

# Role of Soil Microorganisms in Carbon Sequestration

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Human activities, especially the burning of fossil fuels such as coal, oil, and gas, have caused a substantial increase in the concentration of carbon dioxide (CO<sub>2</sub>) in the atmosphere. This increase in atmospheric CO<sub>2</sub>—from about 280 to more than 380 parts per million (ppm) over the last 250 years—is causing measurable global warming (Sundquist et al., 2008). Potential adverse impacts include sea-level rise; increased frequency and intensity of wildfires, floods, droughts, and tropical storms; changes in the amount, timing, and distribution of rain, snow, and runoff; and disturbance of coastal marine and other ecosystems. There is an urgent need of scientific and economically viable strategies to mitigate the consequences of adverse effect of increased atmospheric CO<sub>2</sub>.

Carbon is the major building block for life on earth and found in all living organisms. In the environment, carbon exists in many forms – predominately as plant biomass, soil organic matter, geologic deposits, and as the CO<sub>2</sub> in the atmosphere and dissolved in water bodies (mainly oceans). Carbon sequestration is the long-term storage of carbon in oceans, soils, vegetation (especially forests), and geologic formations. High levels of fossil fuel combustion and deforestation have transformed large pools of carbon from fossils (oil and coal deposits) and forests into atmospheric carbon dioxide. Carbon sequestration and reductions in greenhouse gas emissions can occur through a variety of agriculture practices. Along with anthropogenically enhanced fluxes of other greenhouse gases (e.g. CH<sub>4</sub> and NO<sub>x</sub>), these changes have altered climate on a global scale, possibly moving Earth towards a ‘tipping point’. Although the oceans will ultimately absorb much of the carbon mobilized by humans, the century to millennial time scales involved are too slow to prevent potentially dramatic changes (Schlesinger, 2003).

Soils play significant roles in global carbon cycle. It was estimated that soils have contributed as much as 55 to 878 billion tons (GT) of carbon to the total atmospheric CO<sub>2</sub> (Kimble et al. 2002). The total soil carbon consists of the soil organic and inorganic carbon, estimated to be approximately over 2250 GT in the top 1 meter depth (Batjes, 1996).

## What is soil carbon sequestration?

Soil carbon sequestration is the process of transferring carbon dioxide from the atmosphere into the soil through crop residues and other organic solids, in a form that is not immediately reemitted. This transfer or “sequestering” of carbon helps off-set emissions from fossil fuel combustion and other carbon-emitting activities while enhancing soil quality and long-term agronomic productivity. Soil carbon sequestration can be accomplished by management systems that add high amounts of biomass to the soil, cause minimal soil disturbance, conserve soil and water, improve soil structure, and enhance soil fauna activity. Continuous no-till crop production is a prime example.

## How is carbon sequestered in soils?

Through the process of photosynthesis, plants assimilate carbon and return some of it to the atmosphere through respiration. The carbon that remains as plant tissue is then consumed by animals or added to the soil as litter. Primarily the carbon is stored in the soil as soil organic matter (SOM). SOM is a complex mixture of carbon compounds, consisting of decomposing plant and animal tissue, microbes (protozoa, fungi, and bacteria), and **humus – carbon associated with soil minerals** (Fig. 1). Carbon can either remain stored

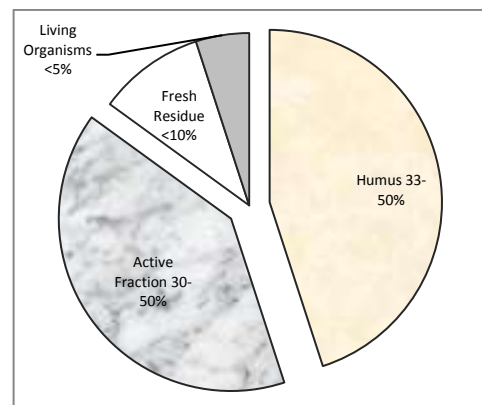


Fig. 1. Soil organic matter components  
Adapted from the Ecological Society of America,  
Ecoed.net, “Carbon Sequestration”  
<http://esa.sdsc.edu>

in soils for millennia, or it can be quickly released back into the atmosphere through respiration by soil microbes. Climatic conditions, natural vegetation, soil texture, drainage, and land use all affect the amount and length of time carbon is stored in soil.

Multiple options for reducing greenhouse gas impacts have been proposed. Some, including those known as geo-engineering, would sequester carbon in the geosphere, oceans and biosphere (Lal, 2008). These can be subdivided into engineered or abiological (deep ocean injection, oil well injection, coal bed etc.) and managed biological approaches (Biofuel production, biologically enhanced carbonate formation, dolomite precipitation, sequestration in biomass and soil organic matter, etc.), each of which has distinct advantages and disadvantages (Lal, 2008; King, 2011).

Both marine and terrestrial ecosystems have been proposed for managed carbon sequestration. Organic carbon production and storage has dominated the research agenda for

**Box 1. Biologically enhanced carbonate formation to sequester carbon**

Several options for sequestering carbon that involve or exploit various properties of bacteria have been proposed. In addition to manipulating bacterial activity to increase soil carbon storage, these include bioengineering bacteria to trap CO<sub>2</sub> using novel complexing agents and manipulating microbial activity in certain hypersaline environments to enhance dolomite (a calcium–magnesium carbonate) formation. Tossel (2009) has speculated that small macrocyclic carbonate receptors similar to those described by Brooks et al. (2006) might be developed for the membranes of living organisms.

Assuming as an example a receptor size of 1 nm and a spherical bacterial cell 0.5 μm in diameter with a volume of  $5.24 \times 10^{-13} \text{ cm}^3$ , a total of 1570 receptors occupying 0.16% of the cell surface would support carbonate binding at 50% of the rate of a 100% sorptive cell surface. If they passively accumulated carbonate at a level of 10 mM,  $1.59 \times 10^{31}$  bacterial cells would be required to sequester the equivalent of 1 Gt of carbon, which is approximately 12% of global annual anthropogenic CO<sub>2</sub> emissions. This cell number exceeds by approximately 50-fold the total number of aquatic and terrestrial bacteria estimated by Whitman et al. (1998), raising serious doubts about the approach.

A very much smaller population of cells might be feasible, however, if carbonate sequestration were coupled efficiently to mineral formation (e.g. calcium carbonates or dolomite) under conditions that required minimal energy input and limited cell turnover. Bioengineering a carbonate sequestration system and operating it at a suitable scale for decadal time periods clearly represent a substantial challenge but merits additional crucial analysis.

these systems, but biologically enhanced carbonate deposition could offer an alternative approach suitable for some situations (Box 1). Manipulation of the marine biosphere has been highly controversial and is not strongly supported (Glibert et al., 2008). By contrast, terrestrial biospheric carbon sequestration might add as much as 100 Pg C (1 Pg = 10<sup>15</sup> g) to existing soil carbon stocks (Houghton, 2007), although the amounts would vary considerably on local to regional scales (Canadell et al., 2007). The 100 Pg C represents only approximately 12 years of current gross total anthropogenic CO<sub>2</sub> emissions (approx. 8.6 Pg C annually) (Houghton, 2007), it represents approximately 60 years of emissions from the transportation sector (Agrawal, 2007). This suggests that terrestrial carbon sequestration can meaningfully contribute to a portfolio of mitigation approaches and potentially offset a significant fraction of diffuse CO<sub>2</sub> sources for which direct capture is not yet feasible.

The potential for carbon storage in soils depends on numerous variables, including intrinsic storage capacities that reflect physical and chemical properties (Lal, 2003). Agricultural and land use practices affect plant and bacterial community composition, pools of organic matter,

soil aggregate abundance and stability and pyrolysed biomass (biochar) formation from plant biomass represents a potentially significant mechanism for increasing sequestered carbon (Fig. 2). The microbial (bacterial and AMF) activities **not only** positively affect plant communities and primary production, but also contribute to carbon storage through organic matter turnover and through production of soil proteins that affect aggregation and formation of degradation resistant carbon pools (Six et al., 2006). Fungi are important for aggregate formation and stabilization (Bossuyt et al., 2001; Gupta and Germida, 1988), and aggregates physically protect plant-derived SOM and microbially derived organic matter (MOM) (Beare et al., 1994; Six et al., 2000). In addition to glomalin, other proteins, such as the hydrophobins and chaplins, might also be important for microaggregate formation (Rillig, 2005). In contrast to glomalin, hydrophobins appear much more widespread in their distribution among fungi, occurring in numerous basidiomycetes and ascomycetes (Claessen et al., 2006; Linder, 2009). Hydrophobins **not only** play important roles in mycelia formation, but they also alter the hydrophobicity of spores, other cell surfaces and presumably various abiological surfaces. Chaplins are produced by streptomycetes (domain Bacteria, phylum Actinobacteria), **but** **however** details of chaplin interactions with organic matter and microaggregates have not yet been explored nor is it known how their properties vary among streptomycetes (Gebbinck et al., 2005). In support of these concepts, Cleveland et al. (2007) have reported that particular members of the *g-Proteobacteria* and *Firmicutes* in rain forest soils rapidly respond to additions of dissolved organic matter derived from plant litter leachate and probably account for changes in respiration. By contrast, changes in storage primarily reflect losses due to physical processes (e.g. erosion and transport) and differences between rates of plant inputs and organic matter mineralization, both of which are complex dynamic processes controlled by many factors. Although the possibility of managing soils for carbon sequestration on decadal time scales has been documented in several studies (Feller and Bernoux, 2008), sequestration on the order of centuries has not been shown, and this is essential for managing CO<sub>2</sub>- related climate change. Long-term carbon sequestration hinges on the lability and long-term accessibility of stored carbon to microbes and on carbon turnover rates. Clearly, a greater understanding of microbiology and ecology can contribute to the success of carbon management.

Carbon stored in soils worldwide represents the 3<sup>rd</sup> largest sink in existence, after oceans and geologic sinks. There is 2-4 times as much **C** stored in soils as there is in the atmosphere and approximately 4 times the C stored in vegetative material (i.e. plants). It is therefore

understandable that the soil C sink is being viewed as one that could potentially have a significant impact on sequestering CO<sub>2</sub> emissions. However, before we consider the feasibility to store extra C in soil, it is essential to understand the forms in which soil C exists.

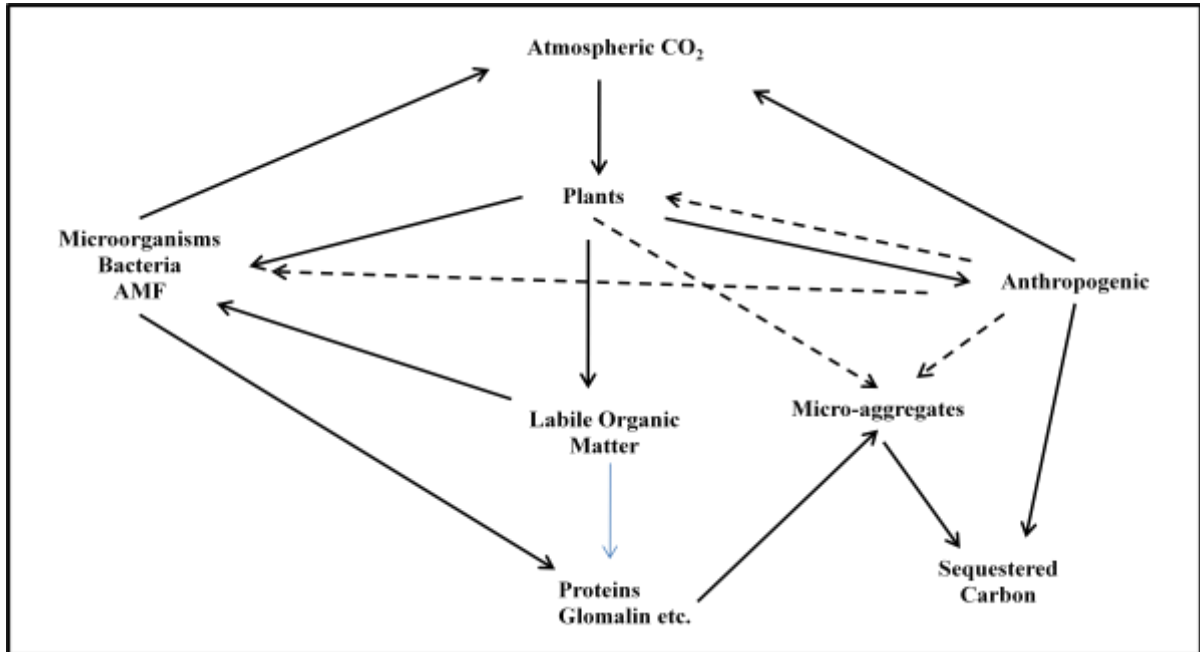


Fig. 2. Overview of carbon flows and selected interactions important for determining the extent and direction of soil carbon sequestration. Soil components subject to human disturbances are indicated with dashed arrows.

Soil C is found as either inorganic (i.e. mineral) or organic materials. Inorganic soil C is generally found as carbonates of calcium (CaCO<sub>3</sub>, or limestone) and magnesium (MgCO<sub>3</sub>). Excluding concentrated deposits of these materials that arose from deposition of the shells of aquatic invertebrates, the majority of these inorganic forms of C are found in alkaline soils. CaCO<sub>3</sub> is effectively insoluble in water at pH > 8.3, so when these extreme conditions are met (typically in subsoils) of the black and grey cracking clays, CaCO<sub>3</sub> precipitates out of the soil solution to form whitish nodules embedded in the clay soil. Whilst these inorganic forms of C can represent a significant amount of the C stored in the profile of these particular soils in some areas, they are a significantly smaller store of C than that found in the organic form. The organic forms of C in soil are a very diverse group of materials that can be defined as “everything in or on the soil that is of biological origin, whether it’s alive or dead”. It therefore includes live plant roots and litter (not shoots), humus, charcoal and other recalcitrant residues of organic matter decomposition. It also includes the organisms that live

in the soil that are collectively called the soil biota (e.g., fungi, bacteria, mites, earthworms, ants and centipedes).

### **Fate of C and nutrients during transformations**

Interestingly, as the microbial decomposition process occurs, C and some nutrients are liberated. The C is released as carbon dioxide (or methane under certain conditions) due to microbial respiration and surplus nutrients are released (mineralised) in inorganic forms suitable for use by other microbes and plants. However, soils are generally nutrient-poor environments so as the decomposition process occurs and the organic materials age in the soil, there is generally more C released as CO<sub>2</sub> than there are surplus nutrients released (i.e. a fungus needs 8 C atoms for every N atom to grow more hyphal threads, but can digest poor quality crop residue with a C:N ratio that starts at 100:1). As a result, surplus C is respired while the N is conserved, and through the aging process in soil the organic materials become increasingly nutrient rich. This enrichment of nutrients in humus and more recalcitrant, charcoal-like materials occurs particularly with N and sulphur (S). The humus that eventually forms from decomposition of, say, cereal straw will have C:N ratio of approximately 12:1, rather than 100:1 in its original form. After so many cycles of digestion and excretion these materials are less readily decomposed than when they entered the soil as plant residue, but the nutrients they contain ensure that they remain a valuable source of nutrients contributing to soil fertility. Bacteria or fungi growing on plant residues are predated by a number of more complex organisms like free living nematodes, which typically have higher C:N ratios than the organisms on which they feed. The excess N in this case will be released in an inorganic form (either ammonium-N or nitrate-N) for use by plants or other organisms. This inorganic N can build up as decomposition continues (if it is surplus to requirements of the microbial community) and will form the basis of the N supplied to the next crop.

### **Relative sizes of the different C pools**

The size of the overall C pool in soils can be misleading as an indicator of how that soil will behave, unless we understand something about the relative proportions of the different 'pools' in which that C can be found. For example, soils that have differing proportions of soil as particulate organic matter (relatively young, labile material), humus and recalcitrant compounds like charcoal will behave very differently – both in terms of properties and the microbial communities (and resulting functions) they can support. Soils supporting open grasslands will have proportionally more char-like material in their native condition, from regular natural burning events, than similar soil types under a rainforest, for example.

Similarly, soils in the sugar industry from areas where regular trash burning occurs can also have high (up to 50%) of soil C as charcoal, compared to much lower proportions with a long term history of trash blanketing (Bell and Lawrence, 2009). Consider the soils shown in the example above (Fig. 3), where soil is examined at two times in its management history – once during the continuous cropping phase and a second occasion after further cropping and then an 8-10 year period under pasture. If simply analysed for total organic C, both soils would appear very similar. The amount of resistant/char like material is unchanged. However, while there has been a large increase in the particulate/labile fraction in response to the pasture, the amount of humus (decades-centuries to form) is much lower and still reflective of the end of the cropping rundown period. This means that (i) the long term soil nutrient stores have not recovered, and (ii) that this soil organic matter will decline much more rapidly if the land is returned to cropping. In fact, the original rate of soil organic matter decline would effectively double in the next cropping phase and much more frequent pasture phases would be needed to maintain soil productivity.

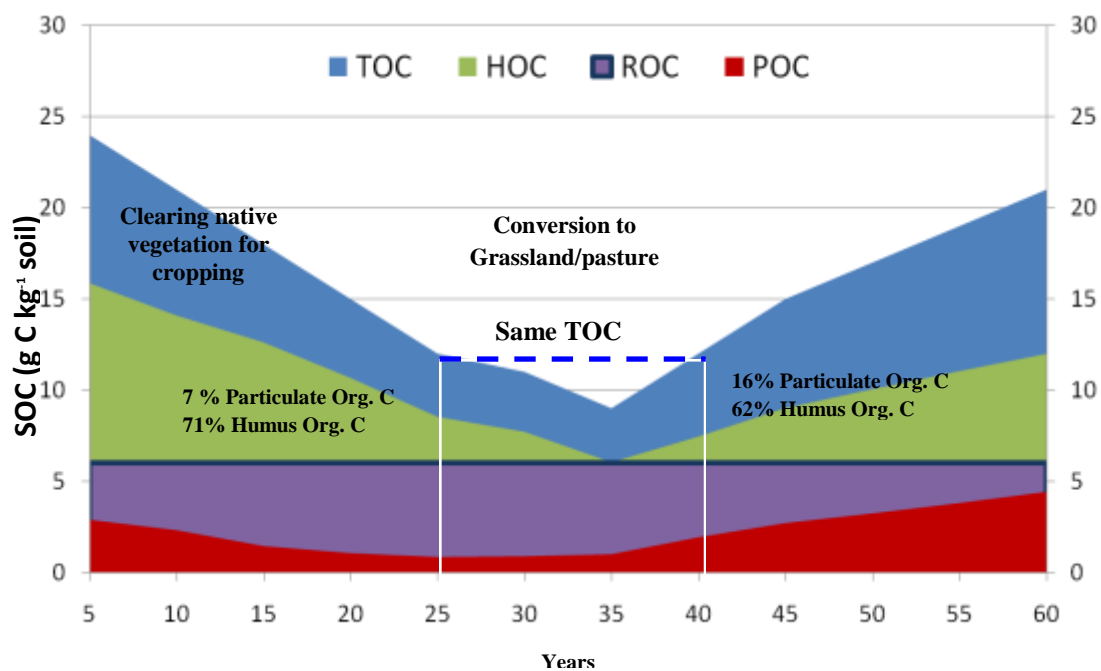


Fig. 3. Relative size of different carbon pools in soil (adapted from Bell and Lawrence, 2009 based on hypothetical data)

Soil C levels are fundamentally determined by the balance between organic matter inputs, primarily as plant residues, roots and root exudates, and organic matter losses due to decomposition, erosion, and leaching. Bacteria and fungi generally comprise >90% of the total soil microbial biomass, and they are responsible for the majority of SOM decomposition. Since soil microbial communities are key regulators of SOM dynamics and

nutrient availability, shifts in microbial community composition and function (e.g., substrate utilization) in response to different agricultural management practices may play an important role in determining rates of C loss from the soil. The ratio of fungal:bacterial biomass has been shown to be particularly sensitive to soil disturbance, with lower ratios associated with increased intensity of cultivation (Bailey et al., 2002), increased grazing pressure (Bardgett et al., 1996) and increased N fertilization inputs (Frey et al., 2004). In addition, fungal:bacterial biomass ratios were found to increase with successional age in a semiarid grassland community (Klein et al., 1996).

Organic C taken up by the microbial biomass is partitioned between microbial cell biomass production, metabolite excretion and respiration. The degree to which MOM accumulates in soil depends on a balance between production and decomposition of microbial products, that is: (1) the microbial growth efficiency (MGE), the efficiency with which substrates are incorporated into bacterial and fungal biomass and byproducts, (2) the degree of protection of microbial biomass in the soil structure, and (3) the rate at which bacterial and fungal byproducts are decomposed by other microorganisms. The proportion of substrate C retained as biomass versus respired as CO<sub>2</sub> depends on MGE and the degree of protection of microbial biomass; the lower the MGE or the less protected the biomass, the more .....

### **Microbial contributions to carbon storage**

Many variables affect soil carbon storage, but microaggregate formation substantially limits decomposition losses (McCarthy, 2008). At large scales (e.g. field to ecosystem), microaggregate formation depends on numerous factors, including organic matter loading rates, mechanical disturbances (e.g. tilling), temperature and water availability. Nonetheless, mycorrhizal fungi (MF), and possibly some bacteria, play additional key roles (Bedini et al., 2009). Of course, MF also respire organic matter and thus participate in several aspects of belowground carbon cycling, the overall balance of which is uncertain.

### ***MF, glomalin and microaggregates***

The details of contributions to microaggregate formation by MF at molecular scales are unclear but probably involve interactions among organic matter, metals, mineral surfaces and specific proteins. The most important of these proteins appears to be glomalin - a glycoprotein. Glomalin, which has been identified as a heat shock protein (Purin and Rillig, 2008), occurs in a phylogenetically narrow range of arbuscular mycorrhizal fungi (AMF) within the order Glomales (Bedini et al., 2010).



The effects of glomalin on microaggregates could be due to its impacts on hydration (water penetration and hydrophobicity) and the resulting accessibility of hydrolytic enzymes to bulk organic matter (Rillig 2005). Soil structure and the pore-size distribution within soil aggregates indirectly influence microbial dynamics by restricting organism movement and thereby modifying the interactions between organisms. The diameters of bacteria, fungal hyphae, protozoa, and nematodes are: 0.15 to 4 (although typically 0.5–1  $\mu\text{m}$ ), 3 to 8, 10 to 100, and  $<50 \mu\text{m}$ , respectively (Coyne, 1999). The larger size of fungi relative to bacteria means that fungi are rarely found in micropores. However, small pores provide refuges for bacteria against attack from larger predators (i.e., protozoa and bacterivorous nematodes) that are typically unable to enter smaller pores. Spatial differentiation of bacterial community composition has been observed between waterstable microaggregate interiors and macroaggregates (Mummey and Stahl, 2004).

The location of bacteria and fungi within the pore network is a key factor in their survival and activity, and the larger size of fungi may make them more vulnerable to predation. Bacterial populations are consistently high in small pores, but highly variable in large pores where they are vulnerable to being consumed (Vargas and Hattori, 1986).

The amount of substrate C that is incorporated into microbial biomass and eventually transferred into the MOM pool is determined by the size of the microbial biomass and its MGE (Fig. 1, Step I). The amount of microbial biomass is not only directly related to the C input level, but is also influenced by other factors including the availability of microbial habitat and consequently, the level of protection provided for the microbial biomass. Microbial biomass can be divided into unprotected and protected pools. Van Veen et al. (1985) split microbial biomass into a protected pool with a death rate of 0.5% per day and an unprotected pool with a death rate of 70% per day.

### **Soil microbial diversity and carbon sequestration**

Soil microbial diversity, defined here as community composition (or taxon richness) and structure (or taxon abundance), has been the subject of numerous analyses (Fierer and Jackson, 2006). The first molecular ecological studies, which were based on small clone libraries and denaturing gel gradient electrophoresis, identified the phylogenetic affiliations and relative abundance of only the dominant community members (Campbell et al., 2008). At present it is uncertain which is the most important for carbon sequestration: the limited genetic potential of a small number of common phylotypes or the greater genetic diversity of individually rare but collectively abundant phylotypes. Yuan et al. (2012) reported that

microbial autotrophy plays a significant role in the sequestration of soil carbon in terrestrial C sequestration in between 2–3 Pg C yr<sup>-1</sup>. Phylogenetic analysis showed that the dominant *cbbL*-containing bacteria were *Azospirillum lipoferum*, *Rhodopseudomonas palustris*, *Bradyrhizobium japonicum*, *Ralstonia eutropha*, and *cbbL*-containing chromophytic algae of the genera *Xanthophyta* and *Bacillariophyta*. Differences in *cbbL* gene diversity were shown to be correlated with differences in SOC. Bacterial *cbbL* abundance was shown to be positively correlated with RubisCO activity ( $r=0.853$ ,  $P < 0.05$ ) and both *cbbL* abundance and RubisCO activity were significantly related to the synthesis rates of <sup>14</sup>C-SOC ( $r=0.967$  and  $0.946$ , respectively,  $P < 0.01$ ).

Although diversity indices, such as the Shannon index, might not be useful in predicting or understanding carbon sequestration, the specific composition of microbial communities could prove much more important. Much of the diversity revealed by molecular ecological approaches is not represented in cultures and often cannot be mapped unambiguously to taxonomic levels below families or genera. As a result, complementary approaches must be pursued, including an emphasis on cultivating the uncultivated.

### **Impacts of key environmental variables on organic matter stabilization**

#### ***Temperature effects***

Numerous factors affect organic matter decomposition, but temperature is often regarded as a master variable (Davidson and Janssens 2006). The effects of rising global temperatures have long been a focus of concern, because they could increase respiration and mobilize soil organic matter resulting in a positive feedback for climate change (Singh et al., 2010). Hawkes et al. (2008) argue, for example, that temperature affects carbon allocation from plant to fungus and allocation within AMF networks, with a possible increase in respiratory carbon losses in a warmer climate that are not offset by increased AMF growth. Nonetheless, much remains unknown about interactions between temperature and managed soil carbon sequestration. Crucial research gaps include the responses of microaggregate formation and destabilization to both rising temperature and increasingly variable climate regimes, that is changes in the frequency of extreme events (e.g. high and low temperatures, precipitation regimes and snowfall).

#### ***Organic matter loading***

Compared with conventional practices, organic farming practices have been shown to promote higher microbial biomass (Lundquist et al., 1999; Petersen et al., 1997) and to alter microbial community composition (Bossio et al., 1998; Petersen et al., 1997). Increased

carbon storage in soils implies either relative or absolute reductions in respiration, increase in organic matter loading or some combination of both. Here, we consider possible impacts of increased loading. Organic matter loading rates have been reported to affect the temperature sensitivity of respiration (Gaumont-Guay et al., 2006). This could occur if, for example diffusion limits substrate availability for respiration and increased loading reduces or eliminates that limitation. In this case, warming would lead to increased carbon turnover until a diffusion limitation was reestablished. Increased availability of labile low molecular weight organics can also affect turnover of bulk organic matter pools through a process referred to as priming (Blagodatskaya and Kuzyakov, 2008). Priming can potentially limit net carbon sequestration by accelerating respiration of bulk organic matter, thus decreasing total soil organic matter concentrations.

### ***Responses to elevated CO<sub>2</sub>***

Responses of both plants and microbes to elevated CO<sub>2</sub> further constrain predictions of soil organic matter dynamics and carbon storage. These responses typically depend on interactions between CO<sub>2</sub> and numerous other variables (Billings and Zeigler, 2008). Nitrogen limitation in some mature, undisturbed soils can significantly diminish the ability of microbes to respond to changes in carbon availability or other environmental variables (Niklaus, 1998), but Barron-Gafford et al. (2005) have observed increased rates of soil respiration in experimental stands of eastern cottonwoods (*Populus deltoides*) exposed to elevated CO<sub>2</sub> and growing on nitrogen-replete soils. Increased microbial respiration occurs when plants are incubated with elevated CO<sub>2</sub> not only due to increased belowground carbon allocation but also as a result of specific interactions between plants, rhizosphere bacteria and MF (Oliver et al., 2008). These interactions are dynamic, varying among plant hosts and as a function of plant physiological status. They include changes in rhizodeposition, microbial growth rates and community structure in response to passive and active chemical signals originating from roots, bacteria and fungi (Barron-Gafford et al., 2005).

Results from a Mojave Desert study (Jin and Evans, 2007) illustrate the complexity of plant–microbe–CO<sub>2</sub> interactions and the need for improved biogeochemical models. Differential impacts were reported for elevated CO<sub>2</sub> on microbial substrate use, nitrogen cycling rates and exoenzymatic activities for soils beneath a dominant shrub and unvegetated interspace soils. Differences were attributed to variations in microbial biomass and turnover times between the two soils. The response of desert soils to climate change and elevated CO<sub>2</sub>, and the ability of these soils to contribute to carbon sequestration, were found to sensitively depend on the

dynamics of plant communities and specific plant–microbe interactions, which at present cannot be sufficiently well modeled to reliably predict outcomes.

Montealegre et al., (2000) observed a shift in the community composition of *R. leguminosarum* bv. *trifolii* occurred as a result of an increased atmospheric CO<sub>2</sub> concentration, and that elevated atmospheric CO<sub>2</sub> affects the competitive ability of root nodule symbionts, most likely leading to a selection of these particular strains to nodulate white clover.

According to the Intergovernmental Panel on Climate Change Fourth Assessment Report of 2007, sequestration and reduction of emissions over the next two to three decades will potentially have a substantial impact on long-term opportunities to stabilize levels of atmospheric CO<sub>2</sub> and mitigate impacts of climate change (Pachauri and Reisinger 2007).

### **Agricultural practices that increase soil carbon sequestration**

Agricultural management practices differ in the type and intensity of disturbance; therefore, the impact on microbial biomass and MOM varies with the management practice employed. In agricultural systems, the amount and length of time carbon is stored in the soil is largely determined by how the soil resource is managed. A variety of agricultural practices that can enhance carbon storage have been proposed. The benefits of these practices as well as their potential hidden costs must be considered when management decisions are made. Though not discussed here, there may also be direct or indirect costs and benefits to farmers implementing these techniques. Removing CO<sub>2</sub> from the atmosphere is only one significant benefit of enhanced carbon storage in soils. Improved soil and water quality, decreased nutrient loss, reduced soil erosion, increased water conservation, and greater crop production may result from increasing the amount of carbon stored in agricultural soils.

Strategies to increase the soil carbon pool include soil restoration and woodland regeneration, no-till farming, cover crops, nutrient management, manuring and sludge application, improved grazing, water conservation and harvesting, efficient irrigation, agroforestry practices, and growing energy crops on spare lands. An increase of 1 ton of soil carbon pool of degraded cropland soils may increase crop yield by 20 to 40 kilograms per hectare (kg ha<sup>-1</sup>) for wheat, 10 to 20 kg ha<sup>-1</sup> for maize, and 0.5 to 1 kg ha<sup>-1</sup> for cowpeas. As well as enhancing food security, carbon sequestration has the potential to offset fossil fuel emissions by 0.4 to 1.2 gigatons of carbon per year, or 5 to 15% of the global fossil-fuel emissions (Lal, 2004). Management techniques that sequester carbon in soils include:

#### ***Conservation tillage***

No-tillage and minimum tillage (MT) systems often exhibit increased C storage compared with CT (West and Post, 2002); however, this difference disappears with the use of fallow rotations (Peterson et al., 1998), demonstrating the importance of using multiple management practices to enhance soil C storage. Minimizes or eliminates plowing of soil for crop production through practices such as no-till (no plow) farming or mulch tillage. These procedures generally reduce soil erosion, improve water use efficiency and increase carbon concentrations in the topsoil leading to a significant increase in soil CO<sub>2</sub> sequestration. Conservation tillage can also lower the amount of fossil fuel consumed since it reduces the operation of farm machinery. Differences in tillage intensity also impact microbial community composition (Jackson et al., 2003; Neely et al., 1991). Frey et al. (1999) observed that a shift toward fungal dominated microbial communities under NT will be most important for residue decomposition and nutrient cycling processes near the soil surface. However, another study found no difference in fungal:bacterial ratio in surface NT and CT soils and a 10% lower fungal:bacterial ratio in NT soil at 6 to 12 cm. Less intensively managed agroecosystems (e.g., NT) bear the closest resemblance to natural ecosystems, which are fungal-dominated (Bardgett, 1996). These fungal-dominated agroecosystems require fewer inputs to sustain organic matter decomposition and nutrient cycling, that is, these systems show greater self-regulation. However, Allison et al. (2005) concluded that an improved metabolic efficiency due to increased relative abundance of AMF and saprophytic fungi does not promote the stabilization of C on cessation of tillage-based agriculture.

### ***Organic Farming and Cover Crops***

Compared with conventional practices, organic farming practices have been shown to promote higher microbial biomass and to alter microbial community composition (Bossio et al., 1998). Cover crops improve carbon sequestration by enhancing soil structure and adding organic matter to the soil (Table 1). Organic farming practices frequently employ cover crops, which can change the soil microbial community and have a variable effect on MOM. The more N supply than C supply controls the effect of cover crops on soil carbohydrate content. More research is needed to investigate relationships between the use of organic farming practices (including cover crops) and soil microbial communities. Organic management appears to increase fungal biomass, which would favor increased soil C sequestration.

### ***Crop rotation***

Crop rotation as a management practice may increase soil C sequestration in comparison with continuous crop management or rotations that include fallow periods and even location specific farming systems (Table 1). More intensive cropping rotations not only increase soil C input but also increase microbial activity and biomass and alter microbial community composition, increasing levels of soil fungi. Effectiveness of crop rotating varies by region, crop type, and crop rotation timing. The hot water extractable carbohydrates are more influenced by crop rotations than are acid hydrolyzable carbohydrates. Since hot water extractable carbohydrates are considered more microbially derived than acid hydrolyzable carbohydrates, the microbially derived carbohydrates seem to be more affected by crop rotations than plant-derived carbohydrates (Six et al., 2006). Varying the type of crops grown can increase the level of soil organic matter.

*Table 1. Agricultural practices for enhancing productivity and increasing the amount of carbon in soils*

<b>Traditional practices</b>	<b>Recommended</b>
Plough till	Conservation till or no-till
Residue removal or burning	Residue return as mulch
Low off-farm input	Judicious use of fertilizers and integrated nutrient management
Regular fertilizer use	Soil-site specific management
No water control	Water management/conservation, irrigation, water table management
Fence-to-fence cultivation	Conversion of marginal lands to nature conservation
Conventional practices	Organic farming (site specific) and cover crops
Land use along poverty lines and political boundaries	Integrated watershed management
Monoculture	Improved farming systems with several crop rotations

### **Epilogue and way forward**

The potential for biospheric carbon sequestration could offset CO<sub>2</sub> emissions from the transportation sector for decades or even longer as biofuels, hydrogen and methane replace petroleum-based fuels. Realizing this potential, however, will require a much more detailed understanding of the specific organisms and mechanisms that can contribute to carbon storage, as well as continued improvement in understanding the responses of soil microbial

communities to carbon loading and organic composition, and to changes in land use, temperature and hydrologic regimes, among other variables. Microbial biomass and community composition as well as MOM concentrations are affected by crop rotations, tillage, organic farming practices and cover crops. All of these management regimes appear to induce shifts in overall microbial community composition and fungal:bacterial biomass ratios. Under NT, especially, higher fungal biomass levels are correlated with quantitative and qualitative improvements in SOM. Shifts in microbial community structure due to soil disturbance have important implications for ecosystem scale processes because bacteria and fungi function differently in the incorporation and turnover of C and in their respective effects on soil physical properties.

**There's** a lot of conceptual advances in recent years about soil carbon sequestration mechanisms that need to be matched by methodological advances. Ways of using field sampling, lab analysis or modeling to understand how soil carbon sequestration changes, specifically in vulnerable to stable soil carbon pools. And it will be important as ever to integrate data that have already been collected into databases where people can use that collected information to understand things at a bigger scale about soil carbon sequestration. Numerous basic challenges remain to be addressed, beginning with an exploration of the extent to which soil organic concentrations represent localized optima. Some of the important challenges are as follows:

1. Develop a specific and predictive understanding of the mechanisms that determine soil carbon concentrations.
2. Identify the most important microbial groups that control soil carbon storage and mobilization.
3. Determine management strategies that can be successfully implemented at suitable spatial and temporal scales to promote contributions of microbes to soil carbon storage by using omics datasets.

To answer these challenges and implement successful long-term carbon management, it will be necessary to couple approaches ranging from molecular to ecosystem scales in scope using nested designs for observation and experimentation to address spatial and temporal hierarchies of bacterial and fungal metabolic activity, hydrolytic enzyme distribution and activity, and microbial community composition and structure.

## **References**

- Agrawal, R. 2007. Sustainable fuel for the transportation sector. *Proceedings of National Academy of Sciences of the United States of America* 104: 4828–4833.
- Allison, V.J., Miller, R.M., Jastrow, J.D., Matamala, R. and Zak, D.R. 2005. Changes in soil microbial community structure in a tall grass prairie chronosequence. *Soil Science Society of America Journal* 69: 1412–1421.
- Bailey, V.L., Smith, J.L. and Bolton, H.Jr. 2002. Fungal-to-bacterial ratios in soils investigated for enhanced C sequestration. *Soil Biology and Biochemistry* 34: 997–1007.
- Bardgett, R.D. 1996. Potential effects on the soil mycoflora of changes in the UK agricultural policy for upland grasslands. p. 163–183. In *Fungi and environmental change* (Ed. J.C. Frankland, N. Magan and G.M. Gadd). Cambridge Univ. Press, Cambridge.
- Bardgett, R.D., Hobbs, P.J. and Frostegard, A. 1996. Changes in soil fungal:bacterial biomass ratios following reductions in the intensity of management of an upland grassland. *Biology and Fertility of Soils* 22: 261–264.
- Barron-Gafford, G., Martens, D., Grieve, K., Biel, K., Kudeyarov, V., McLain, J.E.T., Lipson, D. and Murthy, R. 2005. Growth of eastern cottonwoods (*Populus deltoides*) in elevated CO<sub>2</sub> stimulates stand-level respiration and rhizodeposition of carbohydrates, accelerates soil nutrient depletion, yet stimulates above- and belowground biomass production. *Global Change Biology* 11: 1220–1233.
- Batjes, N.H. 1996. Total carbon and nitrogen in the soils of the world. *European Journal of Soil Science* 47: 151–163.
- Beare, M.H., Cabrera, M.L., Hendrix, P.F. and Coleman, D.C. 1994. Aggregate-protected and unprotected organic matter pools in conventional- and no-tillage soils. *Soil Science Society of America Journal* 58: 787–795.
- Bedini, S., Pellegrino, E., Avio, L., Pellegrini, S., Bazzoffi, P., Argese, E. and Giovannetti, M. 2009. Changes in soil aggregation and glomalin-related soil protein content as affected by the arbuscular mycorrhizal fungal species *Glomus mosseae* and *Glomus intraradices*. *Soil Biology and Biochemistry* 41: 1491–1496.
- Bedini, S., Turrini, A., Rigo, C., Argese, E. and Giovannetti, M. 2010. Molecular characterization and glomalin production of arbuscular mycorrhizal fungi colonizing a heavy metal polluted ash disposal island, downtown Venice. *Soil Biology and Biochemistry* 42: 758–765.
- Bell, M. And Lawrence, D. 2009. *Soil carbon sequestration - myths and mysteries*. The Department of Primary Industries and Fisheries. 1-9.
- Billings, S.A. and Zeigler, S.E. 2008. Altered patterns of soil carbon substrate usage and heterotrophic respiration in a pine forest with elevated CO<sub>2</sub> and N fertilization. *Global Change Biology* 14: 1025–1036.
- Blagodatskaya, E. and Kuzyakov, Y. 2008. Mechanisms of real and apparent priming effects and their dependence on soil microbial biomass and community structure: critical review. *Biology and Fertility of Soils* 45: 115–131.



- Bossio, D.A., Scow, K.M., Gunapala, N. and Graham, K.J. 1998. Determinants of soil microbial communities: Effects of agricultural management, season, and soil type on phospholipid fatty acid profiles. *Microbial Ecology* 36: 1–12.
- Bossuyt, H., Denef, K., Six, J., Frey, S.D., Merckx, R. and Paustian, K. 2001. Influence of microbial populations and residue quality on aggregate stability. *Applied Soil Ecology* 16: 195–208.
- Brooks, S.J., Gale, P.A. and Light, M.E. 2006. Anion binding modes in macrocyclic amidourea. *Chemical Communications* 41: 4344–4346.
- Campbell, J.H., Clark, J.S. and Zak, J.C. 2008. PCR-DGGE comparison of bacterial community structure in fresh and archived soils sampled along a Chihuahuan Desert elevational gradient. *Microbial Ecology* 57: 261–266.
- Canadell, J.G., Pataki, D.E., Gifford, R., Houghton, R.A., Luo, Y., Raupach, M.R., Smith, P. and Steffen, W. 2007. Saturation of the terrestrial carbon sink. In *Terrestrial Ecosystems in a Changing World* (Canadell, J.G. et al., eds), pp. 59–78, Springer-Verlag
- Claessen, D., Jong, W., Dijkhuizen, L. and Wosten, H.A.B. 2006. Regulation of Streptomyces development: reach for the sky! *Trends in Microbiology* 14: 313–319.
- Cleveland, C.C., Nemergut, D.R., Schmidt, S.K. and Townsend, A.R. 2007. Increases in soil respiration following labile carbon additions linked to rapid shifts in soil microbial community composition. *Biogeochemistry* 82: 229–240.
- Coyne, M.S. 1999. *Soil microbiology: An exploratory approach*. Delmar Publishers, Albany, NY.
- Davidson, E.A. and Janssens, I.A. 2006. Temperature sensitivity of soil carbon decomposition and feedbacks to climate change. *Nature (Lond.)* 440: 165–173.
- Feller, C. and Bernoux, M. 2008. Historical advances in the study of global terrestrial soil organic carbon sequestration. *Waste Management* 28: 734–740.
- Fierer, N. and Jackson, R.B. 2006. The diversity and biogeography of soil bacterial communities. *Proceedings of National Academy of Sciences of the United States of America* 103: 626–631.
- Frey, S.D., Elliott, E.T. and Paustian, K. 1999. Bacterial and fungal abundance and biomass in conventional and no-tillage agroecosystems along two climatic gradients. *Soil Biology and Biochemistry* 31:573–585.
- Frey, S.D., Knorr, M., Parrent, J. and Simpson, R.T. 2004. Chronic nitrogen enrichment affects the structure and function of the soil microbial community in a forest ecosystem. *Forest Ecology and Management* 196:159–171.
- Gaumont-Guay, D., Black, T.A., Griffis, T.J., Barr, A.G., Jassal, R.S. and Nesic, Z. 2006. Interpreting the dependence of soil respiration on soil temperature and water content in a boreal aspen stand. *Agricultural and Forest Meteorology* 140: 220–235.
- Gebink, M.F.B.G., Claessen, D., Bouma, B., Dijkhuizen, L. and Wosten, H.A.B. 2005. Amyloids – a functional coat for microorganisms. *Nature Reviews Microbiology* 3: 333–341.

- Glibert, P.M., 2008. Ocean urea fertilization for carbon credits poses high ecological risks. *Marine Pollution Bulletin* 56: 1049–1056.
- Gupta, V.V.S.R. and Germida, J.J. 1988. Distribution of microbial biomass and its activity in different soil aggregate size classes as affected by cultivation. *Soil Biology and Biochemistry* 20: 777–786.
- Hawkes, C.V., Hartley, I.P., Ineson, P. and Fitter, A.H. 2008. Soil temperature affects carbon allocation within arbuscular mycorrhizal networks and carbon transport from plant to fungus. *Global Change Biology* 14: 1181–1190.
- Houghton, R.A. 2007. Balancing the global carbon budget. *Annual Review of Earth and Planetary Sciences* 35: 313–347
- Jackson, L.E., Calderon, F.J., Steenwerth, K.L., Scow, K.M., and Rolston, D.E. 2003. Responses of soil microbial processes and community structure to tillage events and implications for soil quality. *Geoderma* 114: 305–317.
- Jin, V.L. and Evans, R.D. 2007. Elevated CO<sub>2</sub> increases microbial carbon substrate use and nitrogen cycling in Mojave Desert soils. *Global Change Biology* 13: 452–465.
- Kimble, J. M., Lal, R. and Follett, R.R. 2002. Agricultural Practices and policy options for carbon sequestration: what we know and where we need to go. In *Agricultural practices and policies for carbon sequestration in soil* (eds by Kimbel, J.M., R. Lal, and R.F. Follett). New York, Lewis Publishers, p 512.
- King, G.M. 2011. Enhancing soil carbon storage for carbon remediation: potential contributions and constraints by microbes. *Trends in Microbiology* 19: 75-84.
- Klein, D.A., McLendon, T., Paschke, M.W. and Redente, E.F. 1996. Nitrogen availability and fungal-bacterial responses in successional semi-arid steppe soils. *Arid Soil Research and Rehabilitation* 10: 321–332.
- Lal, R. 2003. Global potential of soil carbon to mitigate the greenhouse effect. *Critical Reviews in Plant Sciences* 22: 151–184.
- Lal, R. 2004. Soil carbon sequestration impacts on global climate change and food security. *Science* 304: 1623-1627.
- Lal, R. 2008. Carbon sequestration. *Philosophical Transactions of the Royal Society B: Biological Science* 363: 815–830.
- Linder, M.B. (2009) Hydrophobins: proteins that self assemble at interfaces. *Current Opinion in Colloid & Interface Science* 14: 356–363.
- Lundquist, E.J., Jackson, L.E., Scow, K.M. and Hsu, C. 1999. Changes in microbial biomass and community composition, and soil carbon and nitrogen pools after incorporation of rye into three California agricultural soils. *Soil Biology and Biochemistry* 31: 221–236.
- McCarthy, J.F. 2008. Protection of organic carbon in soil microaggregates via restructuring of aggregate porosity and filling of pores with accumulating organic matter. *Geochimica et Cosmochimica Acta* 72: 4725–4744.

- Montealegre, C.M., Kessel, C.V., Blumenthal, J.M., Hur, H., Hartwig, U.A. and Sadowsky, M.J. 2000. Elevated atmospheric CO<sub>2</sub> alters microbial population structure in a pasture ecosystem. *Global Change Biology* 6: 475-482.
- Mummey, D.L. and Stahl, P.D. 2004. Analysis of soil whole- and inner-microaggregate bacterial communities. *Microbial Ecology* 48: 41-50.
- Neely, C.L., Beare, M.H., Hargrove, W.L. and Coleman, D.C. 1991. Relationships between fungal and bacterial substrate-induced respiration, biomass, and plant residue decomposition. *Soil Biology and Biochemistry* 23: 947-954.
- Niklaus, P.A. 1998. Effects of elevated atmospheric CO<sub>2</sub> on soil microbiota in calcareous grassland. *Global Change Biology* 4: 451-458.
- Oliver, K.L., Hamelin, R.C. and Hintz, W.E. 2008. Effects of transgenic hybrid aspen overexpressing polyphenol oxidase on rhizosphere diversity. *Applied and Environmental Microbiology* 74: 5340-5348.
- Pachauri, R.K. and Reisinger, A. 2007. *Climate Change 2007: Synthesis report*. IPCC, Geneva, Switzerland. pp 104.
- Petersen, S.O., Debosz, K., Schjønning, P., Christensen, B.T. and Elmholt, S. 1997. Phospholipid fatty acid profiles and C availability in wet-stable macro-aggregates from conventionally and organically farmed soils. *Geoderma* 78: 181-196.
- Peterson, G.A., Halvorson, A.D., Havlin, J.L., Jones, O.R., Lyon, D.J. and Tanaka, D.L. 1998. Reduced tillage and increasing cropping intensity in the Great Plains conserves soil C. *Soil & Tillage Research* 47: 207-218.
- Purin, S. and Rillig, M.C. 2008. Immuno-cytolocalization of glomalin in the mycelium of the mycorrhizal fungus *Glomus intraradices*. *Soil Biology and Biochemistry* 40: 1000-1003.
- Rillig, M.C. 2005. A connection between fungal hydrophobins and soil water repellency? *Pedobiologia* 49: 395-399.
- Schlesinger, W. 2003. *Biogeochemistry, An Analysis of Global Change*. Academic Press.
- Singh, B.K., Bardgett, R.D. Smith, P. and Reay, D.S. 2010. Microorganisms and climate change: terrestrial feedbacks and mitigation options. *Nature Review Microbiology* 2: 779-790.
- Six, J., Elliott, E.T. and Paustian, K. 2000. Soil macroaggregate turnover and microaggregate formation: A mechanism for C sequestration under no-tillage agriculture. *Soil Biology and Biochemistry* 32: 2099-2103.
- Six, J., Frey, S.D., Thiet, R.K. and Batten, K.M. 2006. Bacterial and fungal contributions to carbon sequestration in agroecosystems. *Soil Science Society of America Journal* 70: 555-569.
- Sundquist, E., Burruss, R., Faulkner, S., Gleason, R., Harden, J., Kharaka, Y., Tieszen, L. and Waldrop, M. 2008. *Carbon sequestration to mitigate climate change*. U.S. Geological Survey. <http://www.usgs.gov/>.
- Tossel, J.A. 2009. Catching CO<sub>2</sub> in a bowl. *Inorganic Chemistry* 48: 7105-7110.

- Van Veen, A.J., Ladd, J.N. and Amato, M. 1985. Turnover of carbon and nitrogen through the microbial biomass in a sandy loam and a clay soil incubated with  $^{14}\text{C}$  (U) glucose and  $^{15}\text{N}(\text{NH}_4)_2\text{SO}_4$  under different moisture regimes. *Soil Biology and Biochemistry* 17: 747–756.
- Vargas, R. and T. Hattori. 1986. Protozoan predation of bacterial cells in soil aggregates. *FEMS Microbiology Ecology* 38: 233–242.
- West, T.O. and Post, W.M. 2002. Soil organic carbon sequestration rates by tillage and crop rotation: A global data analysis. *Soil Science Society of America Journal* 66: 1930–1946.
- Whitman, W.B., Coleman, D.C. and Wiebe, W.J. 1998. Prokaryotes: the unseen majority. *Proceedings of National Academy of Sciences of the United States of America* 95: 6578–6583.
- Yuan, H., Ge, T., Chen, C., O'Donnell, A.G. and Wu, J. 2012. Microbial autotrophy plays a significant role in the sequestration of soil carbon. *Applied Environmental Microbiology* 78: 2328–2336.