Macromolecular or sorbed or occluded SOC is metabolically non-available (B) but may become available via enzymatic depolymerisation, desorption or exposure (I–III respectively), assuming adequate water, electron acceptors, heat, pH and nutrients for microbial activity.

paddies and peatlands, CO₂ is reduced by hydrogenotrophic archaea in methanogenesis, with the net flux of the methane produced dependent on the relative activity of methanogens (including those fermenting acetate) versus the activity of aerobic methane-oxidising bacteria (methanotrophs) residing in the surface, oxic layers of soil of such wetland systems and also probably the microbial anaerobic oxidation of methane in anoxic layers.

Soil microbes essentially transfer carbon between environmental compartments to fulfill their fundamental goal: survival through reproduction. Thus, microbes utilise different organic and inorganic forms of carbon as carbon and energy sources. However,
Role of soil microbes in global carbon cycle: carbon tracking & agro-cosystem management

the C cycle does not operate independently; it is closely coupled with that of other essential elements for microbial metabolism. This linkage occurs either via the use of the other elements as electron donors and acceptors (e.g. N species ranging from the most reduced, NH$_4^+$, to the most oxidised, NO$_3^-$) in energy transduction or via their immobilisation and mineralisation as part of multiple essential element-containing biomolecules (e.g. proteins, DNA). Hence the availability of other key elements essential for life, particularly N and P, and other environmental factors such as pH, soil texture and mineralogy, temperature and soil water content control the rate at which microbes consume and respire carbon. It is these interactions between environmental conditions and biological processes, including primary production, that are chief in controlling the unequal distribution of organic matter across the world’s soils. The largest global concentration of carbon can be found in wet and cool areas in the northern hemisphere, dominated by deep accumulations of peat and permafrost soils, whereas soils with the lowest carbon content tend to be those of desert biomes, where low mean annual precipitation limits primary production and encourages the prevalence of aerobic soil conditions.

The objectives of this review are (i) to outline the significance of soil microbial communities to global environmental issues of soil organic matter persistence and climate change through carbon cycle feedbacks, (ii) to briefly describe the main techniques that can be used to apportion below-ground utilisation of plant-derived carbon to specific microbial groups and (iii) to discuss whether application of these techniques can provide the information required to underpin the management of agro-ecosystems for carbon sequestration and increased agricultural sustainability.

THE SOIL CARBON CYCLE AND MICROBIAL DECOMPOSERS: SIGNIFICANCE AND ENVIRONMENTAL IMPLICATIONS

Relationship between soil and atmospheric carbon pools

Estimates suggest that global soil organic carbon stocks are equivalent to at least three times the amount of carbon stored in the atmosphere (Table 1). About 8% of the total atmospheric carbon pool is exchanged annually between terrestrial ecosystems and the atmosphere via net primary production and terrestrial heterotrophic (predominantly microbial) respiration (Table 1). In other words, if soil (microbial) respiration ceased, it would only take about 12 years of primary production at current rates to exhaust atmospheric CO$_2$ stocks (if all other components of the carbon cycle are ignored, e.g. oceanic CO$_2$ exchange). At present, terrestrial ecosystems fix, globally, more atmospheric CO$_2$ by photosynthesis than they return to the atmosphere through respiration, which includes removing around 25% of global fossil fuel emissions annually. However, net carbon sequestration varies between locations and is significantly affected by land management. Estimates suggest that 42–78 Gt of carbon have been lost from the world’s degraded and agricultural soils owing to human activity in both pre- and post-industrial times (Fig. 3), and land remediation to ‘restore’ some of this lost carbon could make a significant contribution to offsetting fossil fuel emissions.

Microbial decomposition of plant-derived carbon and persistence of soil organic matter

There are two main routes of input for plant organic carbon to the soil system: (i) above-ground plant litter and its leachates, i.e. dissolved organic carbon washed into the soil from plant material by infiltrating rainfall, and (ii) below-ground root litter.
and exudation, collectively known as rhizodeposition. The relative magnitude of the various inputs from above and below ground will depend upon plant species and, in soils under agriculture, crop management. Rhizodeposition consists of a continuous flow of carbon-containing compounds from roots to soil. Simple molecules such as sugars, amino acids, sugar alcohols, organic acids and more structurally complex secondary metabolites are among the chemical groups that make up the plethora of root exudates that can be rapidly respired following their deposition to soil. By contrast, polymers such as lignin, cellulose and hemicellulose (the typical structural constituents of plant cells) require depolymerisation by extracellular enzymes before they can be taken into the microbial cell and metabolised (Fig. 2). Of particular note in soil carbon cycling is the role of the mycorrhizal fungi. These range from obligate symbionts that can only obtain carbon from the host plant, i.e. the arbuscular mycorrhizal fungi (AMF), to facultative symbionts that can also mineralise organic carbon, e.g. the ectomycorrhizal fungi (ECM). The AMF symbiosis is found in about 85% of all plant families (typically herbaceous, including many crop species, but also woody), and experimental evidence suggests that up to 20% of total carbon assimilated by plants may be transferred to the fungal partner, with the symbiosis having profound effects on rhizodeposition. A proportion of the plant carbon that is transferred to the mycelia is very quickly respired back to the atmosphere, and this represents a short-circuit of the soil carbon cycle.

Soil organic matter (SOM) consists of the continuum from fresh to progressively decomposing plant, microbial and faunal-derived debris and exudates, including the microbial biomass that is responsible for the primary decomposition of the exudate and detrital inputs (Fig. 2). Traditionally, this continuum has been divided into a series of pools with varying decomposition kinetics, ranging from ‘active’ pools that turn over in months to ‘passive’ pools that turn over in thousands of years. In addition to containing fire-derived ‘black carbon’, the ‘passive’ pool has long been thought to be composed of constituents that get their resistance to decomposition from their humified nature, with the formation of the humified substances resulting from spontaneous condensation reactions between reactive microbial products and biochemically altered structural biomolecules. However, recent evidence suggests that environmental and biological factors may exert a far greater control on the long-term persistence of SOM than the molecular structure of plant litter inputs and subsequent formation of humus, forcing a re-evaluation of the concept of ‘recalcitrant’ soil humic substances that underpins predictive models of carbon turnover. Direct, in situ observations have not been able to verify the existence of humic macromolecules in soil, suggesting that the extraction of humic substances from soil may be an artefact of the method used to extract them. Instead, it is suggested that SOM consists of partially decomposed litter and a significant proportion of microbial necromass (i.e. dead biomass residues) and that SOM persists or is ‘passive’ owing to its physical disconnection from, or inaccessibility to, the extracellular enzymes, microorganisms and the optimal environmental conditions (e.g. electron acceptors, water, inorganic nutrients) needed for decomposition as a result of entrapment within soil aggregates and/or sorption to soil mineral phases. Therefore, in addition to their role in the breakdown and release of CO₂ from organic matter, soil microbes contribute to the formation of persistent SOM via their necromass. Additionally, the activities of soil microorganisms may contribute to the stabilisation of soil organic carbon through promotion of the formation of microaggregates, within which SOM may be physically protected from decomposition.

Soil carbon cycle, microbial decomposers and climate change

As mentioned, humans have heavily perturbed the carbon cycle during the industrial period through inputs of CO₂ to the atmosphere, mainly via combustion of fossil fuel and conversion of natural ecosystems to agricultural land (Table 1). The consequences of human actions for the global climate are still uncertain, partly owing to our limited understanding about soil respiration and its representation in Earth system models. Microbial contributions to climate change through carbon cycle feedbacks are far from straightforward, complicated by direct and indirect effects and interactions with other factors (also reviewed in Bardgett et al. and Singh et al.).

An example of a simplified direct positive feedback to global warming is that microbial activity, and therefore organic carbon decomposition and CO₂ released by respiration, may be accelerated in response to an increase in temperature. Analyses of field observations made across the globe point to a link between increased respiration flux from land and increased temperatures. An example of an indirect positive feedback to elevated CO₂ is a consequence of the carbon fertilisation of primary (photosynthetic) production, whereby increased atmospheric CO₂ stimulates photosynthesis and the release of root exudates, which in turn means more labile carbon available for microbial decomposition and respiration. Moreover, increased root deposition

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**Table 1. Estimates of the magnitude of soil carbon pools in relation to the atmospheric carbon pool and annual fluxes**

| Pool                                      | Carbon (Gt or Gt year⁻¹) |
|-------------------------------------------|--------------------------|
| Global soil organic carbon (0–300 cm depth)| 2344                      |
| Northern circumpolar permafrost region soil organic carbon (0–300 cm) | 1024                      |
| Cropland soil organic carbon (0–300 cm)   | 248                      |
| CO₂-C in atmosphere                       | 762                      |
| Net primary production (photo- and chemosynthesis minus autotrophic respiration) | 60d                      |
| Terrestrial heterotrophic respiration      | 55d                      |
| Anthropogenic CO₂-C (fossil, cement, land-use change) | 8c                      |

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a Jobbágy and Jackson (2000). b Tamocai et al. (2009) – a new estimate suggesting significantly more organic carbon in this northern latitude region than reported in previous analysis, e.g. tundra 144 Gt and boreal 150 Gt, by Jobbágy and Jackson (2000). c Solomon et al. (2007) – estimated for the 1990s. d Prentice et al. (2001) – estimated for the 1980s.
of easily available exudates may ‘prime’ the turnover of less available SOM constituents that otherwise would not be subject to decomposition.58–63

Understanding how the balance between terrestrial ecosystem sinks (i.e., photosynthesis) and sources (i.e., respiration, including microbial respiration) of atmospheric CO₂ will be affected in an elevated CO₂ world is still one of the main uncertainties in understanding the coupled carbon–climate system.4,39,41,47,57 The uncertainty increases when soil nitrogen and its availability to plants are taken into consideration, with contradicting indications.64 Some modelling studies have suggested that there may be a point during this century where terrestrial ecosystems shift from a net sink to a net source of atmospheric CO₂,40,54,65 possibly reflecting the scenarios of increased microbial respiration;45 however, these models are still in an early stage of development.53 Questions still remain about the actual temperature sensitivity of soil (microbial) respiration and how this sensitivity is modified by other environmental factors such as changes in soil moisture during droughts and nutrient limitations and physical protection of organic matter in aggregates or by sorption.4,11,30,66,67 This problem is exacerbated by the diversity of soil ecosystems across the world, which vary in their function owing to differences in their forming factors: parent material, topography, climate, organisms and time. Particular concerns have been raised about peatlands and permafrost soils, where climatic conditions that cause the accumulation or preservation of organic material may not be favourable under a future climate, resulting in the release of significant quantities of carbon to the atmosphere.11,39 Further research in this area is an urgent priority if we are to be able to predict impacts and feedbacks between climate change and the global carbon cycle.39,67

METHODOLOGIES FOR TRACKING CARBON FLOW BELOW GROUND TO MICROBIAL GROUPS

Despite the important role of soil microbes in the carbon cycle and the environmental implications of carbon cycle–climate change feedbacks, most carbon cycle models treat the soil microbial biomass as a black box.68 The majority of models calculate soil respiration using first-order kinetics, where decomposition (and CO₂ flux) is proportional to the size of the carbon pool defined by an empirically derived decomposition rate constant that captures the net effect of microbial activity under specific conditions.50,69 Pools typically represent ‘slow’ and ‘passive’ organic matter fractions; and models differ in terms of the number of conceptual pools used (one to nine) and addition of rate modifiers that account for changes in temperature, moisture, etc. (see e.g. FreiDingstein et al.55). Recent studies have called for modifications to these traditional ‘black box’ SOM models to include more explicit representations of microbial community and functions that control decomposition. However, much of this detail is absent because the basic processes are still poorly understood.11

In empirical microbial ecology we are now beginning to be able to unpack the microbial biomass ‘black box’ and to identify which microbial groups are responsible for the turnover of plant-derived inputs to soil. The methods available to do this generally involve the combination of either stable (SIP) or radioactive (RIP) isotope probing and molecular ecology techniques (for a thorough review on methodologies for linking microbial identity to environmental processes, see Gutierrez-Zamora and Manefield90). In SIP or RIP the stable or radioactive isotope (13C or 14C respectively) is used to track the microbial fate of a labelled carbon source (or sources). As a result of anabolic processes, the carbon label becomes incorporated into the biomolecules of those microbes actively decomposing the carbon source of interest. In nucleic acid-SIP the resulting ‘heavier’ 13C DNA or RNA is fractionated by isopycnic density gradient ultracentrifugation from unlabelled nucleic acids and used as a template for downstream analysis. When 14C is used as the label, RIP can be detected with microautoradiography (MAR) and combined with fluorescence in situ hybridisation (FISH), termed FISH-MAR, to enable allocation of carbon utilisation to specific microbial ribotypes with the use of fluorescent rRNA-targeted oligonucleotide probes. The use of an autoradiographic emulsion causes silver grains to form in areas immediately adjacent to radioactive cells (proof of 14C substrate incorporation into the microbial biomass), whereas cell fluorescence after exposure of slides to FISH probes under hybridisation conditions is used for the phylogenetic identification.

Nucleic acid-SIP was first applied over a decade ago, and the majority of studies have focused on non-plant systems. Methanol-utilising microorganisms in soil were studied first with DNA-SIP,71 and subsequent studies have built on this work and used DNA-SIP to link function and identity with respect to the cycling of methane6,7,72,73 and the biodegradation of organic pollutants.74–77

Phenol-utilising microorganisms in wastewater treatment plants were first studied with rRNA-SIP.78 Since then, RNA-SIP has successfully been used in numerous studies to track the carbon utilisation in specific microbial groups in diverse ecosystems (e.g. rivers, tidal flats, aquifers, groundwater) with respect to carbon biogeochemical processes such as methanotrophy,9,72,79 degradation of xenobiotics40–84 and other ecosystem functioning.79,84,85

The application of nucleic acid-SIP to trace, in situ, the microbial fate of rhizodeposit carbon in soil involves the growth of plants in a 13C-CO₂ atmosphere to promote 13C labelling of photosynthate and therefore rhizodeposition. For successful nucleic acid-SIP, a high proportion of 13C-labelled substrate incorporation is required in order to achieve sufficient separation of ‘heavy’ and ‘light’ nucleic acids. With the exception of the use of DNA-SIP to study endophytes that are, by definition, in intimate association with plant root systems,86 this level of labelling is sometimes difficult to achieve when tracking the microbial fate of plant-derived carbon in the rhizosphere because of dilution with plant 12C and the native 12C SOM.90 This sensitivity problem was demonstrated in a study88 where, although the labelling of bacterial nucleic acids with 13C following 13CO₂ incubation of grassland turf occurred, the amount of labelling was indeed too low, preventing the separation of 13C- from 12C-nucleic acids (for reviews on methodological considerations, see Manefield et al.89 and Neufeld et al.90). As RNA is turned over independently of cell replication with a high copy number within the microbial cell, rRNA-SIP has a greater sensitivity than DNA-SIP (reviewed in Whiteley et al.90) and has therefore been used more successfully to directly track plant-derived carbon to microorganisms in the rhizosphere.5,26,29,91–93 The promise of mRNA-SIP has also been recently explored for understanding the links between root exudation and bacterial gene expression in the rhizosphere.94 The SIP approach has been complemented and extended to track plant-derived carbon into biochemical markers other than nucleic acids, such as proteins (protein-SIP,95 for reviews, see Seifert et al.96 and von Bergen et al.97) and phospholipid fatty acids (PLFA-SIP).98–100 Drigo et al.,101 Hannula et al.92 and Dias et al. (2013)101 have recently combined RNA-SIP, neutral lipid fatty acid (NLFA)-SIP...
with neutral (NLFA) and phospholipid (PLFA) lipid fatty acids biomarker analyses and/or PLFA-SIP with real-time polymerase chain reaction (PCR) and community fingerprinting techniques to examine how elevated CO₂ or plant genetic modification alters the destination of photosynthetically fixed carbon with respect to its utilisation by AMF and mycorrhizosphere bacterial and fungal species.

FISH-MAR was first demonstrated in 1999 by two separate research groups that managed to visualise the incorporation of 13C-labelled substrates in probe-detected bacteria under the microscope.102–103 Since then, it has been used mainly to study in situ physiology of bacteria in biofilms104 and activated sewage sludge with enhanced biological phosphorus removal.105–107 Although FISH-MAR has greater sensitivity than DNA- or RNA-SIP, as detection of substrate incorporation is not restricted to analysis of a specific biomolecule (in contrast to nucleic acid-SIP), it is limited, firstly because the microbial groups to be targeted need to be known (and therefore selected with the use of appropriate molecular probes) in advance and secondly owing to the fact that its application is restricted to either single or small clusters of cells (reviewed in Wagner et al.108). Limitations on the number of different fluorophores that can be detected simultaneously also restrict the number of microbial groups that can be targeted at the same time in FISH-MAR.109 However, the development of radioactively labelled RNA-targeted isotope arrays to study multiple microbial populations for their ability to consume a radioactive substrate in activated sludge samples has given promising results.110 The isotope array concept has recently been expanded in isotope rRNA-targeted oligonucleotide microarrays (PhyloChips), containing a much larger number of probes, to reveal substrate consumption profiles of Rhodocyclaceae spp. in activated sludge.111 Isotope arrays have, to our knowledge, not yet been used in soil-based studies.

Returning to in situ hybridisation-based techniques, FISH-MAR has been used in combination with catalysted reporter deposition112 to improve signal detection in oligotrophic prokaryotes, and quantitative (Q)-FISH-MAR104 has been developed to quantify cell-specific carbon uptake in probe-targeted bacterial groups. In addition, the combination of FISH with other methodologies has given birth to further hyphenated techniques such as FISH-SIMS113,114 (the combination of FISH with secondary ion mass spectrometry) and FISH-RAMAN115 (the combination of FISH with Raman microspectroscopy). Both SIMS and Raman microspectroscopy can be applied to characterise cellular incorporation of 13C-labelled substrates, negating the requirement for experiments using 13C-12C pulse chase and attendant safety concerns with respect to use of radioactivity. In FISH-SIMS the FISH-probed identification of microbial cells is coupled to SIMS, which determines the isotopic composition/incorporation of the targeted cells after a caesium ion beam is directed on their surface. In FISH-RAMAN the FISH identification is coupled to highly resolved Raman confocal spectra, and cells that are 13C-labelled through anabolic incorporation of the isotope exhibit key ‘red-shifted’ spectral peaks highly correlated with their 13C content. The FISH-RAMAN method was initially used in naphthalene-degrading groundwater samples115 and can be quantitative with appropriate calibration. The same authors expanded their research by combining RNA-SIP and FISH-RAMAN to fully explore naphthalene degradation of the polluted groundwater.116 Although not yet applied to the root zone, to our knowledge, both FISH-SIMS and FISH-RAMAN have considerable promise for use in rhizosphere carbon flow tracking experiments.

**POTENTIAL FOR MANIPULATING CARBON DYNAMICS IN AGRICULTURAL SYSTEMS**

The intensive cultivation of soils under agriculture results in the loss of soil carbon due to (i) the acceleration of decomposition through improved aeration and the exposure of physically protected organic matter as a result of tillage and drainage and (ii) the reduction of primary production inputs to soil through the removal of plant biomass during harvest. As already mentioned, it is estimated that somewhere in the range of 42–78 Gt of carbon17 that was historically stored in the soil system has been lost as a result of the intensive cultivation of soils, and the capacity for agricultural soils to regain this lost carbon is currently being discussed as one potential contribution to atmospheric carbon remediation and mitigation of climate change.17,116

The size of the C store in soil depends on the interactions between (i) the quantity and quality of primary production inputs and (ii) the fate of these inputs once they have entered the soil in the short and long term. Strategies with respect to the management of soil for C sequestration therefore involve increasing the quantity of primary production, and indeed other organic inputs into the system; this possibility has been widely debated with respect to breeding crop plants with more extensive root systems117 or altered physiological traits, cover- and inter-cropping, increasing the return of crop residues to soil118 and addition of amendments such as compost or biochar.119–121 However, the addition of crop residues and other amendments, while increasing soil organic C, does not usually transfer C additional to that already fixed from the atmosphere to land (depending on the alternative fate of the amendment) and therefore the end point of such practice does not necessarily qualify as ‘soil C sequestration’ under the strictest definitions of this term.118 A second strategy involves manipulating the fate of the inputs once added to the soil. On entering soil, inputs may (after extracellular depolymerisation in the case of macromolecular constituents) be taken up by the soil microbial biomass and the C partitioned for use in the production of biomass (subsequently necromass), excretions and secretions (e.g. extracellular enzymes) and respiration (Fig. 2). The aim of this second strategy is to encourage the processing of plant-derived C to biomass and metabolite precursors of soil organic matter or to secretions that promote the physical protection of C substrates against decomposition rather than to CO₂; in other words, to increase the C use efficiency of the microbial biomass potentially by manipulation of the quality of rhizodeposit inputs or edaphic environmental conditions that have a moderating effect on soil microbial physiology.

We know that climatic and abiotic soil factors (e.g. clay content) influence soil C cycling; however, the identity of soil microorganisms, as the primary decomposers of plant-derived C, is likely to significantly influence the fate of C inputs to soil. The extent to which climatic effects on soil C cycling are confounded with microbial adaptation to certain environmental niches is currently unknown. There is evidence (reviewed in Six et al.122) to suggest that the relative abundance of fungi and bacteria may be important, with more stable carbon being formed in soils with high fungal/bacterial biomass ratios. That fungi have a higher C use efficiency than bacteria and therefore form more biomass per unit of C utilised and also a biomass (subsequently necromass) of a more recalcitrant nature are the suggested mechanisms for the greater accumulation of fungal SOM, although both these mechanisms require further study.122 There have also been some studies...
(reviewed in Nielsen et al.123) that have reported relationships (positive and negative) between soil biodiversity and C cycling processes such as respiration, but these have generally focused on total species richness as the biodiversity measure and not the richness or identity of those species processing the carbon in situ.

At this stage it is not clear how the diversity and identity of those microorganisms using plant C influence the fate of that C. In addition, the edaphic abiotic factors controlling microbial C utilisation efficiency have not been thoroughly characterised.124 Ultimately it is not clear to what extent rhizosphere microbes within agricultural systems can be manipulated for C sequestration. If the community structure of plant C-utilising microbes is important for C fate, then the next step is to understand which are the most important groups that control soil storage with respect to expression of specific functions (e.g. metabolite production) and the proportion of the plant C inputs they are responsible for.

To do this, we need to be able to quantitatively apportion plant C to specific microbial groups in situ and to partition its use (i.e. for biomass/metabolite/CO₂ production) within that group.

We conclude that the methodologies outlined in this paper, although crucial in enabling the identification of plant-derived carbon-utilising microbes, lack the high-throughput ability to do this because of their reliance on extracted biomolecules (nucleic acid-SIP, protein-SIP, PLFA-SIP), precluding the ability to study the partitioning of carbon at the whole cell level, or because they are limited to the study of a small number of cells (FISH-SIMS, in situ quantification of biomass). To address this, it is necessary to develop tools that allow quantification of microbial use efficiency and designation of plant carbon (within phylogenetic groups and the metabolome) to enable a step-change level of understanding. The ultimate benefits from this investment will be the knowledge to inform manipulation of the plant–soil system to favour organisms or physiologies most important for promoting soil carbon storage across the diverse conditions present in the global agricultural land.

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REFERENCES

1 Falkowski PG, Fenchel T and Delong EF, The microbial engines that drive Earth's biogeochemical cycles. Science 320:1034–1039 (2008).
2 Prentice IC, Farquhar GD, Fasham MJR, Goulden ML, Heimann M, Jaramillo VJ, et al., The carbon cycle and atmospheric carbon dioxide, in Climate Change 2001. The Scientific Basis. Contribution of Working Group I to the Third Assessment Report of the Intergovernmental Panel on Climate Change, ed. by Houghton JT. Cambridge University Press, Cambridge, pp. 183–238 (2001).
3 Lu Y and Conrad R, In situ stable isotope probing of methanogenic archaea in the rice rhizosphere. Science 309:1088–1090 (2005).
4 Trumbore S, Carbon respiried by terrestrial ecosystems – recent progress and challenges. Global Change Biol 12:141–153 (2006).
5 Liang C and Balser TC, Microbial production of recalcitrant organic matter in global soils: implications for productivity and climate policy. Nat Rev Microbiol 9:75 (2011).
6 Lu Y, Lueders T, Friedrich MW and Conrad R, Detecting active methanogenic populations on rice roots using stable isotope probing. Environ Microbiol 7:326–336 (2005).
7 Chen Y, Dumont MG, Neufeld JD, Bodrossy L, Stralis-Pavevske N, McNamara NP, et al., Revealing the uncultivated majority: combining DNA stable-isotope probing, multiple displacement amplification and metagenomic analyses of uncultivated Methylocystis in acidic peatlands. Environ Microbiol 10:2609–2622 (2008).
8 Chistoserdova L, Vorholt JA and Lidstrom ME, A genomic view of methane oxidation from aerobic bacteria and anaerobic archaea. Genome Biol 6:208 (2005).
9 Qiu QF, Noll M, Abraham W-R, Lu YH and Conrad R, Applying stable isotope probing of phosphopridic fatty acids and RNA in a Chinese rice field to study activity and composition of the methanotrophic bacterial communities in situ. ISME J 2:602–614 (2008).
10 Gupta V, Smemo KA, Yavitt JB, Fowle D, Branfreun B and Basi-liko N, Stable isotopes reveal widespread anaerobic methane oxidation across latitude and peatland type. Environ Sci Technol 47:8273–8279 (2013).
11 Davidson EA and Janssens IA, Temperature sensitivity of soil carbon decomposition and feedbacks to climate change. Nature 440:165–173 (2006).
12 Schmidt MWI, Torn MS, Abiven S, Dittmar T, Gugenberger G, Janssens IA, et al., Persistence of soil organic matter as an ecosystem property. Nature 478:49–56 (2011).
13 Tarnocai C, Canadell JG, Schuur EAG, Kuhry P, Mazhitova G and Zimov S, Soil organic carbon pools in the northern circumpolar permafrost region. Global Biogeochem Cycles 23:GB2023 (2009).
14 Jobbágy EG and Jackson RB, The vertical distribution of soil organic carbon and its relation to climate and vegetation. Ecol Appl 10:423–436 (2000).
15 Sylvia DM, Fuhrmann J, Hartel PG and Zuberer DA (eds), Principles and Applications of Soil Microbiology. Pearson Education, Upper Saddle River, NJ (2005).
16 Le Quere C, Raupach MR, Canadell JG, Marland G, Bopp L, Ciais P, et al., Trends in the sources and sinks of carbon dioxide. Nature Geosci 2:831–836 (2009).
17 Lal R, Soil carbon sequestration impacts on global climate change and food security. Science 304:1623–1627 (2004).
18 Bais HP, Weir TL, Perry LG, Gilroy S and Vivanco JM, The role of root exudates in rhizosphere interactions with plants and other organisms. Annu Rev Plant Biol 57:233–266 (2006).
19 Muller S, Vandermeer W, Schildknecht H and Visser JH, An automated-system for large-scale recovery of germination stimulants and other root exudates. Weed Sci 41:138–143 (1993).
20 Kowalzki-Knabner I, The macromolecular organic composition of plant and microbial residues as inputs to soil organic matter. Soil Biol Biochem 34:139–162 (2002).
21 Wallenstein MD and Weintraub MN, Emerging tools for measuring and modeling the in situ activity of soil extracellular enzymes. Soil Biol Biochem 40:2098–2106 (2008).
22 Smith SE and Read DJ, The Mycorrhizal Symbiosis. Academic Press, San Diego, CA (2008).
23 Johnson D, Leake JR and Read DJ, Transfer of recent photosynthate and modeling the in situ activity of mycorrhizal mycelium of an upland grassland: short-term respiratory losses and accumulation of 13C. Soil Biol Biochem 34:1521–1524 (2002).
24 Nakano-Hylander A and Olsson PA, Carbon allocation in mycelial of arbuscular mycorrhizal fungi during colonisation of plant seedlings. Soil Biol Biochem 39:1450–1458 (2007).
25 Gavito ME and Olsson PA, Allocation of plant carbon to foraging and storage in arbuscular mycorrhizal fungi. FEMS Microbiol Ecol 45:181–187 (2003).
26 Drigo B, Pijl AS, Duys H, Kisiel AK, Camper HA, Dronkamp M, et al., Shifting carbon flow from roots into associated microbial communities in response to elevated atmospheric CO₂. Proc Natl Acad Sci 107:10938–10942 (2010).
27 Jones DL, Hodge A and Kuziyak Y, Plant and mycorrhizal regulation of rhizodeposition. New Phytol 163:459–480 (2004).
28 Talbot JM, Allison SD and Treseder KK, Decomposers in disguise: mycorrhizal fungi as regulators of soil C dynamics in ecosystems under global change. Funct Ecol 22:953–963 (2008).
29 Van-denkoomenhouye S, Mahé S, Ineson P, Staddon P, Ostle N, Cliquet J-B, et al., Active root-inhabiting microbes identified by rapid incorporation of plant-derived carbon into RNA. Proc Natl Acad Sci USA 107:16970–16975 (2010).
30 Dungait JAJ, Hopkins DW, Gregory AS and Whitmore AP, Soil organic matter turnover is governed by accessibility not recalcitrance. Global Change Biol 18:1781–1796 (2012).

J Sci Food Agric (2014) © 2014 The Authors. wileyonlinelibrary.com/jsfa
31 Wolf DC and Wagner GH, Carbon transformations and soil organic matter formation, in Principles and Applications of Soil Microbiology, ed. by Sylvia DM, Fuhrmann JJ, Hartel PG and Zuberer DA. Pearson Education, Upper Saddle River, NJ, pp. 285 – 332 (2005).
32 Kleber M and Johnson MG, Advances in understanding the molecular structure of soil organic matter: implications for interactions in the carbon cycle. Adv Agron 106:77 – 142 (2010).
33 Kindler R, Mittner A, Thullner M, Richnow H-H and Kätner M, Fate of bacterial biomass derived fatty acids in soil and their contribution to soil organic matter. Org Geochem 40:29 – 37 (2009).
34 Tisdall JM, Possible role of soil microorganisms in aggregation in soils. Plant Soil 159:115 – 121 (1994).
35 Six J, Bossuyt H, Degryze S and van Cleef K, A history of research on the link between (micro)aggregates, soil biota, and soil organic matter dynamics. Soil Tillage Res 79:3 – 31 (2004).
36 Miller RM and Jastrow JD, Mycorrhizal fungi influence soil structure, in Arbuscular Mycorrhizas: Physiology and Function, ed by Kapulnik Y and Douds Jr D. Springer, Kluwer, The Netherlands, pp. 3 – 18 (2000).
37 Rillig MC and Mummey DL, Mycorrhizas and soil structure. New Phytol 171:41 – 53 (2006).
38 Canadell DG, Le Quere C, Rasing MR, Field CB, Buitenhuis ET, Ciais P, et.al., Contributions to accelerating atmospheric CO2 growth from economic activity, carbon intensity, and efficiency of natural sinks. Proc Natl Acad Sci 104:18866 – 18870 (2007).
39 Smith P and Fang C, Carbon cycle: a warm response by soils. Nature 464:499 – 500 (2010).
40 Canadell DG, Kirschbaum MUF, Kurz WA, Sanz MJ, Schlрамnder B and Yamagata Y, Factoring out natural and indirect human effects on terrestrial carbon sources and sinks. Environ Sci Pol 10:370 – 384 (2007).
41 Jin VL and Evans RD, Elevated CO2 increases microbial carbon substrate use and nitrogen cycling in Mojave Desert soils. Global Change Biol 13:452 – 465 (2007).
42 Bardgett RD, Freeman C and Ostle NJ, Microbial contributions to climate change through carbon cycle feedbacks. ISME J 2:805 – 814 (2008).
43 Singh BK, Bardgett RD, Smith P and Reay DS, Microorganisms and climate change: terrestrial feedbacks and mitigation options. Nat Rev Microbiol 8:779 – 790 (2010).
44 Katterer T, Reichstein M, Andreae O and Lomander A, Temperature dependence of organic matter decomposition: a critical review using literature data analyzed with different models. Biol Fertil Soils 27:258 – 262 (1998).
45 Cox PM, Betts RA, Jones CD, Spall SA and Totterdell IJ, Acceleration of global warming due to carbon-cycle feedbacks in a coupled climate model. Nature 408:184 – 187 (2000).
46 Knorr W, Prentice IC and House JI, Long-term sensitivity of soil carbon to warming. Nature 433:298 – 301 (2005).
47 Lopez-Gutierrez JC, Malcolm GM, Koide RT and Eissenstat DM, Cytokinetic responses of photosynthesis, canopy properties and plant production to rising CO2, New Phytol 165:351 – 372 (2005).
48 Heath J, Ayres E, Possell M, Bardgett RD, Black H, Grant H, et al., Rising atmospheric CO2 reduces sequestration of root-derived carbon. Science 309:1711 – 1713 (2005).
49 Blagodatskaya E and Vymazal J, Mechanisms of real and apparent priming effects and their dependence on soil microbial biomass and community structure: critical review. Biol Fertil Soils 45:115 – 131 (2008).
50 Fontaine S, Bardoux G, Abbadie L and Mariotti A, Carbon input to soil may decrease soil carbon content. Ecol Lett 7:314 – 320 (2004).
51 Fontaine S and Barot S, Size and functional diversity of microbial populations control plant persistence and long-term soil carbon accumulation. Ecol Lett 8:1075 – 1087 (2005).
52 Langley JA, McKeel KL, Cahoon DR, Cherry JA and Megonigal JP, Elevated CO2 stimulates marsh elevation gain, counterbalancing sea-level rise. Proc Natl Acad Sci 106:6186 – 6186 (2009).
53 Schleppi P, Bucher-Wallin I, Hagedorn F and Körner C, Increased nitrate availability in the soil of a mixed mature temperate forest subjected to elevated CO2 concentration (canopy FACE). Global Change Biol 18:757 – 768 (2012).
54 Zhu B and Cheng W, Rhizosphere priming effect increases the temperature sensitivity of soil organic matter decomposition. Global Change Biol 17:2172 – 2183 (2011).
55 Langley JA and Megonigal JP, Ecosystem response to elevated CO2 levels limited by nitrogen-induced plant species shift. Nature 466:96 – 99 (2010).
56 Denman KL, Brasseur G, Chidthaisong A, Ciais P, Cox PM, et al., Coupling between changes in the climate system and biogeochemistry in Climate Change 2007: The Physical Science Basis. Contribution of Working Group I to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change, ed. by Solomon S, Qin D, Manning M, Chen Z, Marquis M, Averet KB, et al. Cambridge University Press, Cambridge, pp. 499 – 588 (2007).
57 Schimel J, Balser TC and Wallenstein M, Microbial stress-response physiology and its implications for ecosystem function. Ecology 88:1386 – 1394 (2007).
58 King GM, Enhancing soil carbon storage for carbon remediation: potential contributions and constraints by microbes. Trends Microbiol 19:75 – 84 (2011).
59 Ostle NJ, Smith P, Fischer R, Woodward FI, Fisher JB, Smith JU, et al., Integrating plant – soil interactions into global carbon cycle models. J Ecol 97:851 – 863 (2009).
60 Smith P, Fang CM, Dawson JJC and Moncrieff JB, Impact of global warming on soil organic carbon. Adv Agron 97:1 – 43 (2008).
61 Gutierrez-Zamora M-L and Manefield M, An appraisal of methods for linking environmental processes to specific microbial taxa. Rev Envir Sci Biotechnol 9:153 – 185 (2010).
62 Radajewski S, Ineson P, Parekh NR and Murrell JC, Stable-isotope probing as a tool in microbial ecology. Nature 403:646 – 649 (2000).
63 Hery M, Singer AC, Kumarasen D, Bodrosny L, Stratis-Pavese N, Prosser JI, et al., Effect of earthworms on the community structure of active methanotrophic bacteria in a landfill cover soil. ISME J 2:92 – 104 (2008).
64 Qiu Q, Conrad R and Lu Y, Cross-feeding of methane carbon among bacteria on rice roots revealed by DNA-stable isotope probing. Environ Microbiol Rep 1:355 – 361 (2009).
65 Aitken MD, Jones MD, Singleton DR, Carstensen DP, Powell SN, Swannson JS, et al., Effect of incubation conditions on the enrichment of pyrene-degrading bacteria identified by stable-isotope probing in an aged, PAH-contaminated soil. Microb Ecol 56:341 – 349 (2008).
66 Cupples AM, Luo CL, Xie SG, Sun WM and Li XD, Identification of a novel toluene-degrading bacterium from the candidate phylum TM7, as determined by DNA stable isotope probing. Appl Environ Microbiol 75:4644 – 4647 (2009).
67 Jones MD, Singleton DR, Swannson W and Aitken MD, Multiple DNA extractions coupled with stable-isotope probing of anthracene-degrading bacteria in contaminated soil. Appl Environ Microbiol 77:2984 – 2991 (2011).
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77 Ulrich-O, Jecna K, Mackova M, Vlcek C, Hroudova M, Demmerova K, et al. Biphenyl-Metabolizing Bacteria in the Rhizosphere of Horseradish and Bulk Soil Contaminated by Polychlorinated Biphenyls as Revealed by Stable Isotope Probing. Appl Environ Microbiol. 2009 October 15, 75:6471 – 6477 (2009).

78 Manefeld M, Whiteley AS, Griffiths RI and Bailey MJ, RNA stable isotope probing, a novel means of linking microbial community function to phylogeny, Appl Environ Microbiol 68:5367 – 5373 (2002).

79 Noll M, Frenzel P and Conrad R, Selective stimulation of type I methanotrophs in a rice paddy soil by urea fertilization revealed by RNA-based stable isotope probing. FEMS Microbiol Ecol 65:125 – 132 (2008).

80 Kittelmann S and Friedheim MW, Identification of novel perchloroethene-respiring microorganisms in anoxic river sediment by RNA-based stable isotope probing. Environ Microbiol 10:31 – 46 (2008).

81 Kittelmann S and Friedheim MW, Novel uncultured Chloroflexi dechlorinate perchloroethene to trans-dichloroethene in tidal flat sediments. Environ Microbiol 10:1557 – 1570 (2008).

82 Aburto A and Ball AS, Bacterial population dynamics and separation of active degraders by stable isotope probing during benzene degradation in a BTX-impacted aquifer. Rev Int Contaminación Ambiental 25:147 – 156 (2009).

83 Huang WE, Ferguson A, Singer AC, Lawson K, Thompson IP, Kalin RM, et al., Resolving genetic functions within microbial populations: in situ analyses using rRNA and mRNA stable isotope probing coupled with single-cell Raman-fluorescence in situ hybridization. Appl Environ Microbiol 75:234 – 241 (2009).

84 Langenheder S and Prosser JI, Resource availability influences the diversity of a functional group of heterotrophic soil bacteria. Environ Microbiol 10:2245 – 2256 (2008).

85 Drake HL, Degelmann DM, Kolb S, Dumont M and Murrell JC, Enterobacteriaceae facilitate the anaerobic degradation of glucose by a forest soil. FEMS Microbiol Ecol 68:312 – 319 (2009).

86 Rask S, Fiedler T, Schlotter M, Schauer S, Buegger F, Gattinger A, et al., DNA-based stable isotope probing enables the identification of active bacterial endophytes in potatoes. New Phytol 181:802 – 807 (2009).

87 Manefeld M, Whiteley AS, Ostle N, Ineson P and Bailey MJ, Technical considerations for RNA-based stable isotope probing: an approach to associating microbial diversity with microbial community function. Rapid Commun Mass Spectrom 16:2179 – 2183 (2002).

88 Griffiths RI, Manefeld M, Ostle N, McNamara N, O’Donnell AG, Bailey MJ, et al., 13CO2 pulse labelling of plants in tandem with stable isotope probing: methodological considerations for examining microbial function in the rhizosphere. J Microbiol Meth 58:119 – 129 (2004).

89 Neufeld JD, Dumont MG, Vohra J and Murrell JC, Methodological considerations for the use of stable isotope probing in microbial ecology. Microb Ecol 53:435 – 442 (2007).

90 Whiteley AS, Manefeld M and Luersd T, Unlocking the ‘microbial black box’ using RNA-based stable isotope probing technologies. Curr Opin Biotechnol 17:67 – 71 (2006).

91 Rangel-Castro JI, Kilham K, Ostle N, Nicol GW, Anderson IC, Scrimgeour CM, et al., Stable isotope probing analysis of the influence of liming on root exudate utilization by soil microorganisms. Environ Microbiol 7:828 – 838 (2005).

92 Hannula SE, Boschker HTS, de Boer W and van Veen JA, 13C pulse-labelling assessment of the community structure of active fungi in the rhizosphere of a genetically starch-modified potato (Solanum tuberosum) cultivar and its parental isolate. New Phytol 194:784 – 799 (2012).

93 Lu Y, Bosencourt D, Liesack W and Conrad R, Structure and activity of bacterial community inhabiting rice roots and the rhizosphere. Environ Microbiol 8:1351 – 1360 (2006).

94 Haichar FZ, Roncato MA and Achoaoua W, Stable isotope probing of bacterial community structure and gene expression in the rhizosphere of Arabidopsis thaliana. FEMS Microbiol Ecol 81:291 – 302 (2012).

95 Jehmlíček N, Schmidt F, Hartwich M, von Bergen M, Richnow HH and Vogt C, Incorporation of carbon and nitrogen atoms into proteins measured by protein-based stable isotope probing (Protein-SIP). Rapid Commun Mass Spectrom 22:2889 – 2897 (2008).

96 Seifert J, Taubert M, Jehmlíček N, Schmidt F, Völker U, Vogt C, et al., Protein-based stable isotope probing (protein-SIP) in functional metaproteomics. Mass Spectrom Rev 31:683 – 697 (2012).

97 Von Bergen M, Jehmlíček N, Taubert M, Vogt C, Bastida F, Herbst F-A, et al., Insights from quantitative metaproteomics and protein-stable isotope probing into microbial ecology. ISME J 7:1877 – 1885 (2013).

98 Treonis AM, Ostle NJ, Stott AW, Primrose R, Grayston SJ and Ineson P, Identification of groups of metabolically active rhizosphere microorganisms by stable isotope probing of PLFAs. Soil Biol Biochem 36:533 – 537 (2004).

99 Paterson E, Gubbing T, Abel C, Sim A and Telfer G, Rhizodeposition shapes rhizosphere microbial community structure in organic soil. New Phytol 173:600 – 610 (2016).

100 Shrestha M, Abraham W-R, Shrestha PM, Noll M and Conrad R, Activity and composition of methanotrophic bacterial communities in planted rice soil studied by flux measurements, analyses of pmoA gene and stable isotope probing of phospholipid fatty acids. Environ Microbiol 10:400 – 412 (2008).

101 Dias ACF, Din-IAndreote F, Hannel SE, Andreote FD, Pereira e Silva MdC, Salles JF, de Boer W, van Veen J and van Elsas JD, Different Selective Effects on Rhizosphere Bacteria Exerted by Genetically Modified versus Conventional Potato Lines. PLoS ONE 8:e67948 (2013).

102 Lee N, Nielsen PH, Andreassen KH, Juretischko S, Nielsen JL, Schleifer KH, et al., Combination of fluorescent in situ hybridization and microautoradiography – a new tool for structure–function analysis in microbial ecology. Appl Environ Microbiol 65:1289 – 1297 (1999).

103 Ouvrery MP, M. and Fuhrman JA, Combined microautoradiography – 16S rRNA probe technique for determination of radioisotope uptake by specific microbial cell types in situ. Appl Environ Microbiol 65:1746 – 1752 (1999).

104 Nielsen JL, Christensen D, Klokpenborg M and Nielsen PH, Quantification of cell-specific substrate uptake by probe-defined bacteria under in situ conditions by microautoradiography and fluorescence in situ hybridization. Environ Microbiol 5:202 – 211 (2003).

105 Thomsen BR, Kong Y and Nielsen PH, Ecophysiology of abundant denitrifying bacteria in activated sludge. FEMS Microbiol Ecol 60:370 – 382 (2007).

106 Kong Y, Nielsen JL and Nielsen PH, Microautoradiographic study of Rhodococcus-related polysphosphate-accumulating bacteria in full-scale enhanced biological phosphorus removal plants. Appl Environ Microbiol 70:5383 – 5390 (2004).

107 Nguyen HT, Le VQ, Hansen AA, Nielsen JL and Nielsen PH, High diversity and abundance of putative polysphosphate-accumulating Tetrasphaera-related bacteria in activated sludge systems. FEMS Microbiol Ecol 76:256 – 267 (2011).

108 Wagner M, Nielsen PH, Loy A, Nielsen JL and Daims H, Linking microbial community structure with function: fluorescence in situ hybridization – microautoradiography and isotope arrays. Curr Opin Biotechnol 17:83 – 91 (2006).

109 Aman R, Snaid J, Wagner M, Ludwig W and Schleifer KH, In situ visualization of high genetic diversity in a natural microbial community. J Bacteriol 178:3496 – 3500 (1996).

110 Adamczyk J, Hesselsoe M, Iversen N, Hor M, Lehrer A, Nielsen PH, et al., The isotope array, a new tool that employs substrate-mediated labeling of RNA for determination of microbial community structure and function. Appl Environ Microbiol 69:6875 – 6887 (2003).

111 Hesselsoe M, Fureder S, Schlotter M, Bodrossy L, Iversen N, Roslev P, et al., Isotope array analysis of Rhodococcus uncovers functional redundancy and versatility in an activated sludge. ISME J 3:1349 – 1364 (2009).

112 Teira E, Reinthaler T, Perretal A, Perretal J and Hernd GJ, Combining catalyzed reporter deposition–fluorescence in situ hybridization and microautoradiography to detect substrate utilization by bacteria and archaea in the deep ocean. Appl Environ Microbiol 70:4111 – 4114 (2004).

113 Orphan VJ, House CH, Hinrichs KU, McKeegan KD and DeLong EF, Methane-consuming archaea revealed by directly coupled isotopic and physicochemical analyses. Science 293:484 – 487 (2001).

114 Orphan VJ, House CH, Hinrichs KU, McKeegan KD and DeLong EF, Multiple archaeal groups mediate methane oxidation in anoxic cold seep sediments. Proc Natl Acad Sci 99:7663 – 7668 (2002).
115 Huang WE, Stoeker K, Griffiths R, Newbold L, Daims H, Whiteley AS, et al., Raman-FISH: combining stable-isotope Raman spectroscopy and fluorescence in situ hybridization for the single cell analysis of identity and function. *Environ Microbiol* **9**:1878–1889 (2007).

116 Smith P and Olesen JE, Synergies between the mitigation of, and adaptation to, climate change in agriculture. *J Agric Sci* **148**:543–552 (2010).

117 Kell DB, Breeding crop plants with deep roots: their role in sustainable carbon, nutrient and water sequestration. *Ann Bot* **108**:407–418 (2011).

118 Powlson DS, Whitmore AP and Goulding KWT, Soil carbon sequestration to mitigate climate change: a critical re-examination to identify the true and the false. *Eur J Soil Sci* **62**:42–55 (2011).

119 Sohi SP, Krull E, Lopez-Capel E and Bol R, A review of biochar and its use and function in soil. *Adv Agron* **105**:47–82 (2010).

120 Lal R, Soil carbon sequestration to mitigate climate change. *Geoderma* **123**:1–22 (2004).

121 Smith P, Carbon sequestration in croplands: the potential in Europe and the global context. *Eur J Agron* **20**:229–236 (2004).

122 Six J, Frey SO, Thiet RK and Batten KM, Bacterial and fungal contributions to carbon sequestration in agroecosystems. *Soil Sci Soc Am J* **70**:555–569 (2006).

123 Nielsen UN, Ayres E, Wall DH and Bardgett RD, Soil biodiversity and carbon cycling: a review and synthesis of studies examining diversity–function relationships. *Eur J Soil Sci* **62**:105–116 (2011).

124 Cotrufo MF, Wallenstein MD, Boot CM, Denef K and Paul E, The Microbial Efficiency-Matrix Stabilization (MEMS) framework integrates plant litter decomposition with soil organic matter stabilization: do labile plant inputs form stable soil organic matter? *Global Change Biol* **19**:988–995 (2013).

125 Prosser JI, Microorganisms cycling soil nutrients and their diversity, in *Modern Soil Microbiology*, ed. by Van Elsas JD, Jansson JK and Trevors JT. CRC Press, New York, NY, pp. 237–261 (2007).

126 Solomon S, Qin D, Manning M, Chen Z, Marquis M, Averyt K, et al. (eds), *Climate Change 2007. The Physical Science Basis. Contribution of Working Group I to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change*. Cambridge University Press, Cambridge (2007).