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Editor

Soil Biology and Biochemistry,

I submit a review entitled 'A mechanistic model for the stabilisation of organic carbon in soil aggregates' for publication in Soil Biology and Biochemistry. I request that the review, should it be accepted, be published with the three figures in black and white, with colour used in the web version only. Reference has been made to the PhD thesis of Mukasa Mugerwa (2012). The specific research has been submitted to SBB and is under review. The reference will be replaced should the manuscript be accepted for publication. I am unaware of any conflicts of interest.

I also submitted a proposal for a review to the suggested review editor. As Dr Ward has passed away, may I suggest his name and email address be replaced by the current editor to reduce embarrassment of any reviewing his email address.

Yours sincerely,

Peter McGee

2nd October, 2012.

Highlights

A model of the role of fungi in stabilisation of organic carbon in soil is proposed.

Arbuscular mycorrhizal fungi deposit organic materials.

Organic materials are transformed to aromatic carbon by some saprotrophic fungi.

Aromatic compounds held in anaerobic conditions constitute the stabilised component of soil organic carbon.

A mechanistic model for the stabilisation of organic carbon in soil aggregates.

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Abstract

Soil is a complex and dynamic matrix. The complexity has hindered the development of a clear understanding of how carbon is stored in soil. Processes associated with the stabilisation of organic carbon in soil are examined in this paper, in particular the deposition of aromatic carbon during the formation and maturation of soil aggregates. Two plant-associated groups of fungi render inter-related and critically important roles. Arbuscular mycorrhizal fungi contribute to the development of aggregates and deposition of organic materials in aggregates. Some saprotrophic fungi transform some of this organic material into aromatic compounds such as melanin. Some of the aromatic compounds persist in the aggregate after fungal autolysis because the anaerobic core of aggregates precludes oxidation. Accumulation of aromatic compounds increases the stabilised organic carbon in aggregates and therefore in soil. Differences in carbon stabilisation will arise due to soil characteristics, fungal species and host plant. A mechanistic model of carbon stabilisation in aggregates is proposed. The model indicates a path whereby more predictable ways may be developed for increasing stores of organic carbon in soil.

Keywords: Carbon cycle; Aggregate development; AM fungi; Melanised fungi; Carbon sequestration.

1. Introduction

The decline in the content of organic carbon in soil is a global problem (Banwart, 2011). Organic materials are crucial for the sustainable productivity of agricultural soils (Schmidt et al., 2011). The restoration of stabilised and long-lived organic materials to soil is important for the sustained provision of food and clean water (Daily et al., 1997). Three times more carbon is held in soil than in the atmosphere and terrestrial vegetation combined (Lackner, 2003). Increased carbon storage in soil indicates one mechanism whereby carbon can be removed from the atmosphere (Lal, 2003). Restoration of carbon to soil has immediate and long-term benefits. However the carbon cycle in soil is complex, dynamic, and poorly understood; furthermore, the study of different soil disciplines is poorly integrated (Schmidt et al., 2011). A clearer articulation of the mechanisms that regulate the carbon cycle in soil is essential to realise the potential to restore carbon to soil.

The most significant component of the carbon cycle is biological and our understanding of soil biology is limited (Kleber and Johnson, 2010). Much research is based on correlations and the hypotheses are rarely tested under experimental conditions. Data on subsets of soil biology are also unequal. For instance, relatively extensive data has been collected on the taxonomy and functions of soil bacteria, and much less for fungi (King, 2011). Important misconceptions also surround the flow of carbon in soil and the activity of microbes (Schmidt et al., 2011).

1.1 Current models of carbon sequestration – Misconceptions

Current models of carbon sequestration in soil are beset by a number of misconceptions, and are not assisted by the confusion that arises from the inconsistent use of some terms relating to soil (Schmidt et al., 2011). Important misconceptions include: the fate of plant-derived carbon

in the surface soil, the contribution of aromatic materials to the stores of soil carbon, and the exclusion of microbes from aggregates.

Plant – derived carbon: Perhaps the most limiting misconception is the belief that plants directly contribute to the pool of organic carbon (OC) in surface layers of soil. By OC we mean the stores of aromatic and other organic compounds protected within soil aggregates (Lal, 2004; Six et al., 2004; von Lützow et al., 2008; Schmidt et al., 2011). Plants contribute various forms of organic material to soil (von Lützow *et al*., 2008). Simple root exudates are removed from soil solution within hours (Paterson *et al*., 2008). Roots shed cells to the rhizosphere and the cellular debris is degraded within weeks (Jones *et al*., 2009). Leaf and stem remains are degraded on the soil surface or after incorporation to soil by fauna. Importantly, most plant remains are completely degraded within a short period of time (Baldrian and Lindahl, 2011). While typical root diameters preclude their direct entry into the pores of smaller aggregates, very fine root hairs may penetrate micro-aggregates (Dexter, 1988). The potential for roots to colonise the core of aggregates is therefore extremely limited, and direct contribution to OC by plants is negligible. This speculation is supported by empirical data. A meta-analysis of research on conservation agriculture examined whether management practices influence stabilisation of carbon in cropping soils (Govaerts et al., 2009). Conservation management was associated with increased carbon in 40 of 78 cases, 31 had no change in carbon and 7 had less carbon. Clearly, management of plants and plant remains do not predictably increase stores of OC in soil.

Storage of OC in soil is an integral component of the carbon cycle. Microbes in soil both degrade and transform organic compounds using hydrolysis and/or oxidation. Hydrolysis is the mechanism whereby microbes enzymatically degrade organic materials into smaller molecules and use the resultant nutrients for respiration, growth and reproduction (Daynes et al., 2008).

Carbohydrates including polysaccharides, proteins, DNA and fats are all hydrolysed and thus most plant remains will be hydrolysed. Hydrolysis can take place under aerobic and anaerobic conditions, although the process is slower in the absence of oxygen.

Aromatic compounds, on the other hand, can only be degraded by oxidation resulting in the release of CO_2, either directly through the action of oxygen, or due to the action of oxidative enzymes which also require oxygen. In soil, oxidative enzymes are mostly expressed by fungi (Lundell et al., 2010). In other words, aerobic conditions are required for the degradation of aromatic compounds (Ekschmitt et al., 2008). Lignin is the most common aromatic material entering soil and is degraded as rapidly as the bulk organic materials in soil (Thevenot et al., 2010), even in carbon saturated soils (Carrington et al., 2012). That is, lignin is oxidatively degraded when placed in aerobic locations in soil. Addition of lignin will not increase stores of OC in surface soils. However, OC in soil may be increased by placing aromatic compounds in anaerobic locations: for instance, the core of aggregates is anaerobic (Sexstone et al., 1985) and can be an important location for stabilisation of aromatic carbon.

Aromatic Carbon: Aromatic compounds such as humic materials may be extracted from soil. Humic materials are sometimes termed ‘recalcitrant’: their chemical extraction from soil requires the use of strong acids or alkalis (Kleber, 2010). Chemical recalcitrance was assumed to indicate chemical stability. However measurement of the recalcitrance of free organic materials from soil indicated a more rapid degradation of aromatic compounds than would be expected of recalcitrant carbon (Brodowski et al., 2006; Marschner et al., 2008). The rapid loss of aromatic carbon is due to the relative rate of oxidation of the aromatic components of the organic materials (Thevenot et al., 2010). Recalcitrance in soil is therefore situationally determined and as a result the term has limited use in the description of overall soil properties. ‘Recalcitrance’

96 can only be applied to those aromatic compounds protected from oxidation. Use of the term
97 'stabilised' for aromatic carbon held in the anaerobic core of aggregates allows a clearer
98 consideration of the dynamic and complex processes in soil.

99 'Black carbon' is a term that includes the polyaromatic materials in charcoal, coal dust
100 and deliberately pyrolysed plant materials. Black carbon has been added to soil in an attempt to
101 increase the quantity of carbon stored in the soil. Black carbon was found to be stabilised only
102 when it was located within aggregates: the polyaromatic structure of black carbon by itself was
103 of no particular benefit for long term storage in soil (Brodowski et al., 2006). Location within
104 aggregates will protect the polyaromatic carbon from oxygen and thus oxidation. As with lignin,
105 black carbon is subject to oxidation in aerobic conditions. Black carbon will not make a
106 significant contribution to stores of stabilised OC in soil unless the black carbon is placed in
107 anaerobic conditions.

108 *Exclusion of microbes from aggregates*: The potential role of microbes in carbon
109 sequestration is also misconceived. Soil structure is sometimes suggested to present a physical
110 barrier between the decomposing activities of soil microbes and organic materials (Elliott and
111 Coleman, 1988; Urbanek et al., 2011). Considerable numbers of fungal and bacterial cells have
112 been documented in aggregates (Foster and Martin, 1981) indicating the presence of microbes
113 with the potential to be active. Aggregates have cavities interconnected by pores of varying
114 dimensions (Dexter, 1988; Elliott and Coleman, 1988; Jocteur Monrozier et al., 1991) with water
115 adsorbed on surfaces (Daynes et al., in press) indicating a path whereby microbes may enter an
116 aggregate. Fungal hyphae have diameters ranging from around 1 µm. Fungi will readily access
117 pores as fine as 1 µm. Hydrophobic surfaces are suggested to prevent microbes from penetrating
118 aggregates (Bachmann et al., 2008; Rillig et al., 2010). Some fungi express hydrophobins to soil

119 solution where they attach to surfaces and the surfaces become hydrophobic as the soil dries.
120 Hydrophobic surfaces are no barrier to microbes. Hydrophobins also enable hyphal attachment to
121 hydrophobic surfaces (Sunde et al., 2008), and facilitate growth of hyphae across air-water
122 boundaries (King, 2011), regardless of the origin and composition of the hydrophobic surfaces
123 (Franco et al., 2000). Similarly, bacteria express compounds with detergent properties. Fungi and
124 bacteria will attach to, and remain active on surfaces on and within aggregates so long as the air
125 space has sufficient available water.

126 **1.2 Deposition of Organic Carbon**

127 Transformation of organic materials to aromatic carbon and deposition of the aromatic
128 carbon in the anaerobic core of aggregates appears to be an important mechanism for the
129 stabilisation of OC in surface soils. If plants do not directly deposit OC, the source of aromatic
130 materials and the means whereby aromatic compounds may be stabilised require clarification.
131 Fungi have the potential to elongate through soil and fungi are present in aggregates. The
132 potential contribution of fungi to stabilisation of OC is examined below.

133 Arbuscular mycorrhizal (AM) fungi have been proposed as the primary agents in
134 aggregate formation (Rillig and Mummey, 2006) and carbon sequestration (Jastrow et al., 1998;
135 Wilson et al., 2009). AM fungi constitute by far the most common organic material of microbial
136 origin entering soil. Between 6 and 20% of plant energy is utilised by AM fungi (Smith and
137 Read, 2008) thus determining an indirect route for the entry of plant energy to soil. Up to 20 m
138 of hyphae of AM fungi are found in each gram of soil in undisturbed ecosystems (Olssen et al.,
139 1999). Significant densities of hyphae may even be found in cropping soils (McGee et al., 1997).
140 The movement of large quantities of energy of plant origin to soil provides a significant energy

141 input but does not necessarily lead to stabilisation of significant quantities of OC (Govaerts et al.,
142 2009; Daynes et al., in press). More specifically, glomalin, a putative heat shock protein
143 associated with walls of AM fungi, was hypothesised as the source of stabilised carbon (Wilson
144 et al., 2009). Glomalin is commonly assayed in soil by use of the Bradford reaction.
145 Unfortunately, the stain used in the Bradford reaction (Bradford, 1976) also stains aromatic
146 compounds such as tannins (Kilkowski and Gross, 1999; Whiffen et al., 2007) and humic acid
147 (Whiffen et al., 2007) as well as various amino acids. As a result, correlations between AM fungi
148 and Bradford reactive soil protein need not indicate the presence of glomalin (Gillespie et al.,
149 2011). Indeed, glomalin appears to be a protein, and if so will be subject to hydrolysis. While
150 AM fungi clearly play a significant role in the transfer of plant carbon to soil, the formation of
151 soil aggregates (Rillig, 2004; Daynes et al., in press) and the development of porosity within
152 aggregates (Daynes et al., in press), the potential of AM fungi to directly contribute to stable OC
153 in soil appears limited (Daynes et al., in press) perhaps due to the lack of aromatic components in
154 hyphae.

155 In contrast, fungi using saprotrophic nutrition rely on organic materials in their
156 environment for energy. In soil, nutrients are distributed in a three-dimensional matrix. Over
157 time, each substrate will be successively colonised, exploited and the nutrient depleted. Fungal
158 communities in soil will thus pass through stages of growth and decline, and the species will
159 survive commonly in low densities (Garrett, 1955; Warcup, 1957) except where the fungi are
160 associated with roots. Some saprotrophic fungi have a stage where they associate with the roots
161 of healthy plants as endophytes (Mukasa Mugerwa et al., in press). For instance, various species
162 of *Chaetomium* are readily isolated from a range of organic materials and colonise the shoots and
163 roots of plants (Syed et al., 2009). An endophytic stage may enhance the potential for a

saprotrophic fungus to maintain populations because the fungi spread through the root system (Mukasa Mugerwa et al., in press). Fungal endophytes of roots utilise plant energy while *in planta*. The nutrition of endophytic fungi in soil is unclear. Most may rely on local sources of energy when growing in soil or they may utilise energy from their host plant. However, the importance of the endophytic stage to soil fungi is unknown, likely to be highly variable among the huge diversity of endophytic fungi, and may vary with the age of the host tissue and species of host plant. Our understanding of the nutrition of endophytic fungi in soil remains extremely limited.

If root-associated fungi distribute plant energy through the mycelium, the distribution of plant carbon will lead to stable isotope signatures in soil similar to that of the host plant. The presence of isotope signatures similar to that found in plants has been used to infer plants contribute directly to stabilised carbon in soil (Schmidt et al., 2011; Urbanek et al., 2011). However, because stable isotope analysis is still an imprecise tool (Boecklen et al., 2011), an alternative explanation is that plants provide energy to associated fungi, and the root-associated fungi, especially AM fungi, determine the distribution and deposition of plant energy in aggregates. Limitations to the tool allow us to hypothesise movement of plant energy to aggregates, but will not differentiate between direct and fungus-mediated movement of carbon from plant to aggregate.

Consideration of how OC may be stabilised in aggregates next requires an understanding of the development and maintenance of aggregates. The widely-accepted model of aggregate hierarchy (Tisdall and Oades, 1982) postulates different binding agents at different stages of the aggregate hierarchy. Implicit in the hierarchical model is the sequential formation of each stage (Hadas, 1987; Dexter, 1988). That is, fine structural elements such as clay and organic materials

187 initiate micro-aggregates, which in turn, are bound by mucilage, hyphae and fine roots to form
188 macro-aggregates. Macro-aggregates may also form around organic materials and then fragment
189 along planes of weakness thereby creating micro-aggregates (Oades, 1984; Elliott and Coleman,
190 1988; Six et al., 2000). Fragmentation occurs when energy disrupts the system, such as the
191 shrink-swell process during wet-dry cycles, and elongation, expansion and death of roots (Beare
192 et al., 1994; Angers et al., 1997; Field and Minasny, 1999; Field et al., 2006). Critically, the
193 fragmentation process posits the subsequent enclosure of fine organic materials with encrustation
194 by clay particles, that is the nuclear formation of micro-aggregates (Augustin et al., 1995; Six et
195 al., 2000). Clearly, aggregates may be dynamic and considerable dynamism is likely during
196 aggregate formation and development. Stabilised organic carbon may be enclosed in aggregates
197 of various sizes. The aggregates may be subject to breakdown and re-formation especially in
198 changing soil conditions such as in cultivation. Of the various fractions, micro-aggregate
199 formation is argued to be crucial for the stabilisation of OC in soil (Six et al., 2004; von Lützow
200 et al., 2008) because the micro-aggregate is the stage most resistant to further fragmentation (Zhu
201 et al., 2010).

202 **2. A Mechanistic Model for the Stabilisation of organic Carbon**

203 **2.1 Background**

204 A mechanistic model of carbon sequestration (Elliott and Coleman, 1988) based on the
205 hierarchical model of soil aggregation (Tisdall and Oades, 1982) was proposed whereby organic
206 material is stabilised within micro-aggregates. In this model, and subsequent refinements (Six et
207 al., 2000), the nature and source of the stabilised OC in micro-aggregates remains uncertain
208 (Oades and Water, 1991; Rasse et al., 2005).

We argue that aromatic compounds largely derived from endophytic fungi are the source of stabilised carbon in micro-aggregates. Melanin is an abundant polyaromatic compound produced by many organisms including some fungi (Eisenman and Casadevall, 2012), and degradation of melanin can result in humic materials (Koroloeva et al., 2007). The potential for deposition of OC in soil by melanised endophytic fungi has been investigated. Selected isolates rapidly increased OC by up to 17% in an already well-aggregated carbon-rich soil under experimental conditions (Mukasa Mugerwa, 2012). The next logical question was whether the increased OC might include aromatic compounds. Quantification of aromatic materials deposited in soil is extremely difficult, in part because of the variable composition of aromatic compounds. When compared to gallic acid as the standard (Halvorson et al. 2009), total aromatic materials increased by up to 41% in the bulk soil, and up to 57% in some aggregate fractions (Mukasa Mugerwa, 2012). These results indicate significant and rapid deposition of aromatic materials when soil was colonised by melanised fungi under experimental conditions.

Aromatic carbon increased in aggregates presumably because of the deposition in and differential degradation of organic materials in the aggregate. The hyphae of the melanitic fungi are small enough to penetrate aggregates where they could hydrolyse organic materials, presumably including the hyphal remains of AM fungi. Hyphae of the melanitic fungi autolyse (White et al., 2002; Lucy Liu unpublished data) as nutrients within the aggregate become depleted. During autolysis hydrolysable materials within the mycelium of the melanised fungi are translocated from energy-depleted environs, in a process called autophagy (Shoji and Craven, 2011). However, aromatic compounds will remain within the aggregate because the core of the aggregate is anaerobic (Sexstone et al., 1985). As melanin is likely to be located in or under the wall of hyphae (Eisenman and Casadevall, 2012), hyphal ghosts (Tisdall and Oades, 1982) will

accumulate within the core of the aggregate. Thus the accumulation of aromatic material in mature aggregates will be determined by (1) maintenance of the aggregate integrity, especially the anaerobic core, (2) the on-going addition of organic materials to the aggregate such as from hyphae of AM fungi, followed by the (3) temporary colonisation of the energy-rich aggregate by hyphae that contain aromatic materials.

2.2 The proposed model

The formation and maintenance of aggregates and deposition of stabilised OC are tightly integrated. A mechanistic model for the stabilisation of OC during the initiation, development and maturation of aggregates is proposed. Stabilisation of OC has three critically important stages: (1) initiation of anaerobic micro-sites that then become the core of an aggregate, (2) deposition of aromatic materials during the formation of aggregates, and (3) on-going deposition of aromatic carbon in mature aggregates.

(1). *The initiation of anaerobic micro-sites in soil* (Fig. 1). Initiation of aggregates is influenced by AM fungi, presence of organic materials (Six et al., 2000; Bossuyt et al., 2001) and the presence of plants (Tisdall and Oades, 1982). Primary particles of sand, silt and clay are mixed with organic materials, through which plant roots and hyphae of AM fungi proliferate. Plant energy sustains the AM fungi, and the hyphae of the AM fungi proliferate in response to the organic material (Hodge and Fitter, 2010). The presence of readily available oxygen throughout the amorphous mixture enables hydrolysis and oxidation by extant saprotrophic microbes. Degradation of the organic materials exposes charged surfaces, and initiates potential interactions between organic and clay particles. Pockets of rapid respiration result in the establishment of anaerobic micro-sites where oxidation stops and hydrolysis slows. The

254 differential rates of degradation of organic materials due to pockets of anaerobiosis and
255 subsequent ionic interactions between different particles result in the initiation of aggregates.

256 (2). *The deposition of OC during aggregate development* (Fig. 2). On-going colonisation
257 of the soil matrix by AM fungi adds organic materials to the developing aggregates. Colonisation
258 of the developing structure by melanised saprotrophic fungi results in the transformation of
259 organic materials to include some aromatic compounds. All organic materials will readily
260 degrade (hydrolysis and oxidation) in the aerobic zone that at present surrounds developing
261 aggregates. In anaerobic micro-sites (Tiedje et al., 1984), slow hydrolysis will continue. During
262 autophagy by saprotrophic fungi (Shoji and Craven, 2011), the aromatic materials in the
263 aggregate remain protected from oxidation adding to the plant-based component. The aromatic
264 materials in the aggregate will initially be dominated by those of plant origin. The proportion of
265 aromatic materials will increase in the anaerobic conditions due to accumulation from hyphae
266 that contain aromatic materials.

267 Aggregates develop because of several concurrent processes, including the differential
268 degradation of organic materials in soil, physico-chemical interactions between the components,
269 enmeshment by fungal hyphae and the compressive forces within soil. Complete degradation of
270 the organic materials surrounding anaerobic micro-sites exposes soil particles, resulting in the
271 differentiation of aggregate surfaces within the initially amorphous mixture. Simultaneous and
272 contrasting energy transfer forces also act on the aggregates during development (Six et al.,
273 2000). For instance, on-going colonisation of aggregates by AM fungi may enlarge aggregates,
274 while aggregated structures may shrink as organic materials are removed by hydrolysis from
275 within aggregates. Water ingress and loss may also cause expansion and contraction of
276 aggregates. The matrix supporting aggregates may be altered by various processes, including the

277 growth and degradation of roots, which exert pressures on the aggregate. The developing
278 aggregates are dynamic in structure, size and composition with a key feature being the net
279 accretion of aromatic materials in the anaerobic core.

280 (3). *The maturation of aromatic deposits* (Fig. 3). The final stage of the model involves
281 the on-going deposition of aromatic material within the anaerobic core of the aggregate. Two
282 factors underpin aggregate maturation: on-going deposition of organic materials (Elliot and
283 Coleman, 1988) and autophagy of saprotrophic fungi. Because of their preponderance in soil,
284 AM fungi are likely to continue to colonise aggregates, with hyphal fragments remaining in
285 aggregates. These fragments provide a source of energy that enables the repeated colonisation of
286 aggregates by saprotrophic fungi including some with aromatic components, such as melanin.
287 The repeated colonisation by fungi with aromatic components sustains the on-going deposition
288 of aromatic compounds in the anaerobic core of the aggregate. Aromatic compounds will remain
289 protected from oxidation while anaerobic conditions persist in the core of the aggregate. The
290 accumulation of aromatic compounds in aggregates will contribute to stabilised OC in soil.

291 **2.3 Implications of the Mechanistic Model**

292 Aggregates are dynamic. For instance, aggregates may variably fracture along planes of
293 weakness induced by energy transfer (Oades, 1984). Organic materials on exposed surfaces will
294 be subject to rapid degradation. Subsequent enmeshment of detached fragments by hyphae may
295 re-integrate particles to existing aggregates or initiate the formation of new aggregates, resulting
296 in varying patterns of OC stabilisation across a soil and over time. The rate of turnover of
297 aggregates will influence the age of OC in micro-aggregates. As aggregates mature, the rate of
298 aggregate turnover is likely to slow. While the model of carbon deposition supports the presence

of older carbon in micro-aggregates compared to macro-aggregates (Verchot et al., 2011), the longevity and quantity of OC stored in micro-aggregates remains uncertain. The content of OC in the aggregate will vary over time. Hydrolysable materials will be added to and removed from the aggregate: these materials will constitute a significant proportion of the organic centre of an aggregate. Aromatic carbon will increase in the aggregate while anaerobiosis is maintained, though the upper limit, if any, is unclear (Stewart et al., 2008). Turnover of OC is likely to be slow, and moderated by the composition of the microbial community, environmental conditions and the presence and quality of plant roots as hosts of AM fungi and endophytic fungi that contain aromatic components.

Alternatively, carbon accumulation in aggregates may follow an asymptotic path. That is, aromatic ghosts may slowly accumulate to an extent that precludes further ingress by hyphae of AM fungi. The termination of supplies of fresh nutrients to the aggregate will limit further colonisation by saprotrophic microbes. Thus the content of OC in aggregates may plateau over time. The OC will be protected from oxidation by anaerobic conditions within the aggregate core and the aggregate will be stable while ever soil conditions are stable.

All melanised microbes may contribute aromatic compounds to aggregates. However, plant roots contribute a reliable supply of energy to melanitic endophytic fungi increasing the probability that melanitic endophytic fungi are important contributors of aromatic materials to soil. At the very least, plant roots provide a stable environment from which endophytic fungi will emerge, colonise adjacent soil, and retreat. The plant may supply energy that enables the growth of hyphae of melanitic endophytic fungi beyond the root surface, as applies to AM fungi. Alternatively, while melanitic endophytic fungi will utilise plant energy when *in planta*, beyond the rhizosphere the fungi may rely on local sources of energy (Korkama-Rajala et al., 2008) and

322 therefore be subject to competitive interactions with other saprotrophic microbes. If subjected to
323 competitive interactions, densities in soil of melanitic fungi will always be much lower than AM
324 fungi. The relative densities in surface soil of melanitic and AM fungi are likely to ensure
325 supplies of organic materials from AM fungi will be adequate for the nutrition of the melanitic
326 fungi in aggregates. Rapid increases in OC will therefore rely on species of fungi with aromatic
327 components dominating the community of saprotrophic fungi in roots and soil, and a readily
328 available supply of energy within aggregates.

329 Aromatic carbon may be sequestered in all anaerobic conditions. The deposition of
330 aromatic carbon from plants in anaerobic swamps during the Devonian, Carboniferous and
331 Cretaceous eras accounts for a considerable proportion of the fossil fuels now being used.
332 Aromatic carbon deposited deep in soil is likely to be stabilised because oxygen penetrates
333 limited and variable depths in the profile. Burial of organic matter at depth and in water have
334 limited benefits for agricultural production (Rumpel and Kogel-Knabner, 2011; Kell, 2012), but
335 may make valuable contributions to stabilising OC for other purposes.

336 Many questions remain unanswered. Aggregation is influenced by soil type (Oades and
337 Waters, 1991), host plant and species of AM fungi (Daynes unpublished data) and these factors
338 may influence stabilisation of OC. The micro-site in which anaerobic conditions pertain may
339 vary with edaphic conditions and be difficult to quantify. In the experiments referred to above,
340 melanin was used as an easily recognised polyaromatic material. Even assuming melanin is the
341 dominant aromatic material in fungi, the relative influence of each type of melanised fungus in
342 each set of environmental conditions requires clarification. Melanin is a variable polymer and
343 each fungus will have varying quantities and qualities of melanin. Little is known of the other
344 common aromatic materials formed by fungi and some fungi may express considerable quantities

of aromatic compounds other than melanin. Deposition of stabilised OC in each environment will be determined by the specific contributions of resident fungi, and is unpredictable at the current level of knowledge. The assumption that AM fungi lack aromatic components is also untested. Surveys of AM fungi indicate hyaline hyphae are common. Rare taxa such as *Glomus atrouva* have dark spore and hyphal walls (McGee and Trappe, 2002) which may indicate the presence of melanin (Eisenman and Casadevall, 2012). The potential for melanised AM fungi to directly stabilise aromatic carbon in aggregates remains an intriguing, if local, possibility. Finally, more accurate modelling of carbon stabilisation in soil will rely on integrating many dynamic processes (Feeney et al., 2006) and will be difficult.

3. Conclusions

Loss of organic carbon from soil remains a global problem. The determinants of carbon stabilisation in soil act at a microscopic scale. These differences of scale have profound implications for conceptualising models of carbon sequestration and for developing practical responses to the loss of carbon from soil. The over-emphasis on direct plant contributions to carbon storage, despite overwhelming evidence to the contrary, indicates some of the difficulties to be faced in applying a more logical, methodological and mycological approach to stabilisation of organic carbon. Indeed, as some correlative models of soil carbon are based on the contributions of plant material, especially lignin, to the soil, a considerable restructuring of thinking is essential in order to reflect our developing understanding of the dynamics of the stabilisation of organic carbon in soil.

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374 Figures legends

375 Figure 1. Schematic diagram of the amorphous mixture within which an aggregate may develop.
376 In this diagram, melanin is presented as an example of an aromatic compound. Not to scale.

377

378 Figure 2. Schematic diagram of the development of a soil aggregate. Organic material has been
379 removed from the surface and an anaerobic core emerged within a developing aggregate.
380 Organic material from fungi is added to the aggregate some of which has been hydrolysed.
381 Aromatic materials (M) remain in the anaerobic core. Not to scale. See Fig. 1 for the key.

382

383 Figure 3. Schematic diagram of the mature aggregate. Organic materials of fungal origin
384 continue to be deposited in the aggregate. The organic materials are hydrolysed by fungi.
385 Aromatic materials (M) are deposited and remain in the aerobic core. Not to scale. See Fig. 1 for
386 the key.

387

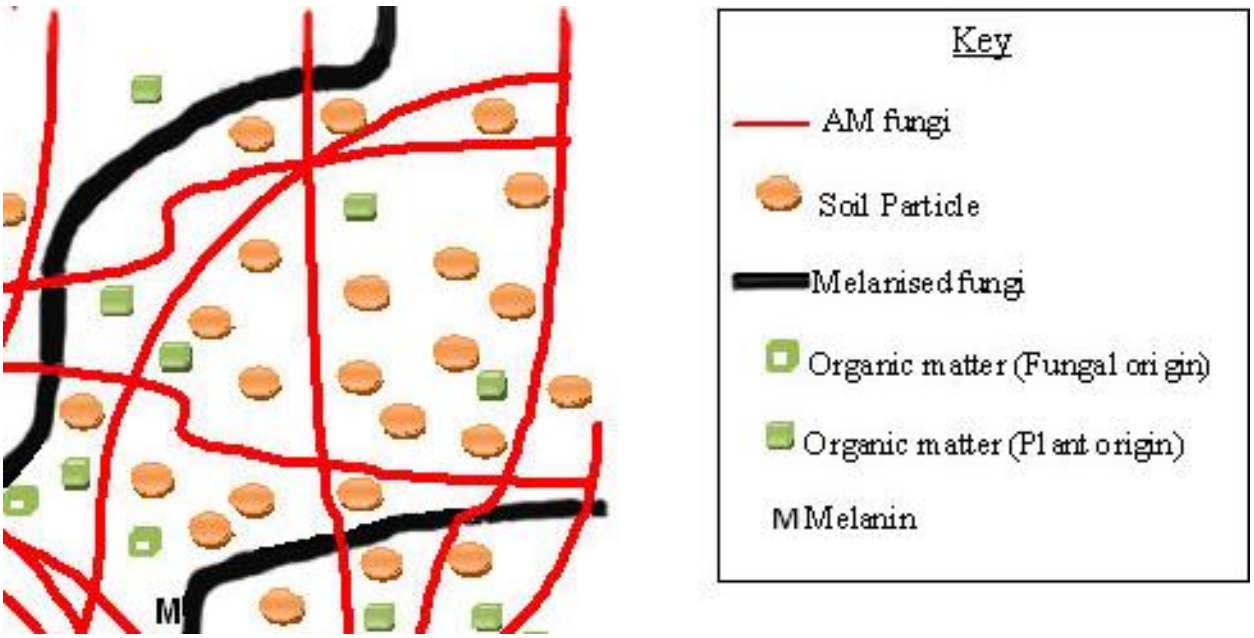


Figure 1. Schematic diagram of the amorphous mixture within which an aggregate may develop. In this diagram, melanin is presented as an example of an aromatic compound. Not to scale.

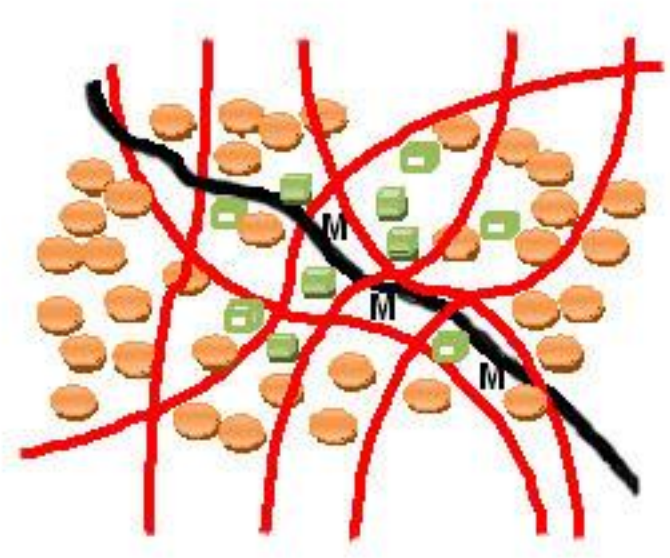
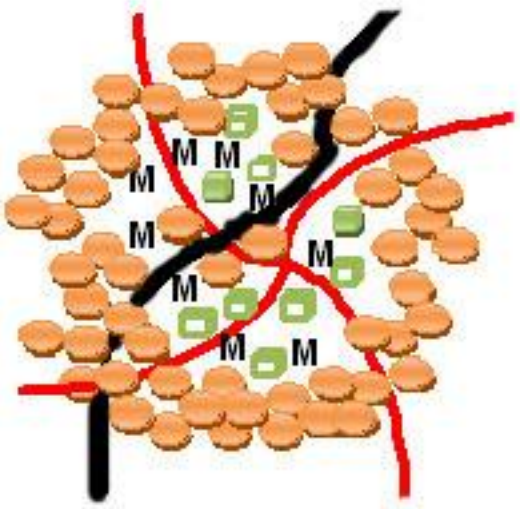


Figure 2. Schematic diagram of the development of a soil aggregate. Organic material has been removed from the surface and an anaerobic core emerged within a developing aggregate. Organic material from fungi is added to the aggregate some of which has been hydrolysed. Aromatic materials (M) remain in the anaerobic core. Not to scale. See Fig. 1 for the key.



401

402 Figure 3. Schematic diagram of the mature aggregate. Organic materials of fungal origin
403 continue to be deposited in the aggregate. The organic materials are hydrolysed by fungi.
404 Aromatic materials (M) are deposited and remain in the aerobic core. Not to scale. See Fig. 1 for
405 the key.

406

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Figure 1

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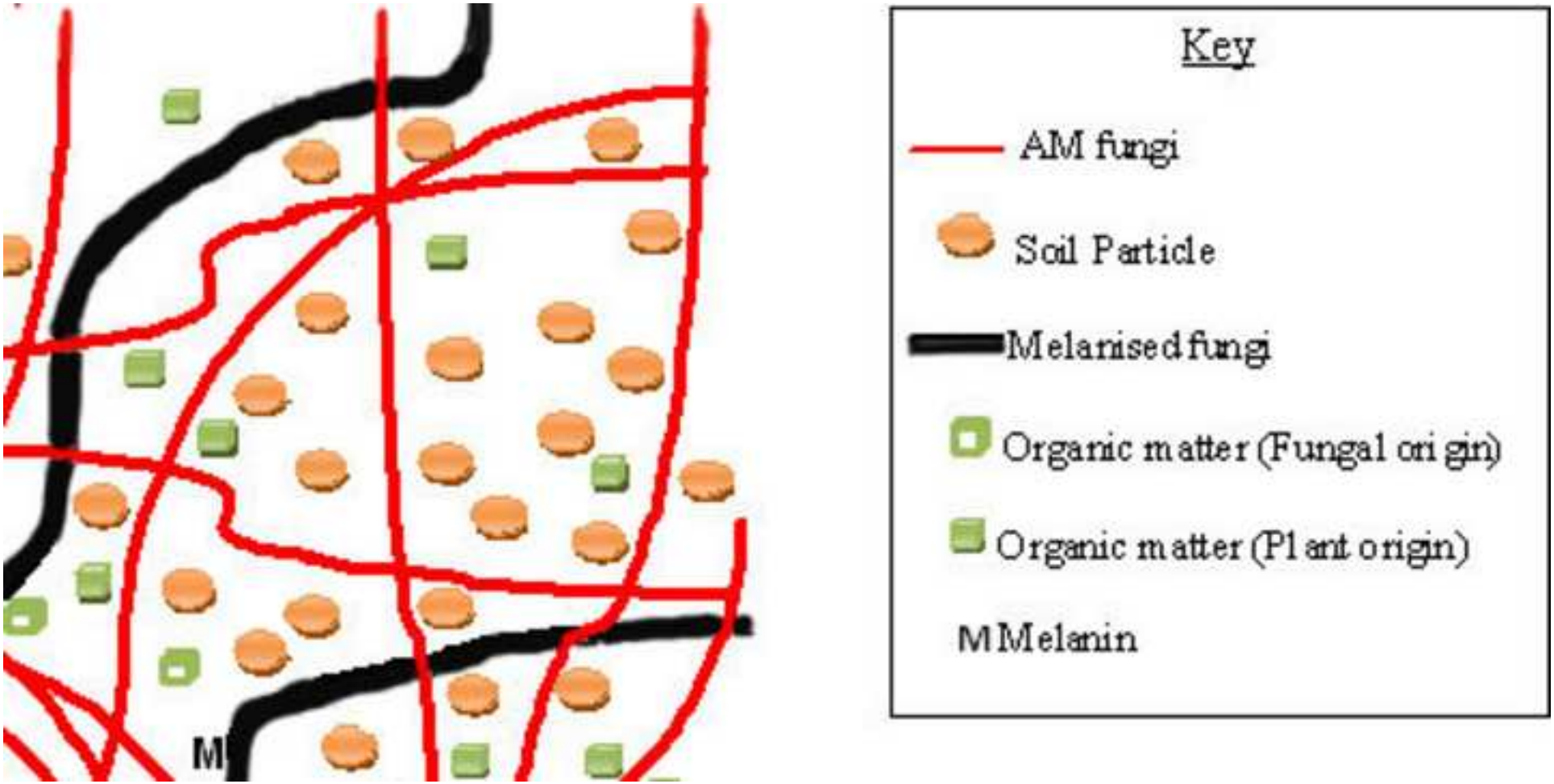

Figure 2

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Figure 3

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