SOIL ORGANIC MATTER DYNAMICS IN SOUTHEASTERN US AGROECOSYSTEMS: AN ANALYSIS OF MANAGEMENT PRACTICES AND EARTHWORM ACTIVITY AS CONTROLLING FACTORS

by

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(Under the Direction of PAUL F. HENDRIX)

ABSTRACT

Understanding soil organic matter (SOM) dynamics and their interactions with different management practices and soil faunal activities is essential in trying to develop sustainable agroecosystems. In this study, the effects of tillage and earthworm activity on SOM and carbon (C) protection were investigated. In the first experiment, the objective was to study the mechanisms by which C is protected under no-tillage (NT) management, using ^{14}C-labeled plant residue. Aggregate-size distribution, total C, and ^{14}C were measured together with different pools of aggregate-associated C and ^{14}C from 21-d laboratory incubations of intact and crushed macro-and microaggregates. The results indicated that (i) more young C (^{14}C) is accumulated in the subsurface soil of conventional tillage (CT) than NT, but this C is not stabilized in the long term, and (ii) short- and long-term stabilization of C is higher in the soil surface layers under NT compared with CT. This C stabilization occurs mainly at the microaggregate level.

The objectives of the next set of experiments were to investigate the effects of different earthworm species (*Aporrotedea caliginosa* and *Lumbricus rubellus*) on aggregation, aggregate-associated C pools and the formation of stable microaggregates within macroaggregates. Two incubations were set up. The first incubation consisted of soil samples crushed < 250 μm to break up all macroaggregates with three treatments: (i) control soil; (ii) soil + ^{13}C-labeled residue; and (iii) soil + ^{13}C-labeled residue + earthworms. After 20 days, aggregate size distribution was measured and microaggregates (53-250 μm) were isolated out of the formed macroaggregates (> 250 μm). A second incubation was conducted to determine protected versus unprotected total C and ^{13}C from 21-day laboratory incubations of intact and crushed macro- and microaggregates. The results indicated that microaggregates are rapidly formed within earthworm casts and showed the direct involvement of earthworms in inducing an important protection of soil C at the microaggregate level. The results also suggested that important interactions between earthworm species take place affecting the incorporation of fresh residue-derived C and the formation of stable microaggregates when fresh residue was placed on the surface.

INDEX WORDS: soil organic matter, soil carbon, tillage, soil aggregation, earthworms, carbon protection

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CHAPTER 1

INTRODUCTION

Soil structure

Soil structure is determined by the presence of soil 'aggregates', which are defined as 'naturally occurring clusters of soil particles in which the forces holding the particles together are much stronger than the forces between adjacent aggregates' (Martin et al., 1955).

Soil aggregation has a great influence on the physical characteristics of the soil. Well-aggregated soils possess a larger pore space and are characterized by a higher infiltration rate and better gaseous exchange between soil and atmosphere, leading to enhanced microbial activity (Lynch and Bragg, 1985). Aggregates are also thought to play an important role in the physical protection of soil organic matter (SOM). The maintenance of SOM and soil carbon is desirable for land use because of the beneficial effects of organic matter on nutrient dynamics. SOM can also increase both soil resistance and resilience to deformation (Kay, 1990), decrease soil compactability (Ball et al., 1988), and improve soil macroporosity (Carter, 1990). Furthermore, the retention of organic carbon in soil is becoming more and more important because of recent concerns over global warming and the increase in atmospheric CO_2.

Soil aggregation

Soil aggregate formation

The aggregation of soil particles occurs on different size scales, resulting in different aggregate size classes, which can be placed in hierarchical order. The lowest order is the combination of single mineral clay particles into domains. These domains can then be combined into the next hierarchical order (Dexter, 1988). A model of aggregate hierarchy has been described by Tisdall and Oades (1982). Mineral particles bind into microaggregates (50-250 μm) and microaggregates into macroaggregates (> 250 μm). Free primary particles are mainly cemented together into microaggregates by persistent binding agents (e.g. clay-polyvalent metal-humified organic matter complexes), characterized as older, more humified, or recalcitrant SOM. The more temporary (e.g., roots and fungal hyphae) and transient (e.g., polysaccharides) agents are thought to bind microaggregates into macroaggregates and are generally considered to be relatively more labile or decomposable (van Veen and Paul, 1981; Elliot, 1986). This model describes the formation of soil aggregates, which occurs mainly through the cementation of smaller units into larger structural elements. For cementation, three main groups of colloidal materials are considered to be important: (i) clay and silt particles (which can be linked together or can be adsorbed to sand particles); (ii) iron and aluminum oxides; and (iii) organic matter. Cementation only, however, produces massive blocks of soil rather than smaller soil aggregates. Therefore, mechanical forces (drying and wetting, action of roots and earthworms) have been considered necessary to mold the soil mass into smaller units (Martin et al., 1955; Baver, 1956).

Models of soil aggregate formation

In the model described by Tisdall and Oades (1982), microaggregates are formed first within the soil and then become the building blocks for the formation of macroaggregates. The conceptual model of Tisdall and Oades (1982) was later modified by Oades (1984), who suggested that microaggregates are predominantly formed within macroaggregates. This modification has formed the basis of several recent studies (Beare et al., 1994a; Gale et al., 2000; Six et al., 1999, 2000). First, macroaggregates (> 250 μm) are formed through the formation of polysaccharides that bind together soil particles (Fig.1.1). These polysaccharides are deposited by microbial populations around fresh residue that serves as a nucleation site. If the macroaggregates are not disturbed, residue decomposes and fragments into finer organic matter that gradually becomes encrusted with clay particles and microbial products, thus forming microaggregates within macroaggregates. When the macroaggregates become destabilized due to degradation of the binding agents, they fall apart and release the stable microaggregates. These microaggregates then form the building blocks for the formation of new macroaggregates, as suggested by Tisdall and Oades (1982).

Soil organic matter dynamics

SOM dynamics are a function of input and output fluxes. SOM input is mainly due to primary production, which in turn depends on vegetation type and landscape, management practices, soil type and climate. SOM loss is due to decomposition and turnover, which is influenced by climate, quality and stability of SOM and soil biological

activity (Jastrow and Miller, 1998; Lavelle and Spain, 2001). Many soils contain SOM pools with different stability and different turnover times (Campbell et al., 1967); defining SOM pools that relate to soil structure is important for a better understanding of SOM dynamics. Cambardella and Elliott (1992) isolated a SOM fraction with an intermediate turnover time, identified as particulate organic matter (POM) from native and cultivated grasslands. To isolate this fraction, soil was dispersed in sodium hexametaphosphate and then passed through a 53 μm sieve. The fraction retained on the sieve is the POM-fraction after correction for mineral particles. Direct microscopic inspection indicated that POM was primarily composed of root fragments in various stages of decomposition. However, most of the organic matter in the soil occurs in a biologically resistant state, often in association with mineral colloids (Paul and Juma, 1981).

Different mechanisms can be responsible for stabilizing SOM: (i) biochemical recalcitrance, due to chemical characteristics of the SOM itself (e.g., lignin compounds); (ii) chemical stabilization, due to chemical binding of SOM to clay particles; and (iii) physical protection, due to the incorporation of labile SOM into aggregates, protecting the SOM against microbial decomposition (Sorensen and Paul, 1971; Christensen, 1996). Indirect evidence of the protective effect on SOM by soil aggregates is obtained from data showing that disruption of soil aggregates results in an increase in the mineralization of both carbon and nitrogen (Elliot, 1986; Gregorich et al., 1989; Beare et al., 1994).

Several investigators used physical fractionations of SOM in order to directly relate soil aggregates and associated SOM fractions (Golchin et al., 1994; Jastrow, 1996; Six et al., 1999). The importance of differentiating the free and intra-aggregate SOM in

conceptual models of physically based SOM pools is described by Elliott et al. (1996) and Christensen (1996). Intra-aggregate organic matter is incorporated and physically protected within macroaggregates (> 250 μm) (Cambardella and Elliott, 1992), whereas free organic matter is found between aggregates. Golchin et al., (1994) used a densimetric method in which soil samples were suspended in sodium polytungstate solution ($\rho = 1.85$). The floating material was defined as the free light fraction (LF) and the heavy fraction was the intra-aggregate organic matter, consisting of particulate organic matter (iPOM) and mineral-associated organic carbon (mSOC). Based on size considerations, Six et al. (1999) differentiated the iPOM in two fractions: coarse iPOM (250-2000 μm) and fine iPOM (53-250 μm).

Effects on soil aggregation and soil organic matter dynamics

Management practices

No-tillage planting is becoming increasingly adopted as an agricultural technique worldwide. The crops are planted and managed without plowing of the soil, which causes a minimal soil disturbance and a placement of plant residues on the soil surface. This has major impacts on soil organic matter, nutrient cycling, soil aggregation and soil organisms. Under conventional tillage (CT) practices, plowing, harrowing, and rotary tillage result in the mixing of the soil profile and the fragmentation and burial of crop residues (Beare et al., 1994). These effects tend to moderate the temperature and water fluctuations in buried residues and increase their proximity to mineral nutrients, thereby enhancing residue decomposition and organic matter transformations (Blevins et al.,

1984; Beare et al., 1992). Conventional tillage is known to disrupt soil aggregates, increase erodibility, lower water infiltration, and reduce organic matter content, due to direct physical disturbance by plowing. Conventional tillage also has several indirect effects on aggregation (Beare et al., 1994; Beare, 1997; Six et al., 1998a), including (i) the continuing exposure of new soil to drying and wetting at the soil surface; (ii) changes in temperature, moisture, and aeration, due to plowing; and (iii) changes in microbial communities.

No-tillage management, on the other hand, can improve soil aggregation, and increase infiltration and SOM content (Carter, 1992; Bruce et al., 1995). Paustian et al. (2000) found an increase in aggregation, simultaneously with an increase in C content in NT compared to CT. The loss of SOM under CT has been correlated with an increased aggregate turnover (Adu and Oades, 1978; Beare et al., 1994; Six et al., 1998; Six et al., 1999). In CT, tillage and aggregate disruption lead to a release and subsequent decomposition of coarse iPOM (Fig.1), resulting in a reduction in formation and stabilization of fine iPOM inside of CT macroaggregates, compared to NT macroaggregates. In NT agroecosystems, aggregates are less susceptible to disruption, resulting in a greater protection of SOM. Since microaggregates have a slower turnover time than macroaggregates (Jastrow et al., 1996), incorporation of fine iPOM into these microaggregates leads to an increased physical protection of carbon and is therefore believed to be an important factor for carbon sequestration.

No-tillage management increases the surface placement of crop residues and reduces soil disturbance compared to CT. These factors can influence the composition, distribution and activity of soil microbial communities (Doran, 1980a; Brussaard et al.,

1990; Beare et al., 1993). Water content, temperature, and nutrient proximity are the most important variables that differ with residue placement (Beare et al., 1992). These variables affect hyphal growth (Magan and Lynch, 1986) and succession of fungal species (Cooke and Rayner, 1984).

Plowing favors food webs comprising soil organisms with short generation times, small body size, rapid dispersal, and omnivorous feeding habits (Steen, 1983). Conventional tillage, where plant residue is mixed with the soil, should promote bacterial activity and encourage colonization by bacteria (Hendrix et al., 1986). Residue carbon and nitrogen are in intimate contact, providing a more favorable environment for bacterial growth relative to fungal growth (Holland and Coleman, 1987).

Aboveground plant residues decompose on the soil surface and in the upper layers in no-tillage systems. The position and relatively large particle size of the residue promote fungal growth (Hendrix et al., 1986) that conserves soil nutrients through fungal immobilization (Beare et al., 1992). Since the residue remains on the soil surface, soil nitrogen and residue carbon are physically separated. Fungal hyphae can form bridges between these two, allowing use of both resources (Holland and Coleman, 1987). It has also been suggested that fungi are more drought resistant than bacteria (Griffin, 1972), which could explain the relatively higher fungal biomass, associated with the dry residue layer.

Earthworms[1]

Formation of casts

The importance of earthworms in soil systems and the formation of soil structure have been recognized since the time of Charles Darwin. In his book, "The formation of vegetable mould, through the action of worms with observations on their habits" (1881), Darwin described the fine soil particles of dark color 'which cover the whole surface of the land in every moderately humid country' as "vegetable mould." He says this mould has passed many times through the 'intestinal canals' of earthworms and could therefore better be called "animal mould."

After Darwin, more and more researchers focused on the activity of earthworms in soil ecosystems. Earthworms are considered to play an important role in the formation and stability of soil aggregates and the cycling of nutrients (Lee and Foster, 1991; Edwards and Bohlen, 1996). They play a key role in the removal of plant litter and other organic materials from the soil surface and the incorporation of these organic materials into soil aggregates (Martin, 1991). Earthworms are often the dominant soil-ingesting animals. The ingested organic matter is mixed with inorganic soil material, passed through their gut and excreted as a cast. Various early researchers (Ponomareva, 1950, 1953; van de Westeringh, 1972) have stated that up to 50% of surface layer soil aggregates in temperate pastures are made up of earthworm casts. In mull-type forest soils (Kubiena, 1953) and in wooded savanna soils at Lamto, Ivory Coast (Lavelle, 1978), the soil top layer consists almost entirely of earthworm casts. Lee (1985) estimated that in an average temperature pasture and grasslands, earthworms cast 40-50 t

[1]Part of: Six, J., H. Bossuyt., S. De Gryze, K. Denef, 2003. A history of research on the link between aggregates, soil biota and soil organic matter dynamics. Invited for *Soil & Tillage Research*, submitted.

ha^{-1} yr^{-1} on the surface and even more below the surface. Activity might be higher in tropical soils and warmer temperate climates. The amount of castings might also depend on feeding activity. When food supply is limited, earthworms seem to ingest more soil in an effort to obtain sufficient food, and consequently cast more frequently (Abbott and Parker, 1981; Martin, 1982). The relative importance of earthworm casts might depend on ecosystem or soil type. For example, earthworm activity seems to be more important in shrub savannas than in grasslands (Blanchart et al., 1989). Also, Oades (1993) found that earthworms are only major soil aggregate formers when soils are not subjected to severe dry-wet cycles.

Earthworms affect both soil aggregate formation and aggregate stability. There are two ways in which earthworms produce soil aggregates: (i) burrows and (ii) casts (Brown et al., 2000). Burrowing produces aggregates on the burrow walls through pressure and deposition of external mucus (Edwards and Bohlen, 1996). Casts can be deposited on burrow walls, within the burrow itself or on the soil surface. Earthworm activity seems to be important in both microaggregate and macroaggregate formation. These microaggregates are usually formed within larger soil aggregates (see also elsewhere in this paper). Barois et al. (1993) detected that the soil microstructure is completely destroyed in the earthworm's gut and that during gut transit old microaggregates are destroyed and new ones are formed. Jongmans et al. (2001) observed the incorporation of fine organic material in an early stage of decomposition into a microstructure into worm casts which was protected from further microbial attack. Earthworm casts are known to contain more water-stable macroaggregates than surrounding soils (Shipitalo and Protz, 1988; Marinissen, 1994). The formation of water-

stable macroaggregates depends primarily on temporary binding agents (Tisdall and Oades, 1982). Earthworms play a role in the formation of these binding agents (Martin and Marinissen, 1993). Various studies showed a higher stability in earthworm casts than in the surrounding soil aggregates (Monnier and Jeanson, 1964; van Rhee, 1977; De Vleesschauwer and Lal, 1981; McKenzie and Dexter, 1987). Other investigators, however, found that the casting activities only enhance aggregate stability if the casts are dried or aged (Shipitalo and Protz, 1988; Marinissen and Dexter, 1990) and that the stability of the casts depends on the quality of the ingested organic matter (Shipitalo and Protz, 1988).

There are several mechanisms to explain the increase of aggregate stability in the presence of earthworms. Stability might arise from mechanical binding by vascular bundles from ingested plant material (Ponomareva, 1953) or from fungal growth after excretion of the casts (Marinissen and Dexter, 1990). It might also originate from the microorganisms which proliferate in ingested materials in the gut (Parle, 1963, Arthur, 1965). The polysaccharides they form in the casts may strengthen bonds between organic and mineral components resulting in a protection against microbial attack (Martin, 1991). One theory suggests aggregate stability is increased by the cementing of soil particles in the worm's gut by calcium humate formed from decomposing organic material and calcite excreted by the worm's calciferous glands (Satchell, 1967).

Interaction of earthworm species

Earthworms are divided into different categories according to their feeding or casting activities, and these categories have distinct effects on soil aggregation and soil

organic matter dynamics. Bouché (1977) recognized three morpho-ecological groups: (i) epigeic species; (ii) anecic species; and (iii) endogeic species. Epigeic species are defined as litter dwellers. They live above the mineral soil surface, mainly in the litter layer of forest soils. Their activity has little or no effect on soil structure and soil aggregation. Anecic species live in burrows in the mineral soil and feed on dead leaves on the soil surface, which they drag into their burrows (Lee, 1985). They are important in burying surface litter. This results in a mixing of organic and mineral components in their casts, which plays an important role in the formation of stable organo-mineral structures (Lavelle and Spain, 2001). Soil organic matter in their casts tends to be more highly humified than the plant material originally ingested. Anecic species also form an extensive network of burrows. These burrows can also contribute to aggregate stability since they are often lined with oriented clays and humic materials which can form a stable structure (Jeanson, 1964). Endogeic species live in mineral soil horizons and feed on soil more or less enriched with organic matter. They are termed "a major agent of soil aggregation and soil organic matter stabilization" (Lavelle and Spain, 2001). Anecic and endogeic earthworm species are considered 'ecosystem engineers' because they produce soil structures which impact soil properties and soil processes beyond their body size and life-time, even up to landscape levels and decades of time. Their activities can significantly change the resource availability to other soil organisms (Jones et al., 1994; Lavelle et al., 1997; Brown et al., 2000).

In tropical agroecosystems, the interaction of endogeic earthworm species with different effects on soil structure and soil aggregation appears to be necessary to sustain physical soil fertility (Blanchart et al., 1997). Blanchart et al. (1998) describe two

different types of endogeic earthworms, depending on their size and casting activity. Large species, such as *Pontoscolex corethrurus* or *Millsonia anomala*, egest large and compact casts (diameter > 5 mm). They are called 'compacting species' and they are known to increase the soil bulk density and proportion of large aggregates. The increase in bulk density is caused by (i) the formation of organo-mineral bonds after mixing and chemical transformations in gut; (ii) reabsorption of water in the latter part of the gut; and (iii) strong compaction due to tail region muscles when casts are expelled (McKenzie and Dexter, 1987). Casts of compacting species might create anaerobic conditions with a slowdown in decomposition and a possible protection of carbon (Blanchart et al., 1993). Smaller earthworm species feed on these large casts and form smaller and more delicate casts (0.5-5 mm). These species are called decompacting species (ex: Eudrilidae). They decrease the soil bulk density and the proportion of large, compact aggregates. When decompacting species ingest casts of compacting species, previously physically-protected C can be mineralized. When, on the other hand, compacting species ingest casts of decompacting species, mineralizable C can be protected in their large casts (Blanchart et al., 1999; Dickschen and Topp, 1987). The activity of only compacting earthworm species can cause the formation of a compact surface crust. This might impede water infiltration when no organic residues are present, but in the presence of organic residues, a favorable macroaggregate soil structure can develop (Blanchart et al., 1997, Blanchart et al., 1999). Barros et al. (2001) described a site after forest clearing where a single species, *Pontoscolex corethrurus*, became very abundant. This species formed a continuous compacted layer, impermeable to water. This layer reached a thickness of 20

cm. The compacting effects here appeared "more pronounced than that of a bulldozer" (Chauvel et al., 1999).

Effects on soil aggregation over time

At the scale of a few hours or days, mineralization inside of earthworm casts is promoted (Brown, 1995; Edwards and Bohlen, 1996, Brown et al., 2000). Unstable aggregates are broken down and microbial activity is stimulated. At the same time, an enhanced formation of microbial byproducts serving as binding agents promotes the formation of new aggregates. A decline of microbial activity is detected in earthworm casts within days of cast excretion and is correlated with the disappearance of labile nutrients (Lavelle et al., 1989; Scheu and Wolters, 1991). After the decline of these labile nutrients, more recalcitrant organic materials become integrated into compact structures as organo-mineral microaggregates (Lavelle et al., 1997) where they are protected from microbial decomposition as long as the structures are maintained (Garnier-Zarli and Harry, 1995). Lierman and Woomer (Unpubl. data, cited by Lavelle et al., 1997) found that the total soil C stock first declined after earthworm introduction. The effect was reversed after 12 years; and after 30 years, C stock was 28% greater in the presence of earthworms. McCartney et al. (1997) found higher pools of organic matter after three years of earthworm reduction. Similarly, Gilot-Villenave et al. (1996) and Pashani et al. (1996) detected a decrease of SOM and an overall acceleration of soil organic matter turnover in the presence of earthworms. Martin and Parton (unpublished data in Lavelle et al., 1998) used the CENTURY model to simulate the effect of earthworm removal on SOM content. The model showed that removal of earthworms

resulted in a reduction of aggregate formation and a significant decrease of SOM after three decades.

Effects of management practices on earthworm abundance

Agricultural management such as tillage can influence earthworm numbers and biomass (Lee, 1985; Hendrix et al., 1987; Lavelle et al., 1989; Hendrix et al., 1992; Fraser, 1994). Tillage can reduce earthworm numbers in different ways. Earthworms may be damaged directly by machinery, exposed to predation or adverse weather, their burrow systems may be disrupted, or the availability of food may be reduced (Edwards and Lofty, 1982; Springett, 1983; Lee, 1985; Dalby, 1996).

Objective of this study

The objective of this study was to investigate important underlying mechanisms of C sequestration and SOM dynamics. Labeled residues (^{13}C or ^{14}C) were used to follow the input of fresh organic materials into different aggregate-associated C fractions and/or layers of the soil profile. While numerous studies have focused on the impacts of tillage on agricultural ecosystems, the exact interactions between SOM dynamics, tillage practices and soil structure remain unknown. And while many researchers have acknowledged the importance of earthworm activity in agricultural fields, it is still unclear how exactly earthworms incorporate organic matter into the soil and can play an important role in the protection and stabilization of C that could otherwise be mineralized. I realize it is extremely important to focus on the global picture of C

sequestration and global warming. I also believe, however, that it is important to investigate the underlying physical mechanisms that will help us understand which factors play an important role in the sequestration of organic matter in agricultural soils.

Thesis outline

Chapter 2 describes an experiment where a single input of ^{14}C-labeled corn residue is traced after 3 years of application in NT and CT experimental plots. Different aggregate-associated C pools are fractionated in different layers of the soil and differences between CT and NT fields are addressed.

Chapter 3 and ***Chapter 4*** describe the effect of earthworms on the formation of soil aggregates, organic residue incorporation and the stabilization of freshly added C inside stable microstructures formed within earthworm casts.

Chapter 5 describes the interactive effects of different earthworm species on the formation of soil aggregates, incorporation of organic matter and formation of stable microstructures within earthworm casts.

References

Abbott, I., and Parker, C.A., 1981. Interactions between earthworms and their soil environment. Soil Biology and Biochemistry, 13: 191-197.

Arthur, D.R., 1965. Form and function in the interpretation of feeding in lumbricid worms. In: Carthy, J.D., Duddington, C.L. (Eds.), Viewpoints in Biology. Butterworths, London. Vol.4, pp. 204-251.

Ball, B.C., O'Sullivan, M.F., and Hunter, R., 1988. Gas diffusion, fluid flow and derived pore continuity indices in relation to vehicle traffic and tillage. Journal of Soil Science, 39: 327-339.

Barois, I., Villemin, G., and Lavelle, P., 1993. Transformation of the Soil Structure through *Pontoscolex corethrurus* (Oligochaeta) intestinal tract. Geoderma, 56: 57-66.

Barros, E., Curmi, P., Hallaire, V., Chauvel, V., and Lavelle, P., 2001. The role of macrofauna in the transformation and reversibility of soil structure of an oxisol in the process of forest to pasture conversion. Geoderma, 100: 193-213.

Baver, L.D., 1956. Soil Physics, 3rd ed. London. Wiley.

Beare, M.H., Pohlad, B.R., Wright, D.H., and Coleman, D.C., 1993. Residue placement and fungicide effects on fungal communities in conventional and no-tillage soils. Soil Science Society of America Journal, 57: 392-399.

Beare, M.H., Hendrix, P.F., and Coleman, D.C., 1994. Water-stable aggregates and organic matter fractions in conventional – and no-tillage soils. Soil Science Society of America Journal, 58: 777-786.

Blanchart, E., Lavelle, P., and Spain, A.V., 1989. Effects of two species of tropical

earthworms (Oligochaete: Eudrilidae) on the size distribution of aggregates in an African soil. Revue d'Ecologie et de Biologie du Sol, 26: 417-425.

Blanchart, E., Bruand, A., and Lavelle, P., 1993. The physical structure of casts of *Millsonia anomala* (Oligochaeta: Megascolecidae) in shrub savanna soils (Côte d'Ivoire). *In*: Brussaard, L. and Kooistra, M.J. (Eds.), International Workshop on Methods of Research on Soil structure/Soil biota Interrelationships. Geoderma 56: 119-132.

Blanchart, E., Lavelle, P., Braudeau, E., Le Bissonnais, Y., and Valentin, C., 1997. Regulation of soil structure by geophagous earthworm activities in humid savannas of Côte d'Ivoire. Soil Biology and Biochemistry, 29: 431-439.

Blanchart, E., Albrecht, A., Alegre, J., Duboisset, A., Gilot, C., Pashanasi, B., Lavelle, P., and Brussaard, L., 1999. Effects of Earthworms on Soil Structure and Physical Properties. *In*: Lavelle, P., Brussaard, L., Hendrix, P. (Eds.), Earthworm Management in Tropical Agroecosystems. CABI Publishing, New York. pp 149-172

Bouché, M.B., 1977. Stratégies lombriciennes. *In*: Lohm, U., Persson, E. (Eds.), Soil Organisms as Components of Ecosystems. Biol. Bull. (Stockholm), 25: 122-132.

Brown, G.G., 1995. How do earthworms affect microfloral and faunal community diversity? Plant and Soil, 170: 209-231.

Brown, G.G., Barois, I., and Lavelle, P., 2000. Regulation of soil organic matter dynamics and microbial activity in the drilosphere and the role of interactions with other edaphic functional domains. European Journal of Soil Biology, 36: 177-198.

Brussaard, L., Bouwman, L.A., Geurs, M., Hassink, J., and Zwart, K.B., 1990. Biomass, composition and temporal dynamics of soil organisms of a silt loam soil under conventional and integrated management. Netherlands Journal of Agricultural Science, 38: 283-302

Cambardella, C.A., and Elliott. E.T., 1992. Carbon and nitrogen distribution in aggregates from cultivated and native grassland soils. Soil Science Society of America Journal, 58: 123-130.

Campbell, C.A., Paul, E.A., Rennie, D.A., and McCallum, K.J., 1967. Applicability of the carbon dating method of analysis to soil humus studies. Soil Science, 104: 217-224.

Carter, M.R., 1990. Relative measures of bulk density to characterize compaction in tillage studies on fine sandy loams. Canadian Journal of Soil Science, 70: 425-433.

Chauvel, A., Grimaldi, M., Barros, E., Blanchart, E., Sarrazin, M., and Lavelle, P., 1999. An amazonian earthworm compacts more than a bulldozer. Scientific Correspondence/ Nature 398: 32-33.

Christensen, B.T., 1996. Matching measurable soil organic matter fractions with conceptual pools in simulation models of carbon turnover: revision of model structure. *In*: Powlson, D.S., and Smith, J.U. (Eds.). Evaluation of soil organic matter models. Springer, New York. NATO ASI Series, vol. I 38. pp 143-159.

Cooke, R.C., and Rayner, A.D.M., 1984. Ecology of saprophytic fungi. Longman, New York.

Dalby, P.R., 1996. Competition between earthworms in high rainfall pastures in the Mt. Lofty Ranges, South Australia. Ph. D. Thesis, University of Adelaide.

Darwin, C., 1881. The Formation of Vegetable Mould, through the Action of Worms with Observations on Their Habits. Murray, London, 298 pp.

De Vleesschauwer, D., and Lal, R., 1981. Properties of worm casts under secondary tropical forest regrowth. Soil Science, 132: 175-181.

Dexter, A.R., 1988. Soil amelioration by natural processes. Proc. Symp. on Soil Management '88. Darling Downs Institute of Advanced Education, Toowoomba, Queensland, 19-21: 431-448.

Dickschen, F., and Topp, W., 1987. Feeding activities and assimilation efficiencies of *Lumbricus rubellus* (Lumbricidae) on a plant-only diet. Pedobiologia, 30: 31-37.

Doran, J.W., 1980. Microbial changes associated with residue management with reduced tillage. Soil Science Society of America Journal, 44: 518-524.

Edwards, C.A., and Lofty, J.R., 1982. The effect of drilling and minimal cultivation on earthworm populations. Journal of Applied Ecology, 17: 533-543.

Edwards, C.A., and Bohlen, P.J., 1996. Biology and Ecology of Earthworms. Chapman and Hall, London, 426 pp.

Elliott, E.T., 1986. Aggregate structure and carbon, nitrogen, and phosphorus in native and cultivated soils. Soil Science Society of America Journal, 50: 627-633.

Elliott, E.T., Paustian, K., and Frey, S.D., 1996. Modeling the measurable or measuring the modelable: A hierarchical approach to isolating meaningful soil organic matter fractions. In: Evaluation of soil organic matter models. NATO ASI Series, vol. I 38. Powlson, D.S., and Smith, J.U. (Eds.) pp 161-179.

Fraser, P.M., 1994. The impact of soil and crop management practices on soil macrofauna. In: Soil Biota. Management in Sustainable Farming Systems. Pankhurst, C.E., Doube, B.M., Gupta, V.V.S.R., and Grace, P.R. (Eds.) pp. 125-132.

Garnier-Zarli E., and Harry, M., 1995. Distribution of humic compounds in mounds of soil soil-feeding termite species of tropical rainforests: Its influence on soil structure stability. Insectes Societaux, 42: 167-185.

Gilot-Villenave D., Lavelle, P., and Ganry, F., 1996. Effects of a tropical geophagous earthworm, *Millsonia anomala*, on some soil characteristics, on maize-residue decomposition and on maize production in Ivory Coast. Applied Soil Ecology, 4: 201-211.

Golchin, A., Oades, J.M., Skjemstad, J.O., and Clarke, P. 1994. Study of free and occluded particulate organic matter in soils by solid state 13C CP/MAS NMR spectroscopy and scanning electron microscopy. Australian Journal of Soil Research, 32: 285-309.

Gregorich, E.G., Kachanoski, R.G., and Voroney, R.P., 1989. Carbon mineralization in soil size fractions after various amounts of aggregate disruption. Journal of Soil Science, 40: 649-659.

Griffin, D.M., 1972. Ecology of soil fungi. Syracuse University press, Syraceuse, New York.

Hendrix, P.F., Parmelee, R.W., Crossley, D.A., Jr., Coleman. D.C., Odum, E.P. and Groffman, P.M., 1986. Detritus food webs in convential and no-tillage agroecosystems. BioScience, 36: 374-380.

Hendrix, P.F., Coleman. D.C., and Crossley, D.A., Jr., 1992. Using knowledge of soil nutrient cycling processes to design sustainable agriculture. Journal of Sustainable Agriculture, 2: 63-82.

Holland, E.A., and Coleman, D.C., 1987. Litter placement effects on microbial and organic matter dynamics in an agroecosystem. Ecology, 68: 425-433.

Jastrow, J.D., 1996. Soil aggregate formation and the accrual of particulate and mineral-associated organic matter. Soil Biology and Biochemistry, 28: 656-676.

Jastrow, J.D., Boutton, T.W., and Miller, R.M., 1996. Carbon dynamics of aggregate-associated organic matter estimated by carbon-13 natural abundance. Soil Science Society of America Journal, 60: 801-807

Jastrow, J.D., and Miller, R.M., 1998. Soil aggregate stabilization and carbon sequestration: feedbacks through organomineral associations. *In*: Lal, R., Kimble, J.M., Follet, R.F., and Stuart, B.A. (Eds.). Soil Processes and the Carbon Cycle. CRC Press, Boca Raton, FL.

Jeanson, C., 1964. Micromorphology and experimental soil zoology: contribution to the study, by means of giant-sized thin section, of earthworm-produced artificial soil structure. *In*: Jongerius, A., (Ed.), Soil Micromorphology. Proc. 2nd Int. Work mett. soil micromophology, Arnhem, the Netherlands, pp 47-55.

Jones, C.G., Lawton, J.H., and Shachak, M., 1994. Organisms as ecosystem engineers. Oikos, 69: 373-386.

Jongmans, A.G., Pulleman, M.M., and Marinissen, J.C.Y., 2001. Soil structure and earthworm activity in a marine silt loam under pasture versus arable land. Biology and Fertility of Soils, 33: 279-285.

Kay, B.T., 1990. Rates of change of soil structure under different cropping systems. Advances in Soil Science, 12:1-41.

Kubiena, W.L., 1953. The Soils of Europe. Murby, London.

Lavelle, P., 1978. Les vers de terre de la savane de Lamto (Côte d'Ivoire). Peuplements, populations et fonctions de l'écosystème. Publ. Lab. Zool. E.N.S., 12: 1-301.

Lavelle, P.I., Barois, I., Martin, A., Zaida, Z., and Schaefer, R., 1989. Management of earthworm populations in agroecosystems: a possible way to maintain soil quality? *In*: Clarholm, M., and Bergstrom, L. (Eds.). Ecology of Arable Land. Kluwer Academic, Boston. pp. 109-122.

Lavelle, P., Schaefer, R., and Zaidi, Z., 1989. Soil ingestion and growth *in Millsonia anomala*, a tropical earthworm, as influenced by the quality of the organic matter ingested. Pedobiologia, 33: 379-388

Lavelle, P., Bignell, D., Lepage, M., Wolters, V., Roger, P., Ineson, P., Heal, O.W., and Dhillion, S., 1997. Soil function in a changing world: the role of invertebrate ecosystem engineers. European Journal of Soil Biology, 33: 159-193.

Lavelle, P., Pashani, B., Charpentier, F., Gilot, C., Rossi, J.P., Derouard, L., Andre, J., Ponge, J.P., and Bernier, N., 1998. Large-Scale Effects of Earthworms on Soil Organic Matter and Nutrient Dynamics. *In*: Edwards, C.A. (Ed.), Earthworm Ecology. St. Lucie Press, Boca Raton, FL. pp. 103-122.

Lavelle,P., and Spain, A.V., 2001. Soil Ecology. Kluwer Academic Publishers, Dordrecht, Netherlands. 654 pp.

Lee, K.E., 1985. Earthworms, Their Ecology and Relationships with Soils and Land Use. Academic Press, Sydney.

Lee, K.E., and Foster, R.C., 1991. Soil fauna and soil structure. Australian Journal of Soil Research, 29: 745-776.

Lynch, J.M., and Bragg, E., 1985. Microorganisms and Soil Aggregate Stability. Advances in Soil Science, 2: 134-170.

Magan, N., and Lynch, J.M., 1986. Water potentials, growth and cellulolysis of fungi involved in decomposition of cereal residues. Journal of General Microbiology, 132: 1181-1187.

Marinissen, J.C.Y., 1994. Earthworms and stability of soil structure: a study in a silt loam soil in a young Dutch polder. Agriculture Ecosystems and Environment, 51: 75-87.

Marinissen, J.C.Y., and Dexter, A.R., 1990. Mechanisms of stabilisation of earthworm casts and artificial casts. Biology and Fertility of Soils, 9: 163-167.

Martin, J.P., Martin, W.P., Page, J.B., Raney, W.A., and De Ment, J.D., 1955. Soil aggregation. Advances in Agronomy, 7: 1-37.

Martin, N.A., 1982. The interaction between organic matter in soil and the burrowing activity of three species of earthworms (Oligochaeta: Lumbricidae). Pedobiologia, 24: 185-190.

Martin, A., 1991. Short- and long-term effects of the endogeic earthworm *Millsonia anomala (Omodeo) (*Megascolecidae, Oligochaeta) of tropical savannas on soil organic matter. Biology and Fertility of Soils, 11: 234-238.

Martin, A., and Marinissen, J.C.Y., 1993. Biological and physico-chemical processes in excrements of soil animals. Geoderma, 56: 331-347.

McCartney, D.A., Stinner, B.R., Bohlen, P.J., 1991. Organic matter dynamics in maize agroecoystems as affected by earthworm manipulations and fertility source. Soil Biology and Biochemistry, 29: 397- 400.

McKenzie, B.M., and Dexter, A.R., 1987. Physical properties of casts of the earthworm *Aporrectodea rosea*. Biology and Fertility of Soils, 5: 328-332.

Monnier, G., and Jeanson, G., 1964. Studies on the stability of soil structure: Influence of moulds and soil fauna. Proc. 11th Easter School in Agricultural Science, Univ. Nottingham

Oades, J.M., 1984. Soil organic matter and structural stability, mechanisms and implications for management. Plant and Soil, 76: 319-337.

Oades, J.M., 1993. The role of biology in the formation, stabilization and degradation of soil structure. *In*: Brussaard, L. and Kooistra, M.J. (Eds.), International Workshop on Methods of Research on Soil struture/Soil biota Interrelationships. Geoderma 56: 377-400.

Pashani, B., Lavelle, P., and Alegre, J., 1996. Effect of inoculation with the endogeic earthworm *Pontoscolex corethrurus* on soil chemical characteristics and plant growth in a low-input agricultural systems of Peruvian Amazonia. Soil Biology and Biochemistry, 28: 801-810.

Parle, J.N., 1963. Micro-organisms in the intestines of earthworms. Journal of General Microbiology, 31: 1-11.

Paul, E.A., and Juma, N.G., 1981. Mineralization and immobilization of soil nitrogen by

microorganisms. *In*: F.E. Clark and T. Rosswall (Eds). Terrestrial Nitrogen Cycles: processes, ecosystem strategies and management impacts. Swedish Natural Science Research Council, Stockholm. pp: 179-195.

Paustian, K., Six, J., Elliott, E.T., and Hunt, W.H., 2000. Management options for reducing CO_2 emissions from agricultural soils. Biogeochemistry, 48: 147-163.

Ponomareva, S.I., 1950. the role of earthworms in the creation of a stable structure in ley rotations. Pochvovedenie, 476-486.

Ponomareva, S.I., 1953. The influence of the activity of earthworms on the creation of a stable structure in a sod-podzolized soil. Trud. Pochv. Inst. Dokuchaeva, 41: 304-378.

Satchell, J.E., 1983. Earthworm Ecology. From Darwin to Vermiculture. Chapman and Hall, London and New York, 495 pp.

Scheu, S., and Wolters, V., 1991. Influence of fragmentation and bioturbation on the decomposition of ^{14}C-labeled beech leaf litter. Soil Biology and Biochemistry, 23: 1029-1034.

Shipitalo, M.J., and Protz, R., 1988. Factors influencing the dispersibility of clay in worm casts. Soil Science Society of America Journal, 52: 764-769.

Six, J., Elliott, E.T., and Paustian, K., 1998. Aggregate and SOM dynamics under conventional and no-tillage systems. Soil Science Society of America Journal, 63: 1350-1358.

Six, J., Elliott, E.T., Paustian, K., and Doran, J.W., 1999. Aggregation and soil organic matter accumulation in cultivated and native grassland soils. Soil Science Society of America Journal, 62: 1367-1377.

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.

Sorensen, L.H., and Paul, E.A., 1971. Transformation of acetate carbon into carbohydrate and amino acid metabolites during decomposition in soil. Soil Biology and Biochemistry, 3: 173-180.

Springett, J.A., 1983. Effect of five species of earthworm on some soil properties. Journal of Applied Ecology, 20: 865-872

Steen, E., 1983. Soil animals in relation with agricultural practices and soil productivity. Swedish Journal of Agricultural Research, 13: 157-165.

Tisdall, J.M., and Oades, J.M., 1982. Organic matter and water-stable aggregates in soils. Journal of Soil Science, 33: 141-161.

van de Westeringh, W., 1972. Deterioration of soil structure in worm free orchards. Pedobiologia, 12: 6-15.

van Rhee, J.A., 1977. A study of the effect of earthworms on orchard productivity. Pedobiologia, 17: 107-114.

Vanveen, J.A., and Paul, E.A., 1981. Organic-carbon dynamics in grassland soils. 1. Background information and computer-simulation. Canadian Journal of Soil Science, 61: 185-201.

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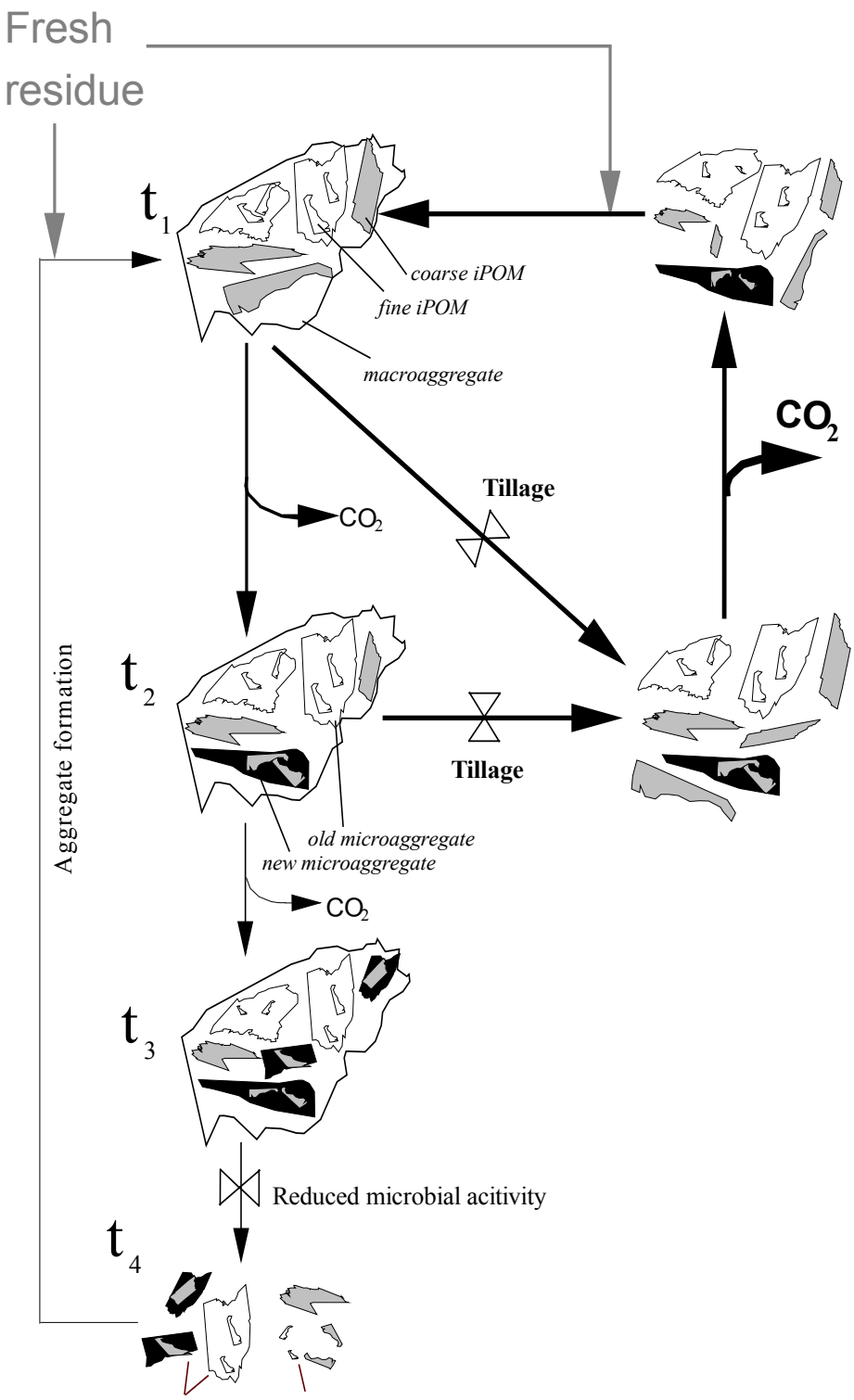
Fresh
residue
t1
coarse iPOM
fine iPOM
macroaggregate
CO2
Increased decomposition
due to reduced physical
protection
Tillage
CO2
t2
Tillage
Aggregate formation
old microaggregate
new microaggregate
CO2
t3
Reduced microbial acitivity
t4

Fig. 1.1

CHAPTER 2

AGGREGATE-PROTECTED CARBON IN NO-TILLAGE AND CONVENTIONAL TILLAGE AGROECOSYSTEMS USING ^{14}C-LABELED PLANT RESIDUE[1]

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Abstract

No-tillage (NT) management can result in higher soil organic matter levels than conventional tillage (CT) practices. The objective was to investigate the underlying mechanisms in which carbon (C) is protected under NT management, using ^{14}C-labeled plant residue as a tracer. Samples were collected from the Horseshoe Bend Research area in Athens, GA. Aggregate size distribution, total C and ^{14}C were measured together with different pools of aggregate-associated C and ^{14}C from 21-day laboratory incubations of intact and crushed macro- and microaggregates. Total C was higher in NT than in CT in all the aggregate size classes of the 0-2.5 cm and 2.5-5 cm layers. Carbon-14 was significantly higher in NT than in CT in all the aggregate size classes of the 0-2.5 cm layer and in the > 2000 μm and 53-250 μm aggregate size classes of the 2.5-5 cm depth. At the 5-15 cm depth, more ^{14}C was found in NT than in CT in the > 2000 μm aggregate size class and more ^{14}C was found in CT than in NT in the 53-250 μm and < 53 μm size classes. Unprotected C and ^{14}C pools, microaggregate-protected and micro- within-macroaggregate-protected C and ^{14}C pools were significantly higher for the 0-2.5 cm and 2.5-5 cm layers in NT than in CT. Carbon-14 pools were generally higher in CT than in NT at the 5-15 cm depth, while total C did not differ between tillage treatments at this depth. The results indicate that NT protects more C than CT in the surface layers of the soil and this protection occurs mainly at the microaggregate level.

Introduction

Maintenance of soil organic matter (SOM) is desirable in agroecosystems because of its beneficial effects on nutrient dynamics and soil structure. Soil organic matter improves soil structural stability, reduces surface crusting and compaction, and increases infiltration, percolation, and water holding capacity of the soil (Tisdale et al., 1993; Stevenson, 1994). The high surface area and chemical nature of SOM buffer pH changes and increase cation exchange capacity (Stevenson, 1994; Sparks, 1995). Sequestration of C in soil is also becoming increasingly important because of the concern over global warming and rising levels of atmospheric CO_2. Soil is estimated to be the largest terrestrial pool of C, containing 1500 Pg or twice as much as the atmosphere (Sundquist, 1993; Schlesinger, 1997).

Management practices, such as no-tillage and reduced tillage, that promote maintenance and accumulation of soil C, are increasingly adopted by farmers because of the growing interest in conservation of SOM (Kern and Johnson, 1993; Burke et al., 1995). No-tillage (NT) management can increase C storage in soils, as well as improve physical, chemical, and biological soil characteristics (Paustian et al., 1997; Hendrix et al., 1998). Soil organic matter may be protected from microbial attack by adsorption to clay minerals (Oades, 1984; Ladd et al., 1985), by the formation of microaggregates (Edwards and Brenner, 1967; Gregorich et al., 1989; Besnard et al., 1996; Six et al., 2000b), by isolation in soil micropores (Adu and Oades, 1978; Foster, 1981), and by physical protection within stable macroaggregates (Elliott, 1986; Gupta and Germida, 1988).

Improvements of soil structure, determined by the level of aggregation, are thought to play an important role in reducing decompositional losses of SOM under no-tillage. Soil aggregates are disrupted by tillage practices and this may lead to an enhanced aggregate turnover and increased decomposition of SOM (Six et al., 1998). The highly dispersible, kaolinitic-clay-based soils of the southeastern USA are very susceptible to aggregate disruption, surface crusting, reduced infiltration, and erosion (Miller and Baharuddin, 1986). These processes can contribute to rapid losses of SOM and a decrease in productivity of agricultural soils in this region (Sanchez et al., 1989; Bruce et al., 1990). Tisdall and Oades (1982) proposed a conceptual model of soil structure describing the binding of mineral particles into microaggregates (50-250 μm) and of microaggregates into macroaggregates (> 250 μm). Free primary particles are mainly cemented together into microaggregates by persistent binding agents (e.g. clay-polyvalent metal-humified organic matter complexes), characterized as older, more humified, or recalcitrant SOM. The more temporary (e.g., roots and fungal hyphae) and transient (e.g., polysaccharides) agents are thought to bind microaggregates into macroaggregates and are generally considered to be relatively more labile or decomposable (van Veen and Paul, 1981; Elliot, 1986). Because of the nature of the binding agents involved, macroaggregates are less stable than microaggregates (Oades, 1984; Beare et al., 1994b) and consequently more susceptible to the disruptive forces induced by cultivation (Tisdall and Oades, 1980, 1982).

The conceptual model of Tisdall and Oades (1982) was later modified by Oades (1984) who suggested that microaggregates are predominantly formed within macroaggregates. This modification has formed the basis of several recent studies (Beare

et al., 1994a; Gale et al., 2000; Six et al., 1999, 2000b). First, macroaggregates (250-2000 μm) are formed around fresh residue. If the macroaggregates are not disturbed (e.g., under NT), residue decomposes and fragments into finer organic matter that gradually becomes encrusted with clay particles and microbial products forming microaggregates within macroaggregates. When the macroaggregates become destabilized due to degradation of the binding agents, they fall apart and release the stable microaggregates. These microaggregates then form the building blocks for the formation of new macroaggregates, as suggested by Tisdall and Oades (1982).

Due to the relatively high background levels of soil carbon, it is usually not possible to detect changes in total carbon resulting from management practices in the short term (3yr.). In this study, we used ^{14}C-labeled plant residue to trace C transformations in soils under CT vs. NT management. It allowed us to follow a one-time organic input of ^{14}C into different soil fractions and layers of the soil profile.

While several studies have investigated the influence of management practices on SOM dynamics and C sequestration, the exact fate of newly added C and the level at which C is protected in NT soils compared to CT soils is still unclear. The objective of this study was to investigate the underlying mechanisms involved in incorporation and protection of C after plant residue application under different management practices. Our main hypothesis is based on the aggregate-turnover model described by Six et al. (2000). Based on this model (described above), we hypothesized that microaggregates within macroaggregates may be important in physically protecting C. Since NT soils contain more stable microaggregates within macroaggregates, we hypothesized that more C would be protected in NT soils than in CT soils. In this study, we looked at the effect of

tillage practices on (i) aggregate size distribution; (ii) total and young (^{14}C) carbon concentrations and; (iii) decomposition and stabilization of aggregate-associated C fractions 3 years after application of ^{14}C-labeled crop residue.

Materials and methods

Site description and soils

Soils were sampled from a set of 16-year-old conventional tillage (CT; moldboard plowed (15 cm depth), disked (10 cm depth) and rotary-tilled (10 cm depth)) and no-tillage (NT; direct-drilled) plots at the Horseshoe Bend Research Area near Athens, GA. The site is located in the Piedmont of the southern Appalachian Mountains (33° 54'N, 83° 24'W). The soil is a Hiawassee series fine loamy, siliceous, thermic, Rhodic Kanhapludult (66% sand, 13% silt, 21% clay). Total C and ^{14}C contents of the soils (measured in Sept, 1999) are given in Table 2.1. Prior to tillage treatments, the plots had been in grass or forage since at least 1938 (Hendrix, 1997). Corn (*Zea Mays* L.) or sorghum [*Sorghum bicolor* (L.) Moench] was no-till planted with an Allis Chalmers seed drill as a summer crop, and rye (*Secale cereale* L.) or crimson clover (*Trifolium incarnatum* L.) was broadcast as a winter cover crop. In November 1996, 20-cm wide acrylic sheets were inserted 15 cm deep to create 1 x 2 meter plots within the larger plots. Carbon-14 (144 Mbq m^{-2}) labeled, dry, corn leaf and stem material were incorporated and the soil turned and pulverized with a shovel to a depth of 15 cm in the CT plots and left on the surface of the NT plots without soil disturbance. General C data from adjacent long-term NT and CT plots suggest that tilling with a shovel was a good simulation of the

CT treatment. A complete description of the labeling technique has been reported by Kisselle et al. (1999). Total C addition averaged 346 g m^{-2} assuming 45 % C in the residue. Cropping and non-labeled residue additions continued as in the larger plots. Crop biomass was mowed and incorporated in the CT plots and left on the surface of NT plots (summer and winter). No crop biomass (including grain and grain heads) was removed. Soil samples were taken from the 3 replicate NT and CT plots on September 29, 1999. No residues were removed prior to soil sampling. Six replicate soil cores were collected randomly from each plot with a 6-cm diameter hammer-driven corer, with a plastic insert, to a depth of 15 cm. The intact samples were sectioned into 0-2.5, 2.5-5 and 5-15 cm depth increments and bulked by depth within plots. All soil samples were sieved (10 mm) in field moist state prior to wet sieving by gently breaking apart the soil along natural planes of weakness.

Aggregate size distributions

Aggregate size distribution of field moist soils was determined by wet sieving, as described by Elliott (1986). A series of three sieves was used to obtain four aggregate size fractions: (i) > 2000 μm (large macroaggregates); (ii) 250-2000 μm (small macroaggregates); (iii) 53-250 μm (microaggregates); (iv) < 53 μm (silt and clay fraction). Following wet sieving, the aggregate size classes > 53 μm were dried on the sieves in a dehumidifying chamber (10°C). Particles < 53 μm were collected in a bucket, total volume was measured, and a subsample of a known volume was taken for analysis. Subsamples from each aggregate size class were ground and analyzed for total C and ^{14}C content.

Carbon analyses

Total organic C content of aggregate size classes was measured on a Carlo Erba, NA 1500, CHN Combustion Analyzer. Carbon-14 was measured using a Harvey Oxidizer (Harvey OX500, R.J. Harvey Instruments Co., Hillsdale, NJ) and a Beckman LS 3801 (Beckman Instruments, Fullerton, CA) liquid scintillation counter. Carbon contents were expressed as amount of C in the aggregate size class per kg of sandfree aggregate. Because of the difference in amount of sand between aggregate size classes, Elliott et al. (1991) suggested that comparisons of C content across aggregate size classes should be based on sand-corrected C data. The sand content of the aggregate size class was determined by dispersing 5 g of the fraction in 20 ml of sodium hexametaphosphate and sieving through a 53 μm sieve. Sand-free C concentration was calculated as follows:

$$\text{Sand-free C (g kg}^{-1}\text{)} = \frac{C_{fraction}\ (\text{g kg}^{-1})}{1\text{-[sand proportion]}_{\ fraction}\ (\text{g kg}^{-1})}$$

Incubations

Five sets of incubations were conducted for each cultivation treatment and for each depth: (i) macroaggregates (large and small mixed); (ii) crushed macroaggregates (large and small mixed) i.e., ground until < 250 μm; (iii) crushed macroaggregates i.e., ground until < 53 μm; (iv) microaggregates and; (v) crushed microaggregates i.e., ground until < 53 μm. Soil samples (25-30 g) to be incubated were weighed into plastic cups and deionized water was added to achieve 55 % water-filled porosity. Plastic cups were

incubated (30°C) in sealed jars containing alkali CO_2 traps (1M NaOH). The CO_2 traps were changed on days 3, 7, 14 and 21 and the respired C was measured by titration with standardized 0.25M HCl after precipitation of carbonates with $BaCl_2$. The respired ^{14}C was measured by adding 0.5 mL NaOH from base traps to 10 mL EcoLite scintillation fluor (ICN biomedicals, Costa Mesa, CA), maintaining samples in the dark for 7 days, and counting for 20 minutes on a Beckman LS 3801 (Beckman Instruments, Fullerton, CA) liquid scintillation counter.

Calculations

The results of the aggregate incubations were used to define five aggregate-associated C pools: (i) unprotected macroaggregate C, (ii) unprotected microaggregate C, (iii) macroaggregate-protected C, (iv) microaggregate-protected C and; (v) micro within macroaggregate-protected C. These different pools were calculated as follows (pools were expressed in g C kg^{-1} sandfree aggregate and kBq ^{14}C kg^{-1} sandfree aggregate):

1) Unprotected macroaggregate C

   = intact macroaggregate C_{min}

2) Unprotected microaggregate C

   = intact microaggregate C_{min}

3) Macroaggregate-protected C

   = < 250 μm crushed macroaggregate C_{min} – intact macroaggregate C_{min}

4) Microaggregate-protected C

   = < 53 μm crushed microaggregate C_{min} – intact microaggregate C_{min}

5) Micro within macroaggregate-protected C

= < 53 μm crushed macroaggregate C_{min} – macroaggregate-protected C –

Unprotected macroaggregate C

[replace macroaggregate-protected C (see equation 3))]

= < 53 μm crushed macroaggregate C_{min} – (< 250 μm crushed macroaggregate

C_{min} – intact macroaggregate C_{min}) – intact macroaggregate C_{min}

= < 53 μm crushed macroaggregate C_{min}– < 250 μm crushed macroaggregate C_{min}

with C_{min} the cumulative C mineralized after 21 days from the intact and crushed aggregate treatments.

Statistical analysis

The data were analyzed, using the SAS statistical package for analysis of variance (ANOVA-PROC MIXED, SAS Institute, 1990). Tillage treatments and depths were considered fixed effects while replicate was considered a random effect (n=3). Separation of means was tested with the DIFF option of the LSMEANS statement with a significance level of $P< 0.05$.

Results

Water-stable aggregates

There was a significant impact of tillage practices on the distribution of water-stable aggregates (WSA) in the surface samples (0-2.5 cm and 2.5-5 cm) (Fig. 2.1). Large macroaggregates (> 2000 μm) made up the largest percentage (~ 50% on average) of the whole soil for NT samples and were on average 2.4 times greater than in CT samples. The smaller aggregate size classes (250-2000 μm, 53-250 μm and < 53 μm) made up a greater proportion of the surface soil in CT than in NT. For the lowest depth (5-15 cm), only the < 53 μm size class was significantly different between CT and NT and was higher in CT than in NT.

Carbon concentrations

Total aggregate-associated C concentrations were significantly influenced by tillage, aggregate size class, depth and their interactions. In the surface layers (0-2.5 cm and 2.5-5 cm), total aggregate-associated C concentrations were significantly higher in NT than in CT for all aggregate size classes (Fig. 2.2). There were no significant differences between tillage treatments for total aggregate-associated C concentrations at the 5-15 cm depth. For all the NT aggregate size classes, the total aggregate-associated C content was higher in the surface layers than in the 5-15 cm layer. There was no significant effect of depth on the total aggregate-associated C concentrations in CT, except for the < 53 μm size class where total aggregate-associated C content was higher in the 2.5-5 cm layer than in the other layers. In the surface layers of NT, total aggregate-

associated C concentrations were significantly higher in the 53-250 μm aggregate size class than in the other classes. The difference in total aggregate-associated C content between the different aggregate size classes was much larger in the NT surface samples than in the CT and the lowest depth of NT.

Unprotected and aggregate-protected C pools

The amount of C mineralized after 21 days is expressed in Table 2.2 as cumulative respired C (g C kg^{-1} sandfree aggregate). There were significant differences between CT and NT in the surface layers for the unprotected, microaggregate-protected and micro-within-macroaggregate-protected C pools. The unprotected macroaggregate C pool was higher in NT than in CT for the 0-2.5 cm layer (1.5 times) and for the 2.5-5 cm layer (2.1 times). The unprotected microaggregate C pool was also higher in NT than in CT for the 0-2.5 cm layer (2.6 times) and for the 2.5-5 cm layer (2.7 times). Macroaggregate-protected C was not significantly different between CT and NT. The microaggregate-protected C pool was 2.9 times greater in NT than in CT in the 0-2.5 cm layer and 2.5 times in the 2.5-5 cm layer. The micro-within-macroaggregate-protected C pool was 4.5 times higher in NT than in CT in the 0-2.5 cm layer. Tillage practices did not have any effect on the C pools at the 5-15 cm layer. Within tillage treatments, there was a significant effect of depth. Within NT, all C pools were highest in the 0-2.5 cm and lowest in the 5-15 cm. Within CT, the unprotected and macroaggregate- protected C pools were higher in the 0-2.5 cm layer than in the 5-15 cm layer, while the microaggregate-protected and micro-within-macroaggregate protected C pools were not significantly different across depths in CT.

Carbon-14 concentrations

Aggregate-associated ^{14}C concentrations were significantly influenced by tillage practice, aggregate size class and their interactions (Fig. 2.3). Aggregate-associated ^{14}C concentrations were significantly higher for all aggregate size classes in NT than in CT in the 0-2.5 cm layer. For the 2.5-5 cm layer, only the aggregate-associated ^{14}C in the large macroaggregate size class (> 2000 μm) and the microaggregate size class (53-250 μm) were higher for NT than for CT. The other aggregate size classes were not significantly influenced by tillage. At the 5-15 cm depth, the 53-250 μm and < 53 μm aggregate size class had a higher aggregate-associated ^{14}C content in CT than in NT whereas the > 2000 μm aggregate size class had a higher aggregate-associated ^{14}C content in NT than in CT. The aggregate-associated ^{14}C content of all the aggregate size classes was significantly lower in the 5-15 cm layer than in the 0-2.5 cm layer of NT. There was no significant effect of depth on the aggregate-associated ^{14}C concentrations in the CT, except for the > 2000 μm aggregate size class where the aggregate-associated ^{14}C content was lower in the 5-15 cm layer than in the other layers. In the surface layers of NT, aggregate-associated ^{14}C concentrations were significantly higher in the 53-250 μm aggregate size class than in the other classes. In the 5-15 cm layer of NT, there were no significant differences between aggregate size classes. In CT, the differences in aggregate-associated ^{14}C concentrations between the aggregate size classes were much smaller than in NT surface samples.

Unprotected and aggregate-protected ^{14}C pools

The amount of ^{14}C mineralized after 21 days is expressed as cumulative respired ^{14}C (g ^{14}C kg^{-1} sandfree aggregate). The unprotected, microaggregate-protected and micro-within-macroaggregate-protected ^{14}C pools were significantly influenced by tillage practices, depth, and their interactions. The unprotected macroaggregate ^{14}C pools were on average 5.8 times higher in NT than in CT in the 0-2.5 cm and 2.5-5 cm layers but did not differ between CT and NT in the 5-15 cm layer. The unprotected microaggregate ^{14}C pool was 8 times higher in NT than in CT in the 2.5-5 cm layer. The macroaggregate-protected ^{14}C pools were not significantly different between CT and NT in the surface layers. The microaggregate-protected ^{14}C pool was 2.6 times higher in NT than in CT in the 0-2.5 cm layer, but no significant differences were found for the 2.5-5 cm layer. The micro-within-macroaggregate-protected ^{14}C pool was 2.7 times higher in NT than in CT for the 0-2.5 cm layer. In the 5-15 cm layer, the macroaggregate-protected ^{14}C pool and microaggregate-protected ^{14}C pool were respectively 6.7 and 8.5 times higher in CT than in NT. Within tillage treatments, there were some significant depth effects. Within NT, the unprotected ^{14}C pool was highest in the 2.5-5 cm layer and lowest in the 5-15 cm layer for both macroaggregates and microaggregates. Within CT, the unprotected macroaggregate ^{14}C pool was significantly lower at the 0-2.5 cm layer than the other layers. The macroaggregate-protected and micro-within-macroaggregate protected ^{14}C pools did not differ significantly across depth in both CT and NT. The microaggregate-protected ^{14}C pool was significantly higher in the 0-2.5 cm layer than in the two other depths in NT and no significant differences were detected across depths in CT for this pool.

Portion of C mineralized

Table 2.3 shows the amount of C mineralized as a percentage of the aggregate-associated C present before the incubation. There were only a few significant differences between CT and NT and these showed a higher percentage of C mineralized in CT samples than in NT samples. Overall, the amount of protected C mineralized is a rather low percentage of the C present. The amount of macroaggregate-protected C mineralized varied between 0.6 and 3.2 %, the amount of microaggregate-protected C mineralized varied between 2.7 and 11% and the amount of micro-within-macroaggregate-protected C mineralized varied between 1.5 and 5.5%.

Methodological issues

It should be noted that crushing of aggregates also breaks up the plant material present within the aggregates. Since it has been suggested that C mineralization increases with decreasing residue particle size, it is possible that only part of the C mineralized is C previously protected physically in the aggregates, and the other part is due to the grinding of the plant material. We do not think that this is an important factor in our experiment. In studies with legume residues, less CO_2 was evolved from soils amended with finely-ground than with coarser material (Stickler and Frederick, 1959; Jensen, 1994). Sörensen et al. (1996) found no significant effect of grinding of the wheat leaf material on the release of $^{14}CO_2$, but found a lower decomposition in soils where ground, compared to unground, subclover leaves were added. They suggested that grinding of plant residues might result in a greater contact of substrate and decomposer

organisms with the soil matrix, resulting in a less extensive decomposition. Sims and Frederick (1970) found that particle size had the greatest effect on CO_2 evolution during the first few days of incubation; after 16 days, soils amended with plant material in the size range of 0.25 to 2.37 mm evolved more CO_2 than did the soils with 4.8 mm size plant materials. The soils with plant material < 250 μm, however, evolved the least CO_2. Bremer et al. (1991) and Anger and Recous (1997) only found a higher C mineralization after 20 days of incubation in soils with ground plant residues when the C/N ratio of the plant material (straw) was high (C/N = 270). When rye residue was added (C/N = 9), the decomposition was higher when residues were larger after 2 days of incubation. Vestergaard et al. (2001) investigated the influence of the size of maize leaves (*Zea Mays* L.) on respiration and they found on average higher respiration rates in soils amended with larger pieces (4-5 mm). Comparing these different studies with our experiment, where soil C/N ~ 12, where aggregates were crushed < 250 μm or < 53 μm, and where ^{14}C containing residue had been applied to the soil 3 years previously, we believe that the C mineralized in the crushed aggregates was due to the breakdown of the aggregates and not to the grinding of the plant material.

Discussion

Results of this study indicate that there are significant differences in aggregate size distribution and aggregate-associated C fractions between NT and CT soils. No-till soils have more large macroaggregates and more C in most aggregate size classes in the surface layers. At the lower depth, however, there are no differences between NT and CT

soils for the aggregate size distribution and the total aggregate-associated C content. Several researchers found more macroaggregates in NT soils compared to CT soils (Carter, 1992; Beare et al., 1994a; Paustian et al., 1997; Six et al., 2000a). Macroaggregates are less stable than microaggregates (Elliott et al., 1986; Cambardella and Elliott, 1993), and therefore more susceptible to the disruptive forces of tillage. In the surface layer of CT soil, new soil is continuously exposed to drying and wetting, which causes the breakdown of larger, less stable aggregates. When these aggregates are disrupted, more organic substrates become available for microbial attack, resulting in an increase in SOM decomposition, and therefore a decrease in C content. Since residues are placed on the surface in NT soils and aggregates are less disturbed, higher C concentrations are found in these surface layers. Beare et al. (1994a), Dick et al. (1997) and Six et al. (1999) also found higher C concentrations in surface samples of NT soils than of CT soils. Similarly to our results, Beare et al. (1994a) and Six et al. (1998) found no significant differences in aggregate size distribution or C content between CT and NT in deeper soil layers. Since the residues are buried in CT soils, there might be a flush of microbial activity at a lower depth, which causes the formation of aggregate binding agents, countering the disruption of large macroaggregates by plowing.

The ^{14}C concentrations followed the same pattern as the total carbon for the surface layers in both CT and NT soils with generally more ^{14}C in the NT aggregate size classes than in CT. At the 5-15 cm depth, however, the microaggregates and clay and silt particles contain more ^{14}C in the CT soils than in the NT soils. This indicates that in the short term, more new C is incorporated in the lower depths in CT soils due to plowing, but this C is not stabilized in the long term since there are no significant differences in

total C content between CT and NT at this depth. Within tillage treatment, the differences between different depths were greater in NT samples than in CT samples. Balesdent et al. (1990) found more than 50 % of new C in the first 4 cm of NT soils and only 20 % below 25 cm, while the new C was homogeneously spread in the ploughed layers of the tilled soils.

Generally, there was more C in the microaggregate fraction in NT soils than in any other fraction. In this experiment, we worked with field moist aggregates that are not slake-resistant. Consequently, the larger aggregates consist mainly of a loose structure that is very easily disrupted by tillage and that contains less C than the smaller fractions. Since these structures are disrupted under CT, the difference in C content between aggregate size classes is much smaller than under NT. Accordingly, Puget et al. (1995) found that intermediate fractions (0.5-0.05 mm) contained most C when the aggregates were dry-sieved (not slake-resistant).

Unprotected C and ^{14}C pools were higher in NT than in CT for the 0-2.5 cm and 2.5-5 cm layer. This demonstrates that, when aggregates were isolated and incubated, NT aggregates contained more labile, readily mineralizable carbon than CT aggregates. Elliott (1986) and Gupta and Germida (1988) reported higher C mineralization in intact aggregates from native sod compared with cultivated soils. No-tillage aggregates in the field hold more labile C that is easily mineralized once these aggregates are isolated in the laboratory. Since the aggregates are regularly disrupted under CT soils, this labile C is decomposed very fast and less of this labile C is still present when the aggregates are isolated and incubated.

Microaggregate-protected and micro-within-macroaggregate-protected C and ^{14}C pools were higher in NT than in CT surface layers, while macroaggregate-protected C and ^{14}C pools did not differ between tillage practices. Other researchers also found that significant amounts of SOM are physically protected in microaggregated structures (Gregorich et al., 1989; Balesdent et al., 2000). This suggests that the protection of SOM in NT soils occurs at the microaggregate level, rather than at the macroaggregate level. However, other studies (Elliott, 1986; Beare et al., 1994b and Balesdent et al., 2000) reported an increase in mineralization when breaking macroaggregates (> 250 μm) into microaggregates (< 250 μm), with a weak or insignificant increase in tilled soils, while it was larger in untilled soils. This indicates that SOM protection might occur at the macroaggregate level in some NT soils.

Although we suggest that the microaggregates offer most of the SOM protection, the stabilization of macroaggregates is important for this protection to occur. When the macroaggregates are not disrupted (e.g., in NT soils), residue that forms the center of a new macroaggregate decomposes and fragments inside of the macroaggregate into finer organic matter which gradually becomes encrusted with clay particles and microbial products, forming microaggregates within macroaggregates (Oades, 1984). This organic matter is then stabilized and protected against further microbial decay. When macroaggregates are disrupted under CT, the organic matter is released and never has the time to fragment and encrust with clay particles and microbial products, resulting in a much smaller amount of microaggregates within macroaggregates (Six et al., 2000b).

Table 2.3 shows the amount of C mineralized as a percentage of the total aggregate-associated C present before the incubation. It shows only a few significant

differences between NT and CT soils where C pools in CT are greater than in NT. Since NT soils generally contained more C, the proportion of the C mineralized during the incubation will usually not be greater than in CT samples where less C is present. The microaggregate- and micro within macroaggregate-protected C pools are on average still less than 5 % of the total C pool (Table 3), indicating that most of the protected C is associated with silt and clay particles.

At the 5-15 cm depth, there were no significant differences for unprotected and protected C pools between CT and NT, whereas macroaggregate-protected and microaggregate-protected ^{14}C pools were higher in CT than in NT, indicating again that more young C is protected under CT soils at this depth, but this C is not stabilized in the long term.

Conclusions

Based on this study we can draw the following conclusions. The impact of tillage practices on aggregate size distribution, C content and organic matter protection is most pronounced in the upper layers of the soil. In these layers, NT soils contain more large macroaggregates and more total and young C. Organic matter is more protected at the surface under NT than under CT and this protection occurs more at the microaggregate level than at the macroaggregate level. At lower depths, more young C is accumulated in CT soils than in NT soils, but this carbon is not stabilized in the long term.

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References

Adu, J.K., and Oades, J. M., 1978. Physical factors influencing decomposition of organic materials in soil aggregates. Soil Biology and Biochemistry, 10: 109-115.

Angers D.A., and Recous, S. , 1997. Decomposition of wheat straw and rye residues as affected by particle size. Plant and Soil, 189: 197-203.

Balesdent, J., Mariotti, A., and Boisgontier, D. ,1990. Effect of tillage on soil organic carbon mineralization estimated from ^{13}C abundance in maize fields. Journal of Soil Science, 41: 587-596.

Balesdent J., Chenu, C., and Balabane, M. , 2000. Relationship of soil organic matter dynamics to physical protection and tillage. Soil and Tillage Research, 53: 215-230.

Beare, M.H., Hendrix, P.F., and Coleman, D.C., 1994a. Water-stable aggregates and organic matter fractions in conventional-tillage and no-tillage soils. Soil Science Society of America Journal, 58: 777-786.

Beare, M.H., Cabrera, M.L., Hendrix, P.F., and Coleman, D.C., 1994b. Aggregate-protected and unprotected organic matter pools in conventional-tillage and no-tillage soils Soil Science Society of America Journal, 58: 787-795.

Besnard, E., Chenu, C., Balesdent, J., Puget, P., and Arrouays, D., 1996. Fate of particulate organic matter in soil aggregates during cultivation. European Journal of Soil Science, 47: 495-503.

Bremer, E., Vanhoutum, W., and Vankessel, C., 1991. Carbon-dioxide evolution from wheat and lentil residues as affected by grinding, added nitrogen, and the absence of soil. Biology and Fertility of Soils, 11: 221-227.

Bruce. R.R., Langdale, G.W., and West, L.T., 1990. Modification of soil characteristics of degraded soil surfaces by biomass input and tillage affecting soil water regime. pp. 4-9. *In*: Trans. Intl. Congr. Soil Sci. 14th, Kyoto, Japan. 12-18 Aug. 1990. Vol. 6. Wageningen, the Netherlands.

Burke, I.C., Elliott, E.T., and Cole, C.V., 1995. Influence of macroclimate, landscape position and management on soil organic matter in agroecosystems. Ecological Applications, 5: 124-131.

Cambardella, C.A., and Elliott, E.T., 1993. Carbon and Nitrogen Distribution in Aggregates from Cultivated and Native Grassland Soils. Soil Science Society of America Journal, 57: 1071-1076.

Carter, M.R., 1992. Influences of reduced tillage systems on organic matter, microbial biomass, macro-aggregate distribution and structural stability of the surface soil in a humid climate. Soil and Tillage Research, 23: 329-335.

Dick, W.A., Edwards, W.M., and McCoy, E.L., 1997. Continuous application of No-tillage to Ohio soils: Changes in crop yields and organic matter-related soil properties. pp. 171-182. *In*: Soil Organic Matter in Temperate Agroecosystems;

Long-term experiments in North America E.A. Paul, K. Paustian, E.T. Elliott, and C.V. Cole (ed.). CRC Press, Ind., Boca Raton, FL.

Edwards, A.D. and Brenner, J.M., 1967. Microaggregates in soil. J. Soil Sci. 18: 64-73.

Elliot, E.T., 1986. Aggregate structure and carbon, nitrogen, and phosphorus in native and cultivated soils. Soil Science Society of America Journal, 4: 518-524.

Elliott, E.T., Palm, C.A., Reuss, D.E., and Monz, C.A., 1991. Organic matter contained in soil aggregates from a tropical chronosequence: correction for sand and light fraction. Agriculture, Ecosystems and Environment 34: 443-451.

Foster, R.C., 1981. Localization of organic materials in situ in ultrathin sections of natural soil fabrics using cytochemical techniques. pp 309-319. *In*: Int. Working Group on submicroscopy, Wageningen, The Netherlands. Bisdom, E.B.A. (Ed.).

Gale W.J, Cambardella, C.A., and Bailey, T.B., 2000. Root-Derived Carbon and the Formation and Stabilization of Aggregates. Soil Science Society of America Journal, 64: 201-207.

Gregorich, E.G., Kachanoski, R.G., and Voroney, R.P.,1989. Carbon mineralization in soil size fractions after various amounts of aggregate disruption. Journal of Soil Science, 40: 649-659.

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.

Hendrix, P.F., 1997. Long-term patterns of plant production and soil carbon dynamics in a Georgia Piedmont agroecosystem. pp. 235-245. *In*: Soil Organic Matter in Temperate Agroecosystems; Long-term experiments in North America. E.A.

Paul, K. Paustian, E.T. Elliott, and C.V. Cole (ed.). CRC Press, Ind., Boca Raton, FL.

Hendrix. P.F., Franzluebbers, A.J., and McCracken, D.V., 1998. Management effects on C accumulation and loss in soils of the southern Appalachian Piedmont of Georgia. Soil and Tillage Research, 47: 245-251.

Jensen, E.S., 1994. Mineralization and immobilization of nitrogen in soil amended with low C/N ratio plant residues with different particle sizes. Soil Biology and Biochemistry, 26: 519-521.

Kern, J.S., and Johnson, M.G., 1993. Conservation tillage impacts on national soil and atmospheric carbon levels. Soil Science Society of America Journal, 57: 200-210.

Kisselle, K.W., Garrett, C.J., Hendrix, P.F., Crossley, D.A., and Coleman, D.C., 1999. Method for C-14-labeling maize field plots and assessment of label uniformity within plots. Communications in Soil Science and Plant Analysis, 30: 1759-1771.

Ladd, J.N., Amato, M., and Oades, J.M., 1985. Decomposition of plant material in Australian soils. III. Residual organic and microbial biomass C and N from isotope labeled plant material and organic matter decomposing under field conditions. Australian Journal of Soil Research, 23: 603-611.

Miller, W.P. and Baharuddin, M.K., 1986. Relationship of soil dispersibility to infiltration and erosion of southeastern soils. Soil Science, 142: 235-240.

Oades, J.M., 1984. Soil organic matter and structural stability: Mechanisms and implications for management. Plant and Soil, 76: 319-337.

Paustian K., Collins, H.P., and Paul, E.A., 1997. Management controls in soil carbon. pp. 15-49. *In*: Soil Organic Matter in Temperate Agroecosystems; Long-term experiments in North America E.A. Paul, K. Paustian, E.T. Elliott, and C.V. Cole (ed.). CRC Press, Ind., Boca Raton, FL.

Puget, P., Chenu, C., and Balesdent, J., 1995. Total and young organic-matter distribution in aggregates of silty cultivated soils. European Journal of Soil Science, 46: 449-459.

Sanchez, P.A., Palm, C.A., Szott, L.T., Cuevas, E., and Lal, R., 1989. Organic input management in tropical agroecosystems. pp 125-152. *In*: Dynamics of soil organic matter in tropical ecosystems. D.C. Coleman et al. (ed). Univ. of Hawaii Press, Honolulu.

SAS Institute, 1990. SAS/STAT User's guide. Vol. 2. Version 6 ed. SAS Inst., Cary, NC.

Schlesinger, W.H., 1997. Biogeochemistry: An analysis of global change. 2nd ed. Academic Press, San Diego, CA.

Sims, J.L., and Frederick, L.R., 1970. Nitrogen immobilization and decomposition of corn residue in soil and sand as affected by residue particle size. Soil Science, 109: 355-361.

Six, J., Elliott, E.T., and Paustian, K., 1998. Aggregate and SOM dynamics under conventional and no-tillage systems. Soil Science Society of America Journal, 63: 1350-1358.

Six, J., Eliott, E.T., Paustian, K., and Doran, J.W., 1999. Aggregation and soil organic matter accumulation in cultivated and native grassland soils. Soil Science Society of America Journal, 62: 1367-1377.

Six, J., Paustian, K., Elliott, E.T., and Combrick, C., 2000a. Soil structure and organic matter. I. Distribution of aggregate-size classes and aggregate-associated carbon. Soil Science Society of America Journal, 64: 681-689.

Six, J., Elliott, E.T., and Paustian, K., 2000b. Soil macroaggregate turnover and microaggregate formation: a mechanism for C sequestration under no-tillage agriculture. Soil Biology and Biochemistry, 32: 2099-2103.

Sörensen P., Ladd, J.N., and Amato, M., 1996. Microbial assimilation of C-14 of ground and unground plant materials decomposing in a loamy sand and a clay soil. Soil Biology and Biochemistry, 28: 1425-1434.

Sparks, D.L., 1995. Environmental soil chemistry. Academic Press, San Diego, CA.

Stevenson, F.J., 1994. *Humus Chemistry: Genesis, Composition, Reactions*. 2nd edition. John Wiley and Sons, New York, 496 pp.

Stickler, F.C., and Frederick, L.R., 1959. Residue particle size as a factor in nitrate release from legume tops and roots. Agronomy Journal, 51: 271-274.

Sundquist, E.T., 1993. The global carbon dioxide budget. Science 259: 936-961.

Tisdale, S.L., Nelson, W.L., Beaton, J.D., and Havlin, J.L., 1993. Soil fertility and fertilizers. 5th ed. MacMillan Publishing Co., New York, NY.

Tisdall, J.M., and Oades, J.M., 1980. The effect of crop rotation on aggregation in a redbrown earth. Australian Journal of Soil Research, 18: 423-433.

Tisdall, J.M., and Oades, J.M., 1982. Organic matter and water-stable aggregates in soils. Journal of Soil Science, 33: 141-161.

van Veen, J.A., and Paul, E.A., 1981. Organic carbon dynamics in grassland soils. I. Background information and computer simulations. Canadian Journal of Soil Science 61: 185-201.

Vestergaard, P., Ronn, R., and Christensen, S., 2001. Reduced particle size of plant material does not stimulate decomposition, but affects the microbivorous microfauna. Soil Biology and Biochemistry, 33: 1805-1810.

Table 2.1. Total carbon (C) and Carbon-14 (^{14}C) contents of conventional tillage (CT) and no-tillage (NT) soils at the Horseshoe Bend Experimental Area.

Depth	Organic C		^{14}C	
	NT	CT†	NT	CT
cm	g kg^{-1}		Bq g^{-1}	
0-2.5	22.7	10.1*	95.8	45.9*
2.5-5	17.6	10.1*	55.4	44.3*
5-15	7.2	7.7	18.3	28.9*

†Values followed by * are significantly different between tillage treatments within depth.

Table 2. Aggregate-associated carbon (C) and Carbon-14 (^{14}C) pools of conventional tillage (CT) and no-tillage (NT) soils at the Horseshoe Bend Experimental Area.

	t otal carbon mineralized					
	0-2.5 cm		*2.5-5 cm*		*5-15 cm*	
	NT	CT †	NT	CT	NT	CT
	g kg^{-1} sandfree aggregate					
1) Macroaggregates						
Unprotected C	8.0a	5.2a*	4.8b	2.3b*	1.9c	1.9b
Protected C	1.6a	0.9a	0.8a	0.4b	0.4a	0.3b
2) Microaggregates						
Unprotected C	13.7a	5.3a*	8.5b	3.1c*	1.7c	1.5c
Protected C	5.9a	2.0a *	4.7a	1.9a*	0.9b	3.4a
3) Micro within macroaggregates						
Protected C	4.5a	1.0a*	1.6b	0.7a	1.6b	1.1a
	^{14}Carbon mineralized					
	0-2.5 cm		*2.5-5 cm*		*5-15 cm*	
	NT	CT	NT	CT	NT	CT
	kBq kg^{-1} sandfree aggregate					
1)Macroaggregates						
Unprotected C	1.1b	0.1b*	1.5a	0.6a*	0.3c	0.6a
Protected C	0.2a	0.4a	0.1a	0.1a	0.06a	0.4a*
2)Microaggregates						
Unprotected C	1.1a	0.3a	2.3a	0.3a*	0.2b	0.6a
Protected C	3.3a	1.3a*	1.2b	1.3a	0.2b	1.7a*
3)Micro within macroaggregates						
Protected C	1.7a	0.5a*	0.7b	0.3a	0.4b	0.5a

†Values followed by * are significantly different between tillage treatments within depth. Values followed by a different lowercase letter among depths within tillage treatments are significantly different.

Table 3. Portions of aggregate-associated carbon (C) mineralized of conventional tillage (CT) and no-tillage (NT) soils at the Horseshoe Bend Experimental Area.

Portion of C mineralized

	0-2.5 cm		*2.5-5 cm*		*5-15 cm*	
	NT	CT †	NT	CT	NT	CT
	% C respired/total C present					
1) Macroaggregates						
Unprotected C	9.8	17.0 *	4.4	5.0	5.0	4.2
Protected C	2.0	3.2	0.8	0.9	1.1	0.6
2) Microaggregates						
Unprotected C	10.2	15.8 *	4.5	9.1 *	7.6	5.5
Protected C	4.4	5.6	2.7	5.5	4.1	11.0 *
3) Micro within macroaggregates						
Protected C	5.5	3.6	1.5	1.6	3.9	2.2

†Values followed by * are significantly different between tillage treatments within depth.

List of figures

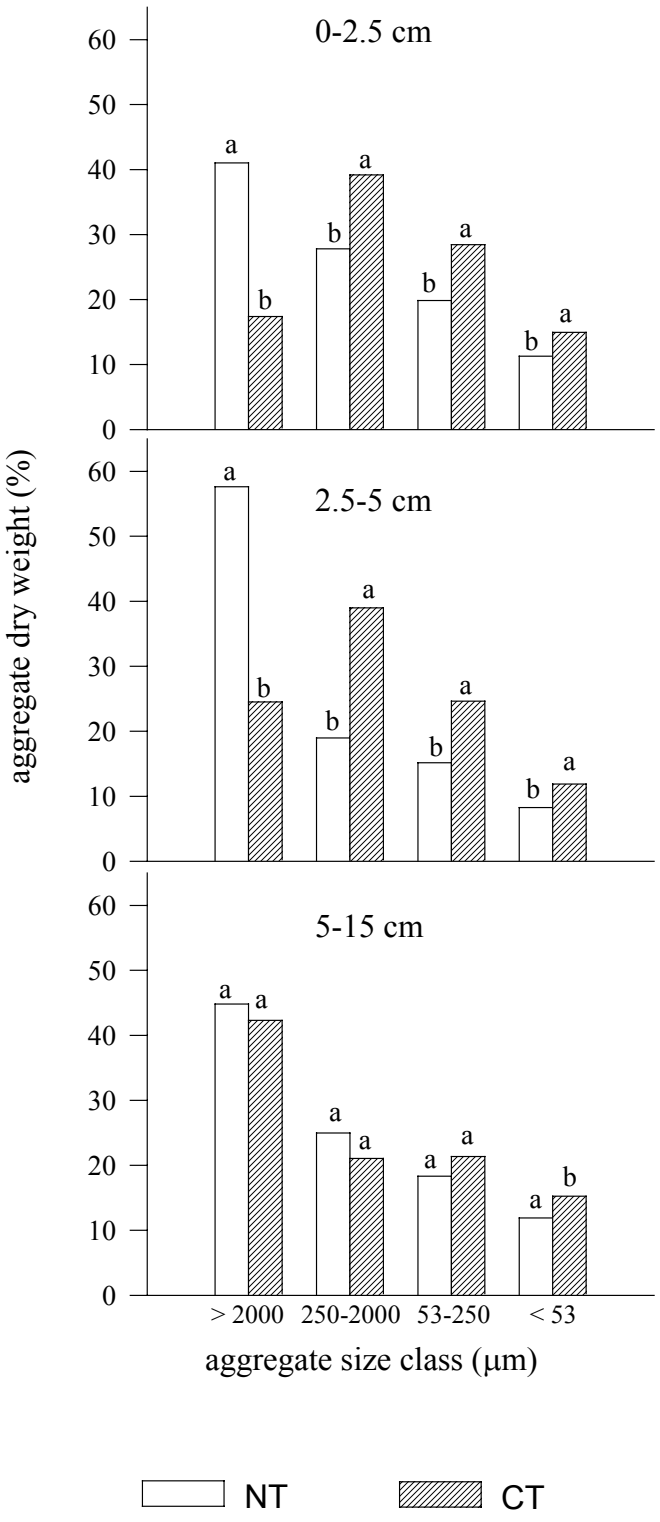
0-2.5 cm
2.5-5 cm
5-15 cm
aggregate dry weight (%)
0
10
20
30
40
50
60
a
b
> 2000
250-2000
53-250
< 53
aggregate size class (μm)
NT
CT

Fig. 2.1

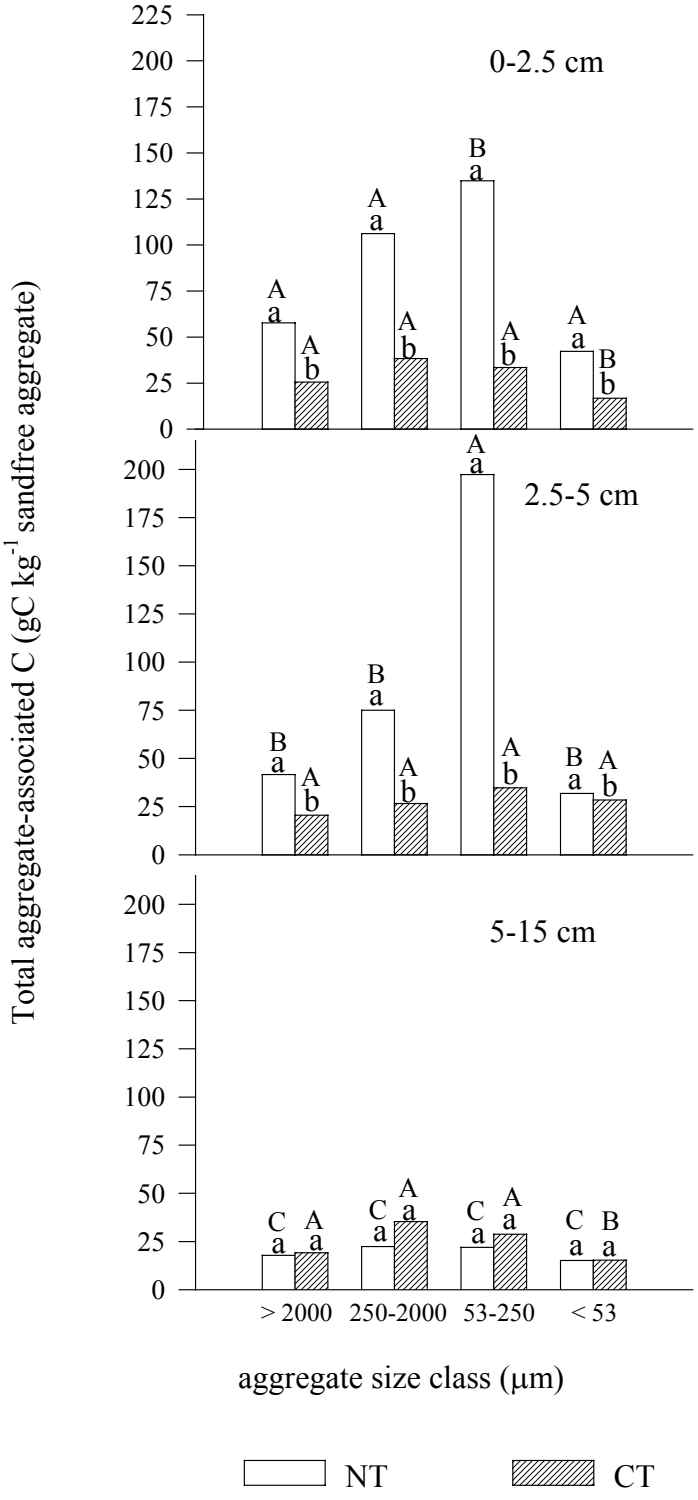
0-2.5 cm
2.5-5 cm
5-15 cm
Total aggregate-associated C (gC kg-1 sandfree aggregate)
> 2000
250-2000
53-250
< 53
aggregate size class (μm)
NT
CT

Fig. 2.2

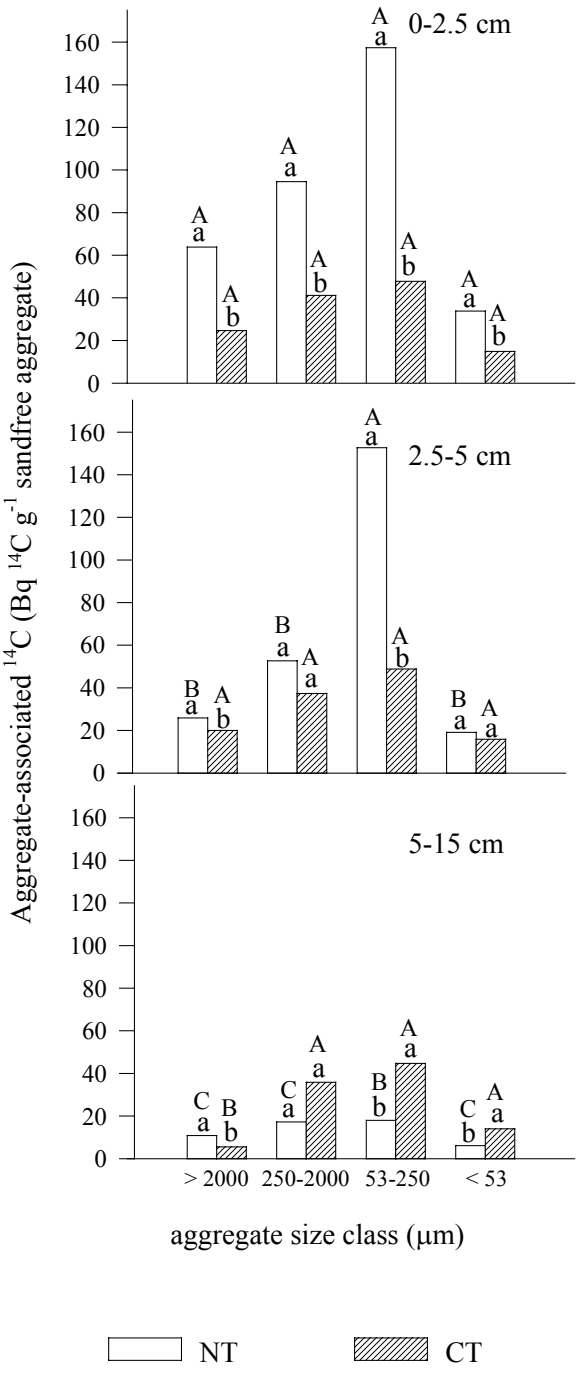
0-2.5 cm
2.5-5 cm
5-15 cm
Aggregate-associated ^{14}C (Bq ^{14}C g^{-1} sandfree aggregate)
0
20
40
60
80
100
120
140
160
> 2000
250-2000
53-250
< 53
aggregate size class (μm)
NT
CT

Fig. 2.3

CHAPTER 3

RAPID INCORPORATION OF FRESH RESIDUE-DERIVED CARBON INTO NEWLY FORMED STABLE MICROAGGREGATES WITHIN EARTHWORM CASTS[1]

[1]Bossuyt, H., J. Six, P.F. Hendrix, 2003. Submitted to *European Journal of Soil Science*, 12/23/2002

Abstract

In this study, the effect of earthworm activity on the formation of stable microaggregates inside of newly formed macroaggregates and the distribution of soil C was investigated. Samples were collected from the Horseshoe Bend Research area in Athens, GA. The incubation consisted of soil samples crushed (< 250 μm) with three treatments: (i) soil + ^{13}C-labeled residue + earthworms; (ii) soil + ^{13}C-labeled residue; and (iii) control soil. After 20 days, aggregate size distribution, total C and ^{13}C were measured and microaggregates (53-250 μm) were isolated out of the formed macroaggregates (> 250 μm). This microaggregate fraction was dispersed to separate the free fine particulate organic matter (inter-POM) and intra-microaggregate fine particulate organic matter (intra-POM). The ^{13}C-signature of the isolated fractions was used to calculate residue-derived C content of the fractions. The results showed that earthworms had a positive influence on the formation of large macroaggregates (> 2000 μm). These large macroaggregates contained 4 times more stable microaggregates than those from samples without earthworms. Both intra- and inter-aggregate POM-C were higher in the presence of earthworms and the differences between the treatments were even more pronounced for the residue-derived C. The higher amounts of organic matter (OM) inside of stable microaggregates in earthworm casts after 12 days of incubation indicates that these microaggregates are rapidly formed around freshly incorporated residue within casts. In conclusion, earthworm activity has a direct impact on the formation of stable microagregates and the incorporation of OM inside of these microaggregates. These processes are expected to be of great significance for the long-term stabilization of soil C.

Introduction

The highly dispersible, kaolinitic-clay-based soils of the southeastern USA (Miller & Baharuddin, 1986) are very susceptible to structural degradation, surface crusting, reduced infiltration, and erosion when cultivated. These factors can contribute to a rapid loss of soil organic matter (SOM) (Sanchez et al., 1989; Bruce et al., 1990). The maintenance of SOM has beneficial effects on soil structural stability and nutrient dynamics, generally resulting in sustainability of agricultural productivity.

Soil aggregates are thought to play an important role in the physical protection of SOM by making it inaccessible for further decomposition. Since this protection will only exist as long as the aggregate remains stable, stable aggregates will provide a higher degree of protection than unstable ones. Different models have been proposed for the formation of soil aggregates. Tisdall & Oades (1982) suggested that aggregates are formed by the binding of mineral particles into microaggregates (50-250 μm) and of microaggregates into macroaggregates (> 250 μm). Free primary particles are mainly cemented together into microaggregates by persistent binding agents (e.g. clay-polyvalent metal-humified organic matter complexes), characterized as older, more humified, or recalcitrant SOM. The more temporary (e.g., roots and fungal hyphae) and transient (e.g., polysaccharides) agents are thought to bind microaggregates into macroaggregates and are generally considered to be relatively more labile or decomposable (van Veen & Paul, 1981; Elliot, 1986). Because of the nature of the binding agents involved, macroaggregates are less stable than microaggregates (Oades, 1984; Beare et al., 1994b). This model was later modified by Oades (1984) who suggested that microaggregates are

predominantly formed within macroaggregates. This modification has formed the basis of several recent studies (Beare et al., 1994a; Gale et al., 2000; Six et al., 1999a, 2000). First, macroaggregates (250-2000 μm) are formed around fresh residue. If the macroaggregates are not disturbed (e.g., under NT), residue decomposes and fragments into finer organic matter that gradually becomes encrusted with clay particles and microbial products forming microaggregates within macroaggregates. When the macroaggregates become destabilized due to degradation of the binding agents, they fall apart and release the stable microaggregates. These microaggregates then form the building blocks for the formation of new macroaggregates, as suggested by Tisdall & Oades (1982).

Earthworms might have a pronounced effect on SOM dynamics. They are known to play an important role in the process of litter incorporation into soil macroaggregates (Martin, 1991). They ingest large amounts of organic matter, mix it with inorganic soil material, pass this mixture through their gut and excrete it as a cast (Guggenberger et al., 1996; Scullion & Malik, 2000). Earthworm casts are known to contain more water-stable macroaggregates than surrounding soils (Shipitalo & Protz, 1988; Marinissen, 1994).

On a smaller scale, earthworms also play an important role in the formation of microaggregates. Shipitalo & Protz (1988) and Barois et al. (1993) detected that the soil microstructure is completely destroyed in the earthworm's gut and that during gut transit old microaggregates are destroyed and new ones are formed. Jongmans et al. (2001) observed the incorporation of fine organic material in an early stage of decomposition into the microstructure within worm casts. The formation of these stable

microaggregates inside of earthworm casts might be an important mechanism in protecting labile SOM.

We hypothesized that earthworms would be partly responsible for the fast formation of stable microaggregates inside macroaggregates and sequestration of organic C in these microaggregates.

The objectives of this study were to investigate the effects of earthworms on (i) soil aggregate formation, (ii) soil organic matter incorporation and (iii) microstructure formation and C content in a short-term laboratory experiment using grain ^{13}C-labelled sorghum plants (*Sorghum bicolor (L.) Moench*).

Materials and methods

Site description and soils

Surface no-tillage soil samples were collected with a shovel from the long-term agricultural experimental site (Horseshoe Bend) near Athens, GA. The site is located in the Piedmont of the southern Appalachian Mountains (33° 54'N, 83° 24'W). The soil is a well-drained sandy clay loam (66% sand, 13% silt, 21% clay) in the Hiwassee series (fine kaolinitic thermic typic Kanhapladult). The area receives a mean annual precipitation of 1270 mm. The experimental plots (0.1ha) were established in 1978 with replicated tillage treatments assigned in a completely randomized design. Details on the treatment histories at the Horseshoe Bend Research Area Site can be found in Beare et al. (1994a) and Hendrix (1997).

After collection, the soil was air-dried (moisture content after air-drying 1-2 %) and pushed through a 250 μm sieve. The 250-1000 μm sized sand plus particulate organic matter (POM) fractions were kept and re-mixed with the soil after sieving. Stones larger than 1000 μm were discarded.

Incubation

From the 250 μm sieved, air-dried soil, 12 subsamples (150 g) were subjected to 3 treatments, each in 4 replicates (n=4): (i) ^{13}C labeled plant material and 6 earthworms; (ii) ^{13}C-labelled plant material, but no earthworms; and (iii) no plant material and no earthworms. ^{13}C-labeled grain sorghum (*Sorghum bicolor (L.) Moench*) leaves ground to a size of 500-1000 μm were used as the labeled plant material. The earthworm species was *Aporrectodea caliginosa* (Savigny 1826). In the first two treatments, 1.2 g of plant material was mixed in 150 g soil; the mixture was brought to field capacity (11% water content) and put in glass jars with lids containing septa for gas sampling. The jars were held overnight at 4°C to equilibrate and then incubated at 20°C for 20 days. Earthworms were added after 8 days of incubation. At day 20, the earthworms were taken out of the jars and the soil was air-dried for 3 days to allow worm casts to stabilize (Marinissen & Dexter, 1991). After being dried, the soil was slowly rewetted, held overnight at 4°C and aggregate size distribution was determined by wet sieving as described by Elliott (1986). Briefly, a series of three sieves was used to obtain four aggregate size fractions: (i) > 2000 μm (large macroaggregates); (ii) 250-2000 μm (small macroaggregates); (iii) 53-250 μm (microaggregates); (iv) < 53 μm (silt and clay fraction). Following wet sieving, the aggregate size fractions > 53 μm were dried on the sieves in a dehumidifying

chamber (10°C). Particles < 53 μm were collected in a bucket, total volume was measured and a subsample of a known volume was taken for analysis.

Isolation of microaggregates within macroaggregates

Subsamples from the small and large macroaggregate fractions were taken to isolate the microaggegates held within macroaggregates following the method described by Six et al. (2000). A device was used to completely break up macroaggregates while minimizing the break down of the released microaggregates. Macroaggregates (10 g) were immersed into deionized water on top of a 250 μm mesh screen and gently shaken with 50 glass beads (dia. = 4 mm). Continuous and steady water flow through the device ensured that microaggregates were immediately flushed onto a 53 μm sieve and not further disrupted by the glass beads. After all macroaggregates were broken up, the sand and coarse particulate organic matter (coarse POM) retained on the 250 μm sieve were washed off and collected. The material collected on the 53 μm sieve was sieved according to Elliott (1986) to ensure that the isolated microaggregates were water-stable. The proportion of microaggregate weight (53-250 μm) within macroaggregates (250-2000 μm) was calculated as:

$$\frac{\text{(microaggregate weight – weight of 53-250 μm sized sand)}}{\text{(macroaggregate weight – weight of 250-2000 μm sized sand)}}$$

The weights of macro- and microaggregates were corrected for the sand content of the same size as the aggregates because sand of the same size as an aggregate has a higher probability of not being a part of the aggregate and should consequently not be weighed as an aggregate.

Inter-microaggregate particulate organic matter and intra-microaggregate particulate organic matter contents

The inter-microaggregate particulate organic matter (inter-POM) that was retained on the 53 μm sieve together with the microaggregates was isolated by density flotation with 1.85 g cm^{-3} sodium polytungstate (Six et al., 1998). After density flotation, the microaggregates were dispersed by shaking overnight (18 h.) in 5 g L^{-1} hexametaphosphate and intra-microaggregate particulate organic matter (intra-POM) was separated from the clay-and silt fraction (< 53 μm) by sieving. To avoid cross contamination of C, and especially ^{13}C, among samples, sodium polytungstate was recycled according to Six et al. (1999b).

Analyses

Total C and ^{13}C from the aggregate size fractions and from the coarse POM, fine inter-POM and intra-POM were determined using a Finnigan Delta C Mass Spectrometer coupled to a Carlo Erba, NA 1500, CHN Combustion Analyzer via Finnigan's Conflo II Interface.

Results are expressed as:

$$^{13}C‰ = \left[\left(\frac{^{13}R_{sample}}{^{13}R_{standard}}\right) - 1\right] \times 1000, \quad \text{where } ^{13}R = {}^{13}C/{}^{12}C$$

and the standard is the international Pee Dee Belemnite (PDB).

Statistical analysis

The data were analyzed, using the SAS statistical package for analysis of variance (ANOVA-PROC GLM, SAS Institute, 1990). Separation of means was tested with the DIFF option of the LSMEANS statement with a significance level of $P < 0.05$.

Results

Aggregate size distribution and total carbon concentrations

In table 3.1, the effects of earthworm activity on the formation of water-stable aggregates and the distribution of C within these aggregates are shown. Significant impacts of earthworm activity are mainly seen in the formation of large macroaggregates and the C content of those macroaggregates. The soil samples with earthworms contained 3.6 times more large macroaggregates (> 2000 μm) than the soil samples

without earthworms. Large macroaggregates were not formed when earthworms and residue were absent. Instead, smaller aggregate size classes (250-2000 um, 53-250 um and < 53 um) were found in higher percentages.

The large macroaggregates (> 2000 μm) contained significantly more total aggregate-associated C (4.26 g C kg^{-1} soil) in the presence of earthworms while total aggregate-associated C concentrations were generally higher in the samples without earthworms for the smaller aggregate size classes. The silt- and clay fraction (< 53 μm) contained significantly more total C in the absence of earthworms and residue but no other significant differences were found in total aggregate-associated C concentrations between the samples without earthworms and without residue.

Coarse free total POM-C concentration was significantly higher in the presence of earthworms in the large macroaggregates (> 2000 μm) and was ~ 1.5 times higher than in the samples without earthworms (data not shown). For the small macroaggregates, there was no significant difference in total coarse POM-C concentration between the samples with and without earthworms or without residue. Earthworm activity had no significant impact on residue-derived coarse free POM-C for both large and small macroaggregates.

Formation of microaggregates inside of macroaggregates

The formation of water-stable microaggregates inside of macroaggregates was significantly influenced by earthworm activity (Fig. 3.1). The proportion of microaggregates within large macroaggregates was ~4 times higher in the presence of earthworms. There was no difference in microaggregate proportion between samples with and without earthworms for the small macroaggregates (250-2000μm).

Total C and ^{13}C distribution in aggregate-associated fractions

Total C concentrations of the fractions inside of large macroaggregates (fine intra-POM and fine inter-POM) were significantly higher in the presence of earthworms (Fig. 3.2). The C concentrations were higher for fine intra- and inter-POM without earthworms in the small macroaggregates, but the differences were not as pronounced. In general, intra-POM total C concentrations were higher than fine inter-POM C concentrations for both earthworm and no-earthworm samples. The residue-derived POM-C was significantly higher in the samples with earthworms than in those without earthworms for the large macroaggregates (Fig. 3.3) and this difference was more pronounced than the differences in total C between the treatments. Approximately 15% of the total fine intra-POM C in the presence of earthworms in large macroaggregates was residue-derived C while only ~ 6 % when no earthworms were present. For the fine inter-POM C, 11% was residue-derived C in the presence of earthworms compared to 15% when no earthworms were present. No significant differences between treatments were detected for the small macroaggregates.

Figure 3.4 shows the ratio of fine intra-POM-^{13}C to fine inter-POM-^{13}C that is free between microaggregates. For both large and small macroaggregates, more fine POM is incorporated into microaggregates in the presence of earthworms and this ratio is approximately 1.9 times higher for the large macroaggregates and 1.5 times higher for the small macroaggregates.

Discussion

In this study, we were mainly interested in the role of earthworm activity in the formation of stable microaggregates within their casts and the distribution of newly added C within and between formed microaggregates or casts. As shown by several other researchers, we found that earthworm activity has a positive impact on the formation of large macroaggregates (Blanchart et al., 1989; Martin & Marinissen, 1993; Guggenberger et al., 1996; Marinissen & Hillenaar, 1996; Scullion & Malik, 2000). These large macroaggregates contained significantly more C (both old and new) in the presence of earthworms, as reported by numerous other researchers (Shipitalo & Protz, 1989; Mulongoy & Bedoret, 1989; Daniel & Anderson, 1992; Barois et al., 1993; Buck et al., 1999).

While almost all of the related research has been focused on the impact of earthworm activity on the production of macroaggregates and C within these macroaggregates, this study concentrated on the formation of microaggregates within earthworm casts and the organic matter within and between these microaggregates. A much higher proportion of microaggregates within large macroaggregates was found in the presence of earthworms. Since no macroaggregates (> 250 μm) were present at the start of the incubation, all of the microaggregates within macroaggregates were newly formed microaggregates or old microaggregates incorporated inside of macroaggregates. Shipitalo & Protz (1988) and Barois et al. (1993) found that the soil microstructure was completely destroyed in the earthworm's gut and that during gut transit old microaggregates were destroyed and new ones formed.

In our study, these water-stable microaggregates inside of large macroaggregates also contained more total C and residue-derived C. This shows that earthworms can play a very important role in incorporating fresh organic matter inside of microaggregates within macroaggregates. The ratio of fine intra-POM to fine inter-POM was higher in the presence of earthworms for both large and small macroaggregates, showing again that earthworms have a direct involvement in the incorporation of free fresh residues into stable microaggregates within their casts. Jongmans et al. (2001) also observed fine organic material in an early stage of decomposition in the microaggregates within worm casts. Likewise, Pulleman et al. (submitted) studied earthworm casts and bulk soil aggregates in a pasture soil greatly affected by earthworms and found a higher amount of stable microaggregates in macroaggregates and a higher amount of fine POM inside of these microaggregates. Winsome & McColl (1998) analyzed the microaggregates within earthworm casts and bulk soil with an electron microscope and found that interior surfaces of earthworm casts contained tight aggregations of organic materials, clay domains and filaments while the interior surface of uningested soil aggregates consisted of mineral grains embedded in clay- and silt particles.

Microaggregates are known to be more stable than macroaggregates and organic matter incorporated into microaggregates is thought to be more resistant to decomposition than organic matter in macroaggregates (Tisdall & Oades, 1982; Oades, 1984). The formation of stable microaggregates inside of macroaggregates might be a very important mechanism in protecting organic matter at the microaggregate level. When macroaggregates fall apart due to a disturbance or natural turnover, the organic matter within the macroaggregates but between microaggregates would become available

for microbial attack while the organic matter inside of microaggregates would still be protected.

Only few researchers have focused on the formation of microaggregates inside of earthworm casts and the incorporation of organic matter inside of these microaggregates and these studies generally looked at earthworm casts formed in the field or in longer term microcosms, making it impossible to determine how fast these microaggregates are formed and how fast organic matter is incorporated inside of them. In current conceptual models of aggregate and SOM dynamics (e.g. Six et al., 1998; Gale et al., 2000), the stabilization of C occurs in newly formed microaggregates within macroaggregates by ageing, microbial activity and exudation. It is suggested that microaggregates within macroaggregates are formed by microbial decomposition of organic residues inside of macroaggregates that become gradually encrusted with microbial mucigels and clay particles. This study, however, showed that after only 12 days of incubation, earthworm activity already had a large impact on the formation of these microaggregates within macroaggregates. Newly formed earthworm casts already contained significantly more earthworm-created microaggregates than bulk soil aggregates, and earthworms incorporated considerably more organic matter inside of their casts. Since C associated with microaggregates is known to turn over slower than C associated with macroaggregates (Six & Jastrow, 2002), the formation of these microaggregates within macroaggregates might be an important mechanism for the long-term protection of C.

Conclusions

Based on the results of this study, we can conclude that earthworms induce a rapid formation of stable microaggregates inside of large macroaggregates and enhance the incorporation of fresh residues inside of these microaggregates. While researchers have previously reported the importance of microaggregate formation inside of macroaggregates to stabilize and protect organic matter, none have investigated how fast earthworms are able to form these microaggregates and how fast they incorporate organic matter at this microaggregate level. This fast incorporation and consequent protection of organic matter inside of stable microaggregates is expected to be a very important factor in the sequestration of soil C.

Since certain management practices, such as tillage, are known to have an adverse effect on earthworm abundance, the implementation of conservation tillage or no-tillage, that lead to higher earthworm populations, may therefore lead to increased levels of OM and soil C content

Further studies are needed to determine the longer-term stability of these microaggregates under field conditions, especially where soils are continuously processed by earthworms and where interactions of different earthworm species may be important.

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References

Barois, I., Villemin, G., and Lavelle, P., 1993. Transformation of the soil structure through *Pontoscolex Coretrhrurus* (Oligochaeta) intestinal tract. Geoderma, 56: 57-66.

Beare, M.H., Hendrix, P.F., and Coleman, D.C., 1994a. Water-stable aggregates and organic matter fractions in conventional – and no-tillage soils. Soil Science Society of America Journal, 58: 777-786.

Beare, M.H, Cabrera, M.L., Hendrix, P.F., and Coleman, D.C., 1994 b. Aggregate-protected and unprotected organic matter pools in conventional and no-tillage Soils. Soil Science Society of America Journal, 58: 787-795.

Blanchart, E., Lavelle, P., and Spain, A.V., 1989. Effects of two species of tropical earthworms (Oligochaete:Eudrilidae) on the size distribution of aggregates in an African soil. Revue d'Ecologie et de Biologie du Sol. Sol, 26: 417-425.

Bruce. R.R., Langdale, G.W. and West, L.T., 1990. Modification of soil characteristics

of degraded soil surfaces by biomass input and tillage affecting soil water regime. pp. 4-9. *In*: Trans. Intl. Congr. Soil Sci. 14th, Kyoto, Japan. 12-18 Aug. 1990. Vol. 6. Wageningen, the Netherlands.

Buck, C., Langmaack, M., and Schrader, S., 1999. Nutrient content of earthworm casts influenced by different mulch types. European Journal of Soil Biology, 35: 23-30.

Daniel, O. and Anderson, J.M., 1992. Microbial biomass and activity in contrasting soil materials after passage through the gut of the earthworm *Lumbricus rubellus*. Soil Biology and Biochemistry, 24: 465-470.

Elliott, E.T., 1986. Aggregate structure and carbon, nitrogen, and phosphorus in native and cultivated soils. Soil Science Society of America Journal, 50: 627-633.

Gale, W.J., Cambardella, C.A. and Bailey, T.B., 2000. Surface residue- and root-derived carbon in stable and unstable aggregates. Soil Science Society of America Journal, 64: 196-201.

Guggenberger, G., Thomas, R.J. and Zech, W., 1996. Soil organic matter within earthworm casts of an anecic-endogeic tropical pasture community, Colombia. Applied Soil Ecology, 3: 263-274.

Hendrix, P.F., 1997. Long-term patterns of plant production and soil carbon dynamics in a Georgia Piedmont agroecosystem. pp. 235-245. *In*: Soil Organic Matter in Temperate Agroecosystems; Long-term experiments in North America. E.A. Paul, K. Paustian, E.T. Elliott, and C.V. Cole (ed.). CRC Press, Ind., Boca Raton, FL.

Jongmans, A.G., Pulleman, M.M., and Marinissen, J.C.Y., 2001. Soil structure and earthworm activity in a marine silt loam under pasture versus arable land. Biology and Fertility of Soils, 33: 279-285.

Marinissen, J.C.Y., 1994. Earthworms and stability of soil structure: a study in a silt loam soil in a young Dutch polder. Agriculture, Ecosystems & Environment, 51: 75-87.

Marinissen, J.C.Y., and Dexter, A.R., 1990. Mechanisms of stabilisation of earthworm casts and artificial casts. Biology and Fertility of Soils, 9: 163-167.

Marinissen, J.C.Y., and Hillenaar, S.I., 1996. Earthworm induced distribution of organic matter in macro-aggregates from differently managed arable fields. Soil Biology and Biochemistry, 29: 391-395.

Martin, A., 1991. Short- and long-term effects of the endogeic earthworm *Millsonia anomala (Omodeo) (Megascolecidae, Oligochaeta*) of tropical savannas on soil organic matter. Biology and Fertility of Soils, 11: 234-238.

Martin, A., and Marinissen, J.C.Y., 1993. Biological and physico-chemical processes in excrements of soil animals. Geoderma, 56: 331-347.

Miller, W.P., and Baharuddin, M.K., 1986. Relationship of soil dispersibility to infiltration and erosion of southeastern soils. Soil Science, 142: 235-340.

Mulongoy K., and Bedoret, A., 1989. Properties of worm casts and surface soils under various plant covers in the humid tropics. Soil Biology and Biochemistry, 21: 197-203.

Oades, J.M., 1984. Soil organic matter and structural stability, mechanisms and implications for management. Plant and Soil, 76: 319-337.

Pulleman, M.M., Marinissen, J.C.Y., Jongmans, A.G. Six, J., and Uijl, A., 2003. Macro- and microaggregate structure and soil organic matter dynamics as affected by earthworm activity in different farming systems. Biology and Fertility of Soils, submitted.

Sanchez, P.A., Palm, C.A., Szott, L.T., Cuevaa, E., and Lal, R., 1989. Organic input management in tropical agroecosystems. pp 125-152. *In*: Dynamics of soil organic matter in tropical ecosystems. D.C. Coleman et al. (ed). Univ. of Hawaii Press, Honolulu.

SAS Institute, 1990. SAS/STAT User's guide. Vol. 2. Version 6 ed. SAS Inst., Cary, NC.

Scullion, J., and Malik, 2000. Earthworm activity affecting organic matter, aggregation and microbial activity in soils restored after open cast mining for coal. Soil Biology and Biochemistry, 32: 119-126.

Shipitalo, M.J., and Protz, R., 1988. Factors influencing the dispersibility of clay in worm casts. Soil Science Society of America Journal, 52: 764-769.

Shipitalo, M.J., and Protz, R., 1989. Chemistry and micromorphology of aggregation in earthworm casts. Geoderma, 45: 357-374.

Six, J., Elliott, E.T., and Paustian, K., 1998. Aggregate and SOM dynamics under conventional and no-tillage systems. Soil Science Society of America Journal, 63: 1350-1358.

Six, J., Eliott, E.T., Paustian, K., and Doran, J.W., 1999a. Aggregation and soil organic matter accumulation in cultivated and native grassland soils. Soil Science Society of America Journal, 62: 1367-1377.

Six, J., Schultz P.A., Jastrow J.D., and Merckx R., 1999b. Recycling of sodium polytungstate used in soil organic matter studies. Soil Biology and Biochemistry, 31:1193-1196.

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: 2099-2013.

Six, J. & Jastrow, J.D. 2002. Soil organic matter turnover. In R.Lal (Ed.) Encyclopedia of Soil Science. Marcel Dekker, NY.

Tisdall, J.M., and Oades, J.M., 1982. Organic matter and water-stable aggregates in soils. Journal of Soil Science, 33: 141-161.

Van Veen, J.A., and Paul, E.A., 1981. Organic carbon dynamics in grassland soils. 1. Background information and computer simulations. Canadian Journal of Soil Science, 61: 185-201.

Winsome, T., and McColl, J.G., 1998. Changes in chemistry and aggregation of a California forest soil worked by the earthworm *Argilophilus papilifer Eisen* (Megascolecidae). Soil Biology and Biochemistry, 30: 1677-1687.

Table 3.1. Aggregate size distribution and total-aggregate associated C concentrations in samples with and without earthworms and/or added residue.

aggregate size(μm)	+ earthworms† + residue	- earthworms + residue	- earthworms - residue
	aggregate dry weight(%)		
> 2000	36.8a	10.1b	0.00c
250-2000	28.7b	40.7a	42.9a
53-250	31.3b	44.5a	50.2a
< 53	3.16c	4.78b	6.88a

aggregate size(μm)	+ earthworms† + residue	- earthworms + residue	- earthworms - residue
	C (g kg^{-1} soil)		
> 2000	6.31a	2.05b	0.00c
250-2000	3.70a	4.80a	4.82a
53-250	4.44b	6.21a	7.03a
< 53	1.11c	1.69b	2.47a

† Values followed by a different lowercase letter are significantly different between sample treatments

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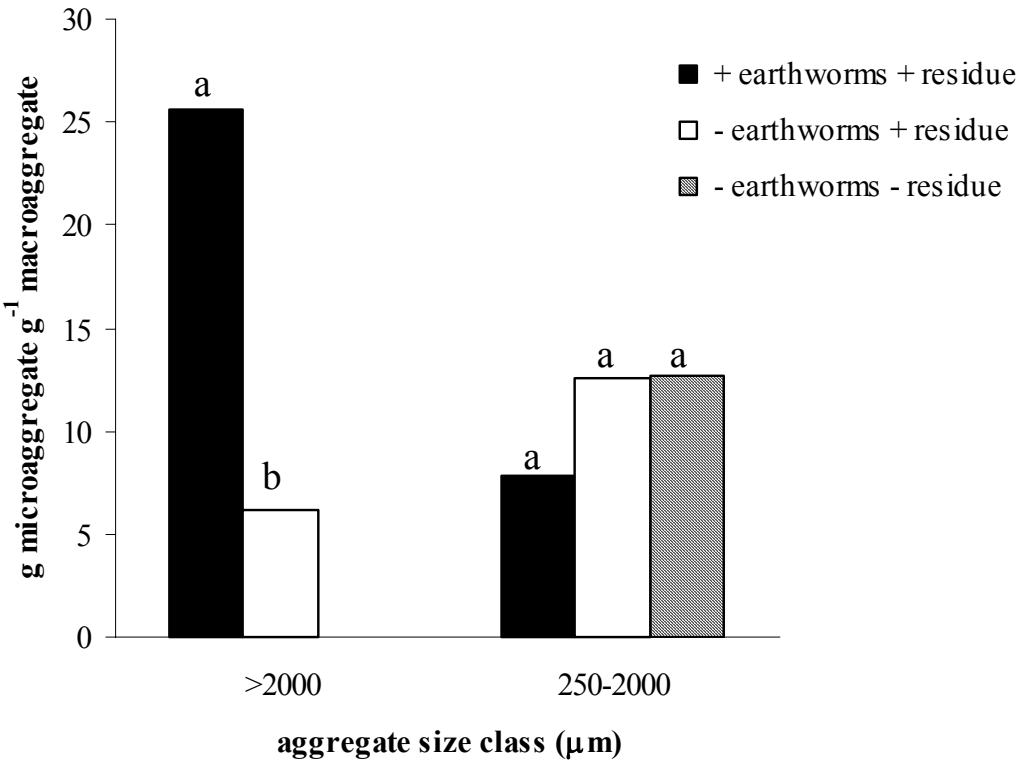
30
25
20
15
10
5
0
g microaggregate g-1 macroaggregate
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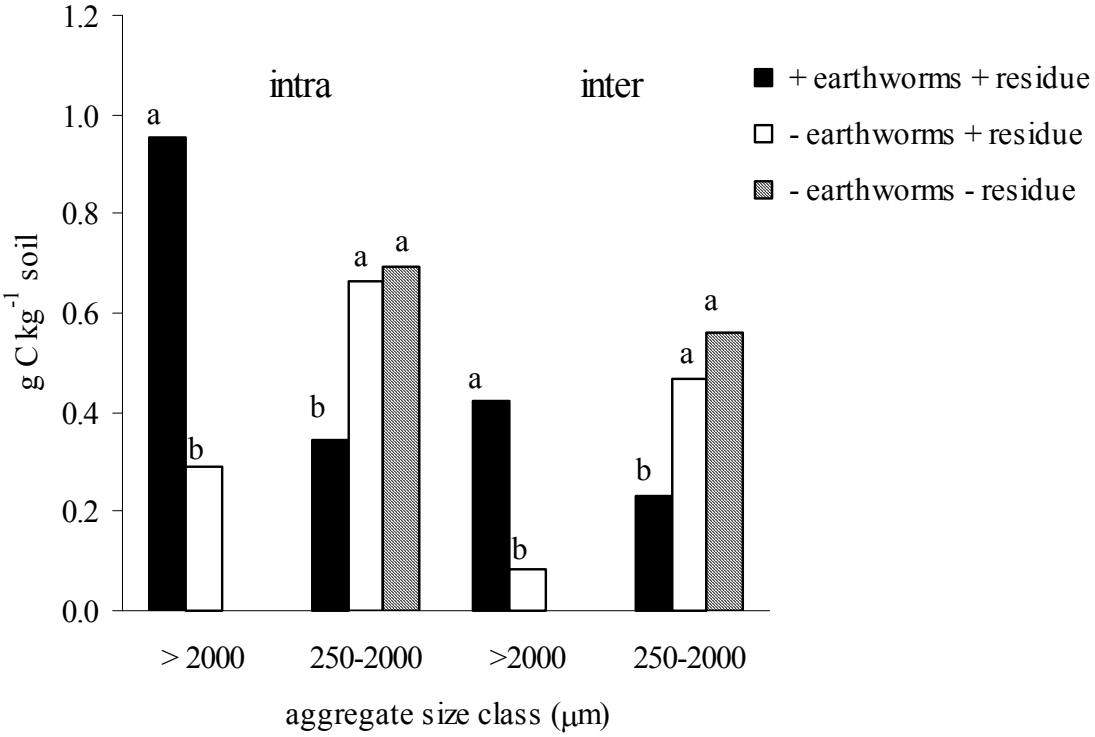
b
a
a
a
+ earthworms + residue
- earthworms + residue
- earthworms - residue
>2000
250-2000
aggregate size class (µm)

Fig. 3.1


1.2
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0.4
0.2
0.0
g C kg-1 soil
intra
inter
+ earthworms + residue
- earthworms + residue
- earthworms - residue
a
b
a
a
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b
> 2000
250-2000
>2000
250-2000
aggregate size class (μm)

Fig. 3.2

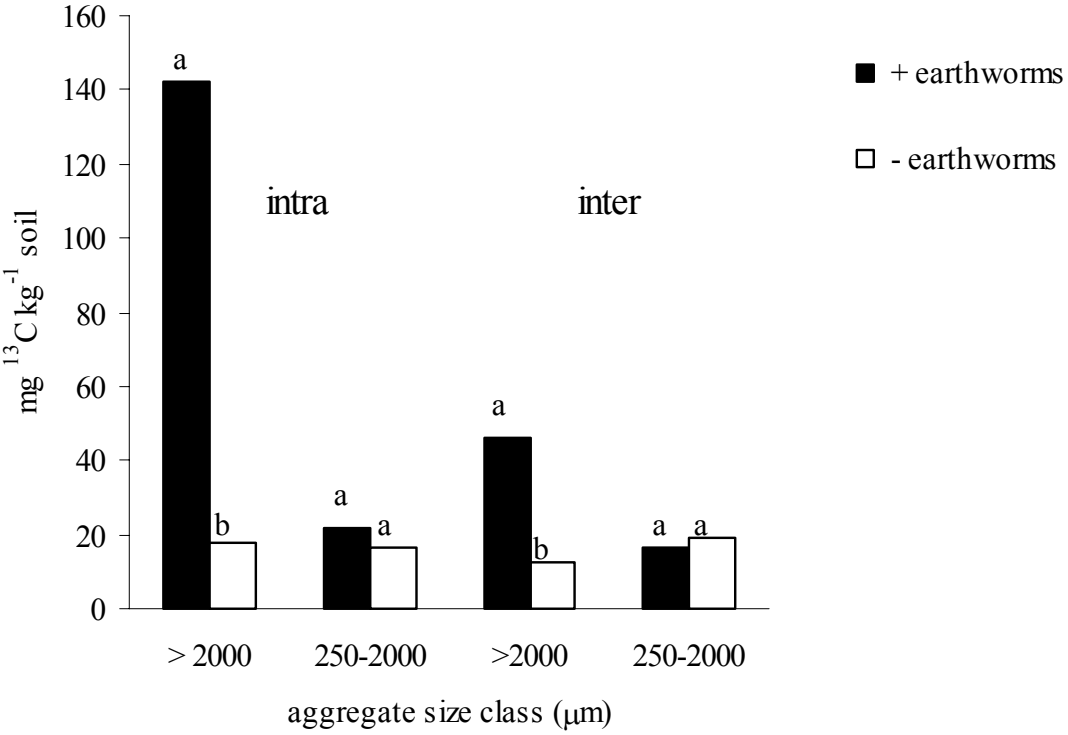
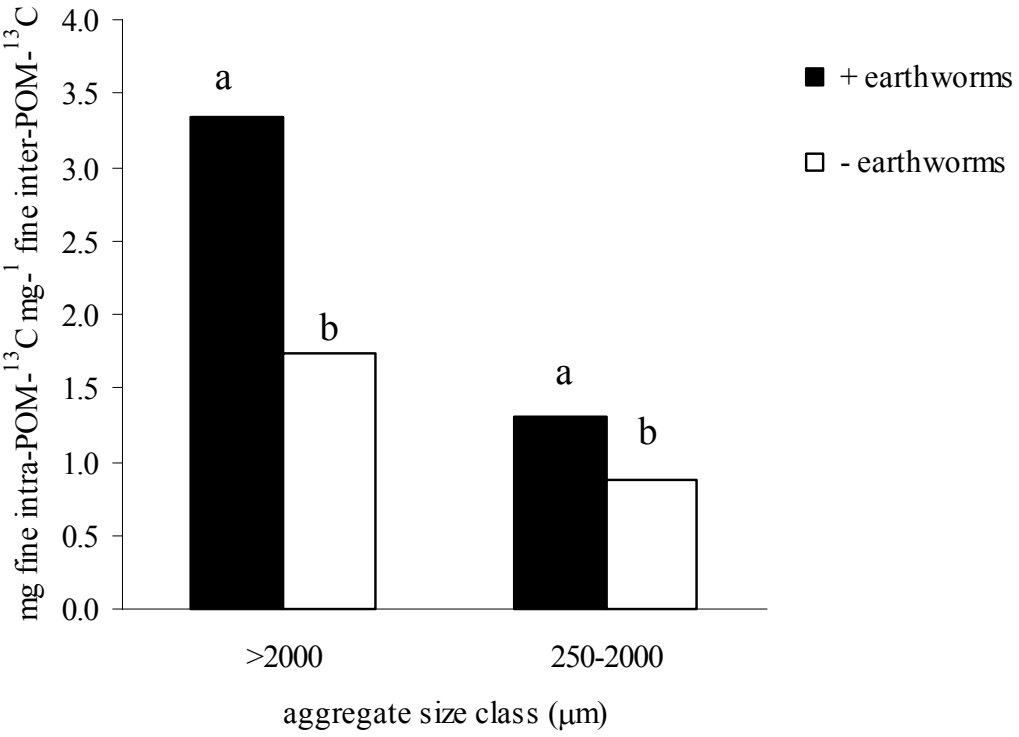

160
140
120
100
80
60
40
20
0
mg ^{13}C kg^{-1} soil
a
b
a
a
a
b
a
a
intra
inter
+ earthworms
- earthworms
> 2000
250-2000
>2000
250-2000
aggregate size class (μm)

Fig. 3.3


mg fine intra-POM-^{13}C mg-^{1} fine inter-POM-^{13}C
4.0
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250-2000
aggregate size class (μm)
+ earthworms
- earthworms

Fig. 3.4

CHAPTER 4

SOIL CARBON PROTECTION AT THE MICROAGGREGATE LEVEL WITHIN EARTHWORM CASTS[1]

[1]Bossuyt, H., J. Six, P.F. Hendrix, 2003. Submitted to *Soil Biology and Biochemistry*, 12/23/2002

Abstract

Earthworms are known to play a role in aggregate formation and soil organic matter (SOM) protection. However, it is still unclear at what scale and how fast earthworms induce a protection of SOM. The objective was to investigate the effects of *Aporrectodea caliginosa* on aggregation and aggregate-associated C pools using ^{13}C-labeled sorghum (*Sorghum bicolor (L.) Moench*) leaves residue. Two incubations were set up. The first incubation consisted of soil samples crushed < 250 μm to break up all macroaggregates with three treatments: (i) control soil; (ii) soil + ^{13}C-labeled residue; and (iii) soil + ^{13}C-labeled residue + earthworms. After 20 days, aggregate size distribution was measured together with total C and ^{13}C in each aggregate fraction. A second incubation was conducted to determine protected versus unprotected total C and ^{13}C from 21-day laboratory incubations of intact and crushed macro- and microaggregates. Different pools of aggregate-associated C were quantified: (i) unprotected macroaggregate-C, (ii) unprotected microaggregate-C, (iii) macroaggregate-protected C, (iv) microaggregate-protected C, and (v) microaggregate within macroaggregate-protected C. In the presence of earthworms, a higher proportion of large macroaggregates was newly formed and these aggregates contained more C and ^{13}C compared to bulk soil. There were no significant differences between the samples with or without earthworms for the macroaggregate-protected, microaggregate-protected and microaggregate within small macroaggregate-protected pools. In the presence of earthworms, the microaggregate within large macroaggregate-protected C pool was a significant pool and 22% of this C pool was newly added C. In conclusion, our results

clearly indicate the direct involvement of earthworms in inducing a longer-term protection of soil C at the microaggregate level.

Introduction

Soil aggregation has a great influence on the physical characteristics of the soil. Well-aggregated soils possess a larger pore space and are characterized by a higher infiltration rate and better gaseous exchange between soil and atmosphere, leading to enhanced microbial activity (Lynch and Bragg, 1985). Soil aggregation and soil organic matter (SOM) dynamics are closely linked. Aggregates are thought to play an important role in the physical protection of SOM and at the same time, SOM binds with mineral particles to form aggregates of different sizes (Tisdall and Oades, 1982). The preservation of SOM is desirable for land use since SOM is widely recognized as a key component in nutrient cycling. Furthermore, the retention of organic C in soil is becoming more important since the rise in atmospheric CO_2 and global warming are recent concerns (Schlesinger, 1997).

Earthworms are considered to improve soil aggregation and they are known to promote the cycling of nutrients (Lee and Foster, 1991; Edwards and Bohlen, 1996). They play a crucial role in the removal of plant litter and other organic materials from the soil surface and the incorporation of these organic materials into soil aggregates (Martin, 1991). Earthworms ingest organic matter and mix it with inorganic soil material. This mixture passes through their gut and is excreted as a cast (Parmelee et al., 1998). Earthworms contribute to soil aggregation mainly through the production of casts. Casts

occur mostly in the upper 0-20 cm of the soil (Lee and Foster, 1991) and contain more water-stable aggregates than surrounding soils (Shipitalo and Protz, 1988; Marinissen, 1994). The formation of water-stable macroaggregates (> 250 μm) depends primarily on temporary binding agents (Tisdall and Oades, 1982). Earthworms play a role in the formation of these binding agents (Martin and Marinissen, 1993) through secretion of mucus in their gut. Earthworm activity increases the mean diameter of dry aggregates in bulk soil and the stability of aggregates of certain size classes. Microbial polysaccharides and other organic products in the casts may strengthen bonds between organic and mineral components, resulting in a protection against microbial attack. Martin (1991) found a decrease in SOM decomposition in the long term when earthworms were present, possibly due to the physical protection of SOM in water-stable aggregates.

It has been suggested that earthworms also have a profound effect on soil aggregation and structure at the microaggregate scale. Several studies have suggested that during gut transit of the soil, the old microstructure is completely destroyed, but new microaggregates are formed within the casts (Shipitalo and Protz, 1988; Barois et al., 1993; Jongmans et al., 2001). In an incubation study, Bossuyt et al. (2003) confirmed that new microaggregates are very rapidly formed (< 20 days) within newly excreted casts. However, these studies have not investigated the C protective capacity of these newly formed microaggregates.

The objective of this study was to investigate the effect of earthworms on (i) soil macro-and microaggregate formation, and (ii) protection of C at a microaggregate scale inside of their casts. In this study, we used ^{13}C-labeled sorghum leaves, to follow the incorporation of newly added residue into different aggregate-associated C fractions.

Carbon mineralization rates of formed intact aggregates and crushed aggregates were determined to assess the level of C protection exerted by the different aggregate size classes.

Materials and methods

Site description and soils

Surface no-tillage soil samples were collected with a shovel from a long-term agricultural experimental site (Horseshoe Bend) near Athens, GA. The site is located in the Piedmont of the southern Appalachian Mountains (33° 54'N, 83° 24'W). The soil is a well-drained sandy clay loam (66% sand, 13% silt, 21% clay) in the Hiwassee series (fine kaolinitic thermic typic Kanhapladult). The area receives a mean annual precipitation of 1270 mm. The experimental plots (0.1ha) were established in 1978 with replicated tillage treatments assigned in a complete randomized block design. Details on the treatment histories at the Horseshoe Bend Research Area Site can be found in Beare et al. (1994a) and Hendrix (1997).

After collection, the soil was air-dried (moisture content after air-drying 1-2 %) and pushed through a 250-μm sieve. The 250-1000 μm sized sand plus particulate organic matter (POM) fractions were kept and re-mixed with the soil after sieving. Stones larger than 1000 μm were discarded.

Incubation 1

This incubation was conducted as described by Bossuyt et al. (2003). Briefly, the 250 μm sieved soil was subjected to 3 treatments, each in 4 replicates (n=4): (i) no plant material and no earthworms; (ii) ^{13}C labeled plant material, but no earthworms; and (iii) ^{13}C labeled plant material and 6 earthworms. Carbon-13 labeled sorghum (*Sorghum bicolor (L.) Moench*) leaves were used as the labeled plant material. The earthworm species was *Aporrectodea caliginosa* (Savigny 1826). In the first two treatments, 1.2 g of plant material was mixed in 150 g soil; the mixture was brought to field capacity (11% water content) and put in glass jars. Field capacity was determined on pressure plate extractors. Samples were incubated at 20°C for 20 days. Earthworms were added after 8 days of incubation. Respiration (total CO_2 and $^{13}CO_2$) was measured every day during the first week and every other day afterwards up to 20 days. At day 20, the earthworms were taken out of the jars and the soil was air-dried for 3 days to allow earthworm casts to stabilize (Marinissen and Dexter, 1991). Aggregate size distribution was determined by wet sieving the slowly rewetted soil as described by Elliott (1986). Briefly, a series of three sieves is used to obtain four aggregate size fractions: (i) > 2000 μm (large macroaggregates); (ii) 250-2000 μm (small macroaggregates); (iii) 53-250 μm (microaggregates); (iv) < 53 μm (silt and clay fraction). Following wet sieving, the aggregate size fractions > 53 μm were dried on the sieves in a dehumidifying chamber (10°C). Particles < 53 μm were collected in a bucket, total volume was measured and a subsample of a known volume was taken for analysis.

Incubation 2

A second incubation was done to biologically determine the macro- and microaggregate-associated C and ^{13}C pools in the formed aggregates. Different sets of incubations were conducted: (i) large and small macroaggregates, (ii) < 250 μm large and small crushed macroaggregates, (iii) < 53 μm large and small crushed macroaggregates; (iv) microaggregates and, (v) < 53 μm crushed microaggregates. From all the dry aggregate size classes from incubation 1, except the < 53 μm aggregate size class, subsamples (12-15 g) to be incubated were weighed into plastic cups and deionized water was added to achieve field capacity. Subsamples were incubated (30°C) in sealed jars with lids containing septa for gas sampling. Gas samples were taken on days 3, 11, and 21.

Analyses

Total CO_2 evolved during the incubations was analyzed on a Varian Star 3600CX (Varian Analytical Instruments, Sugar Land, Texas) gas chromatograph with a thermal conductivity detector. Variations in ^{13}C of the CO_2 evolved during the incubations were determined using a micromass VG optima mass spectrometer (Micromass UK Ltd., Manchester, UK). Results are expressed as:

$$^{13}C‰ = \left[\left(\frac{^{13}R_{sample}}{^{13}R_{standard}}\right) - 1\right] \times 1000, \quad \text{where } {}^{13}R = {}^{13}C/{}^{12}C$$

and the standard is the international Pee Dee Belemnite (PDB).

The amount of CO_2-C derived from the sorghum residue (Q_w) is calculated using the following mass balance :

$$Q_t \times \delta_t = Q_w \times \delta_w + Q_s \times \delta_s + Q_b \times \delta_b$$

where Q_t = the total amount of CO_2-C; δ_t = its isotopic composition; Q_w = the amount of CO_2-C derived from the wheat; δ_w = its isotopic composition (296 ± 2.7‰); Q_s = the amount of CO_2-C derived from the soil; δ_s = its isotopic composition (-24.76 ± 0.18‰); Q_b = blank CO_2-C amount; δ_b = its isotopic composition (-7.5 ± 0.59‰). The control samples (no sorghum added) are used to measure Q_s and δ_s ; with the assumption of no priming effect.

Total C and ^{13}C from the aggregate size fractions was determined using a Finnigan Delta C Mass Spectrometer coupled to a Carlo Erba, NA 1500, CHN Combustion Analyzer via Finnigan's Conflo II Interface.

Calculations

The results of the aggregate incubations were used to define five aggregate-associated carbon pools (Bossuyt et al., 2002): (i) unprotected macroaggregate C, (ii) unprotected microaggregate C, (iii) macroaggregate-protected C, (iv) microaggregate-protected C and; (v) microaggregate within macroaggregate-protected C. These different pools were calculated as follows:

2) Unprotected macroaggregate C

= intact macroaggregate C_{min}

2) Unprotected microaggregate C

= intact microaggregate C_{min}

3) Macroaggregate-protected C

= < 250 μm crushed macroaggregate C_{min} – intact macroaggregate C_{min}

4) Microaggregate-protected C

= < 53 μm crushed microaggregate C_{min} – intact microaggregate C_{min}

5) Microaggregate within macroaggregate-protected C

= < 53 μm crushed macroaggregate C_{min} – macroaggregate-protected C –

Unprotected macroaggregate C

[replace macroaggregate-protected C (see equation 3))]

= < 53 μm crushed macroaggregate C_{min} – (< 250 μm crushed

macroaggregate C_{min} – intact macroaggregate C_{min}) – intact

macroaggregate C_{min}

= < 53 μm crushed macroaggregate C_{min}– < 250 μm crushed

macroaggregate C_{min}

with C_{min} the cumulative C mineralized after 21 days of incubation. The macroaggregate-associated C pools were calculated for both large and small macroaggregates.

Statistical analysis

The data were analyzed, using the SAS statistical package for analysis of variance (ANOVA-PROC GLM, SAS Institute, 1990). Separation of means was tested with the DIFF option of the LSMEANS statement with a significance level of $P< 0.05$.

Results

Incubation I

Water-stable aggregates

There was a significant impact of earthworm activity on the distribution of water-stable aggregates (Fig. 4.1). Large macroaggregates (> 2000 μm) made up the largest percentage (~ 37% on average) of the whole soil for samples with earthworms and were on average 3.6 times greater than samples without earthworms. When no earthworms and no residue were added, no large macroaggregates were formed. The smaller aggregate size classes (250-2000 μm, 53-250 μm and < 53 μm) made up a greater proportion of the soil without earthworms and/or residue.

Respiration

There was no significant difference in cumulative respiration between samples with or without earthworms, for both total and residue-derived respiration (Table 4.1). The residue-derived respiration accounted for approximately 70 % of the total respiration.

Total C and ^{13}C concentrations

Total aggregate-associated C concentrations were significantly influenced by earthworm activity. Total large macroaggregate-associated C concentrations were significantly higher in the presence of earthworms (Fig. 4.2). There was no significant difference in total small macroaggregate-associated C. For the other aggregate size

classes, total aggregate-associated C concentrations were higher in the absence of earthworms. There were no significant differences in total aggregate-associated C concentrations between the control samples and the samples without earthworms, except for the silt – and clay fraction (< 53 μm) where total aggregate-associated C was significantly higher when no residue was added.

Aggregate-associated ^{13}C concentrations were significantly influenced by earthworm activity. Large macroaggregate-associated ^{13}C concentrations were significantly higher in the samples with earthworms (Fig. 4.3). All the other aggregate size classes had higher ^{13}C concentrations without earthworms added, except for the 250-2000 μm where there was no significant difference in ^{13}C concentration between the samples with and without earthworms. The aggregate-associated ^{13}C concentrations were following the same trend as the total aggregate-associated C concentrations.

Incubation II

Unprotected and aggregate-protected C and ^{13}C pools

The amount of C mineralized after 21 days is shown in Table 4.2 as cumulative respired C (mg C kg^{-1} soil). The unprotected C pools were the largest C pools and were always significantly larger than the corresponding protected C pools. The unprotected small macroaggregate C pool was highest when no earthworms were present and lowest when no residue was added. The unprotected microaggregate C pool was significantly higher in the samples without earthworms than in those with earthworms. In control soil, this pool was not significantly different than the other treatments. The small

macroaggregate-protected, microaggregate-protected and microaggregate within small macroaggregate-protected C pools did not differ between any of the treatments and were relatively non-existing or small pools. A large pool in the samples with earthworms is the microaggregate within large macroaggregate protected C pool which is about 2.5 times higher than the microaggregate within small macroaggregate protected C pool. This pool was not detectable in the samples without earthworms or without residue because only a small amount of large macroaggregates were present.

The amount of ^{13}C or residue-derived C mineralized after 21 days is shown in Table 4.2 as cumulative respired ^{13}C (mg ^{13}C kg^{-1} soil). The largest residue-derived C pools are the unprotected pools. The unprotected small macroaggregate pool is higher in the absence of earthworms. There is no difference between the treatments for the unprotected microaggregate ^{13}C pool. There is no residue-derived C found in the large macroaggregate-protected, small macroaggregate-protected or microaggregate within small macroaggregate-protected pools. The microaggregate within large macroaggregate-protected ^{13}C pool is a significant pool in the presence of earthworms. Twenty-two percent of the C in microaggregate within large macroaggregates is newly added residue C. Earthworm activity has no effect on the incorporation of residue into free microaggregates.

Discussion

Incubation I

Water-stable aggregates

This study indicates that the presence of earthworms has a significant impact on the formation of large macroaggregates. In the samples where earthworms were added, higher amounts of water-stable large macroaggregates (> 2000 μm) were found. Several researchers described the positive influence of earthworms on the formation and stability of soil aggregates. Blanchart et al. (1989) found that in 33 days, 42.4% of the soil was aggregated in the presence of earthworms compared to 13.3 % in the control soil. Martin and Marinissen (1993) described how earthworms play an important role in the production of binding agents responsible for the formation of water-stable macroaggregates. Various studies showed a higher stability in earthworm casts than in the surrounding soil aggregates (Monnier and Jeanson, 1964; van Rhee, 1977; De Vleesschauwer and Lal, 1981; McKenzie and Dexter, 1987). Earthworms ingest large quantities of organic materials that are mixed and excreted as casts (Parmelee et al., 1990; Martin and Marinissen, 1993; Jégou et al., 1998) and improve stable macroaggregation (Guggenberger et al., 1996; Marinissen and Hillenaar, 1996; Scullion and Malik, 2000). The aggregates formed in this incubation were dried for 3 days to allow casts to stabilize. This was done because several researchers found that the casting activities only enhance aggregate stability if the casts are dried or aged (Shipitalo and Protz, 1988; Marinissen and Dexter, 1990).

Respiration

There was no effect of earthworm activity on overall soil respiration or residue-derived respiration. Earthworms are known to have a significant impact on microbial activity by causing favorable conditions. The general contribution, however, to total respiration is usually low. Petersen and Luxton (1982) reported that the soil fauna generally appears to be responsible for less than 5% of total decomposer respiration. Several other researchers also found that micro-organisms are the major contributors to soil respiration being responsible for 80 to 95% of the total CO_2 respired (Satchell, 1971; Persson and Lohm, 1977; Lamotte, 1989). Zhang and Hendrix (1995), on the other hand, saw an increase in microcosm respiration up to ~22% when earthworms were present compared to control soil. Haimi and Huhta (1990) observed that 15 to 18% of microbial respiration was due to earthworm activity in raw humus forest soil.

Total C and ^{13}C concentrations

This study showed that earthworms induced a redistribution of total C and residue-derived C into the large macroaggregates or the earthworm casts. Total and residue-derived aggregate-associated C concentrations in large macroaggregates were respectively 3 and 2 times higher in the presence of earthworms. Zhang and Schrader (1993) found an increase of 20-37 % of total C in casts compared to bulk soil and Lee (1985) concluded that C content of casts is usually about 1.5 to 2 times higher than the surrounding soil. Various other researchers reported a higher amount of total or organic C in earthworm casts (Shipitalo and Protz, 1988; Mulongoy and Bedoret, 1989; Daniel and Anderson, 1992; Barois et al., 1993; Buck et al., 1999). A selective feeding of

particles high in organic matter may result into a higher amount of C in the earthworm casts. Bhandari et al. (1967) suggested that the increase in microbial activity and an increase in polysaccharide production in casts caused a higher OM concentration compared to bulk soil.

Incubation II

Unprotected and aggregate-protected C and ^{13}C pools

Since the grinding of aggregates potentially breaks up the plant material present within the aggregates it possibly increases C mineralization beyond the level caused by physical protection. Bossuyt et al. (2002), however, reviewed literature on the effects of grinding on plant residue mineralization and concluded that the grinding of organic particles is probably not an important factor contributing to increased C mineralization. Therefore, our methodology to assess physical protection exerted by aggregates seems to be valid.

The largest pools were unprotected pools. This suggests that after 20 days of incubation, most of the aggregate-associated C is still in an unprotected form and easily mineralizable by the microbial population. Crushing the macroaggregates to the size of microaggregates made macroaggregate-protected C and ^{13}C available for microbial mineralization. These pools were very small or non-existent after 20 days of incubation, showing that, in the short term, almost no C or ^{13}C is protected at a macroaggregate scale in both the samples with or without earthworms or the control soil.

When aggregates are ground until < 53 μm, however, a significant amount of C is mineralized from the large macroaggregates in the presence of earthworms. This showed that C protection due to microaggregates within macroaggregates is much more important than C protection at a macroaggregate level and that earthworms play a very important role in protecting C inside of microaggregates within macroaggregates. This pool was not measurable and therefore insignificant in the absence of earthworms because the amount of large macroaggregates was ~ 4 times lower than in the earthworm treatment. In current aggregate-SOM concepts (e.g. Gale et al., 2000; Golchin et al., 1994; Six et al., 1998), the formation of stable microaggregates within macroaggregates and the concomitant protection of C is understood as a longer-term aging and stabilization process of organo-mineral complexes mediated by microbial activity and exudation. However, Bossuyt et al. (2003) showed that earthworms induce a fast formation of these microaggregates and here we show that these microaggregates do protect a significant amount of C after 12 days of incubations.

Twenty-two percent of the total C protected inside of microaggregates within large macroaggregates is residue-derived C, showing that a significant amount of freshly added residue is already incorporated and protected inside microaggregates within macroaggregates. The formation of microaggregates inside of earthworm casts already takes place before the cast is excreted (Shipitalo and Protz, 1989; Barois et al., 1993) since earthworms incorporate fresh organic residues directly into the soil matrix without the requirement of microbial decomposition for microaggregate formation (Pulleman et al., submitted).

Conclusions

Based on the results of this study, we can conclude that earthworms have a significant impact on the distribution of soil aggregates and aggregate-associated C pools. The proportion of large macroaggregates is significantly higher in the presence of earthworms and these aggregates contain significantly more total C and residue-derived C compared to bulk soil. Earthworms were responsible for the formation of a significant pool of microaggregate within macroaggregate-protected C after 12 days of incubation and the fast protection of C at this microaggregate level is much larger than at the macroaggregate level.

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References

Barois, I., Villemin, G., and Lavelle, P., 1993. Transformation of the Soil Structure through *Pontoscolex corethrurus* (Oligochaeta) intestinal tract. Geoderma, 56: 57-66.

Blanchart, E., Lavelle, P., and Spain, A.V., 1989. Effects of two species of tropical earthworms (Oligochaete: Eudrilidae) on the size distribution of aggregates in an African soil. Revue d' Ecologie et de Biologie de Sol, 26: 17-425.

Bhandari, G.S., Randhawa, N.S., and Maskin, M.S., 1967. On the polysaccharide content of earthworm casts. Curriculum of Science (Bangalore), 36: 519-520.

Bossuyt, H., Six, J., and Hendrix, P.F., 2002. Aggregate-Protected Carbon in No-Tillage and Conventional Tillage Agroecosystems Using Carbon-14 Labeled Residue. Soil Science Society of America Journal, 66: 1965-1973.

Bossuyt, H., Six, J., and Hendrix, P.F., 2003. Rapid incorporation of fresh residue-derived carbon into newly formed stable microaggregates within earthworm casts. European Journal of Soil Science, submitted.

Buck, C., Langmaack, M., and Schrader, S., 1999. Nutrient content of earthworm casts influenced by different mulch types. European Journal of Soil Biology, 35: 23-30.

Daniel, O., and Anderson, J.M., 1992. Microbial biomass and activity in contrasting soil materials after passage through the gut of the earthworm *Lumbricus rubellus*. Soil Biology and Biochemistry, 24: 465-470.

De Vleesschauwer, D., and Lal, R., 1981. Properties of worm casts under secondary tropical forest regrowth. Soil Science, 132: 175-181.

Edwards, C.A., and Bohlen, P.J., 1996. Biology and Ecology of Earthworms. Chapman and Hall, London.

Elliott, E.T., 1986. Aggregate structure and carbon, nitrogen, and phosphorus in native and cultivated soils. Soil Science Society of America Journal, 4: 518-524.

Guggenberger, G., Thomas, R.J., and Zech, W., 1996. Soil organic matter within earthworm casts of an anecic-endogeic tropical pasture community, Colombia. Applied Soil Ecology, 3: 263-274.

Haimi, J., and Huhta, V., 1990. Effects of earthworms on decomposition processes in raw humus forest soil: A microcosm study. Biology and Fertility of Soils, 10: 178-183.

Hendrix, P.F., 1997. Long-term patterns of plant production and soil carbon dynamics in a Georgia Piedmont agroecosystem. pp. 235-245. *In*: Soil Organic Matter in Temperate Agroecosystems; Long-term experiments in North America. E.A. Paul, K. Paustian, E.T. Elliott, and C.V. Cole (ed.). CRC Press, Ind., Boca Raton, FL.

Jégou, D. Cluzeau, D., Balesdent, J., and Tréhen, P., 1998. Effects of four ecological categories of earthworms on carbon transfer in soil. Applied Soil Ecology, 9: 249-255.

Jongmans, A.G., Pulleman, M.M., and Marinissen, J.C.Y., 2001. Soil structure and earthworm activity in a marine silt loam under pasture versus arable land. Biology and Fertility of Soils, 33: 279-285.

Lamotte, M., 1989. Place des animaux détritivores et des microorganismes décomposeurs dans les flux d'énergie des savanes africaines. Pedobiologia, 33: 17-35.

Lee, K.E., 1985. Earthworms: Their Ecology and Relationships with Soils and Land Use. Academic Press, Sydney, 411pp.

Lee, K.E., and Foster, R.C., 1991. Soil fauna and soil structure. Australian Journal of Soil Research, 29: 745-776.

Lynch, J.M., and Bragg, E., 1985. Microorganisms and Soil Aggregate Stability. Advances in Soil Science, 2: 134-170.

Marinissen, J.C.Y., 1994. Earthworms and stability of soil structure: a study in a silt loam soil in a young Dutch polder. Agriculture, Ecosystems & Environment, 51: 75-87.

Marinissen, J.C.Y., and Dexter, A.R., 1990. Mechanisms of stabilization of earthworm casts and artificial casts. Biology and Fertility of Soils, 9: 163-167.

Marinissen, J.C.Y., and Hillenaar, S.I., 1996. Earthworm induced distribution of organic matter in macro-aggregates from differently managed arable fields. Soil Biology and Biochemistry, 29: 391-395.

Martin, A., 1991. Short- and long-term effects of the endogeic earthworm *Millsonia anomala (Omodeo) (Megascolecidae, Oligochaeta*) of tropical savannas on soil organic matter. Biology and Fertility of Soils, 11: 234-238.

Martin, A., and Marinissen, J.C.Y., 1993. Biological and physico-chemical processes in excrements of soil animals. Geoderma, 56: 331-347.

McKenzie, B.M., and Dexter, A.R., 1987. Physical properties of casts of the earthworm *Aporrectodea rosea*. Biology and Fertility of Soils, 5: 328-332.

Monnier, G., and Jeanson, G., 1964. Studies on the stability of soil structure: Influence of moulds and soil fauna. Proc. 11th Easter School in Agricultural Science, Univ. Nottingham

Mulongoy K., and Bedoret, A., 1989. Properties of worm casts and surface soils under various plant covers in the humid tropics. Soil Biology and Biochemistry, 21: 197-203.

Parmelee, R.W., Beare, M.H., Cheng, W., Hendrix, P.F., Rider, S.J., Crossley, D.A., and Coleman, D.C., 1990. Earthworms and enchytraeids in conventional and no-tillage agroecosystems. A biocide approach to assess their role in organic matter breakdown. Biology and Fertility of Soils, 10: 1-10.

Parmelee, R.W., Bohlen, P.J., and Blair, J.M., 1998. Earthworms and Nutrient Cycling Processes: Integrating Across the Ecological Hierarchy. *In*: Earthworm Ecology. Edwards, C.A. (Ed.) pp 123-146.

Persson, T., and Lohm, U., 1977. Energetical Significance of the Annelids and Arthropods in a Swedish Grassland Soil, NFR, Stockholm.

Petersen, H., and Luxton, M., 1982. A comparative analysis of soil fauna populations and their role in decomposition processes. Oikos, 39: 288-388.

Pulleman, M.M., Marinissen, J.C.Y., Jongmans, A.G. Six, J., and Uijl, A., 2003. Macro- and microaggregate structure and soil organic matter dynamics as affected by earthworm activity in different farming systems. Biology and Fertility of Soils, submitted.

SAS Institute, 1990. SAS/STAT User's guide. Vol. 2. Version 6 ed. SAS Inst., Cary, NC.

Satchell, J.E., 1971. Feasibility study of an energy budget for Meathop Wood. In: Productivité des Ecosystèmes Forestiers. (ed. P. Duvigneaud), UNESCO, Paris, pp. 619-630.

Scullion, J., and Malik, A., 2000. Earthworm activity affecting organic matter, aggregation and microbial activity in soils restored after open cast mining for coal. Soil Biology and Biochemistry, 32: 119-126.

Schlesinger, W.H., 1997. Biogeochemistry: An analysis of global change. 2nd ed. Academic Press, San Diego, CA.

Shipitalo, M.J., and Protz, R., 1988. Factors influencing the dispersibility of clay in worm casts. Soil Science Society of America Journal, 52: 764-769.

Shipitalo, M.J., and Protz, R., 1989. Chemistry and micromorphology of aggregation in earthworm casts. Geoderma, 45: 357-374.

Six, J., Eliott, E.T., Paustian, K., and Doran, J.W., 1999. Aggregation and soil organic matter accumulation in cultivated and native grassland soils. Soil Science Society of America Journal, 62: 1367-1377.

Six, J., and Jastrow, J.D. 2002. Soil organic matter turnover. In R.Lal (Ed.) Encyclopedia of Soil Science. Marcel Dekker, NY.

Tisdall, J.M., and Oades, J.M., 1982. Organic matter and water-stable aggregates in soils. Journal of Soil Science, 33: 141-161.

van Rhee, J.A., 1977. A study of the effect of earthworms on orchard productivity. Pedobiologia 17, 107-114.

Zhang, Q.L., and Hendrix, P.F., 1995. Earthworm (*Lumbricus rubellus* and *Aporrectodea caliginosa*) Effects on Carbon Flux in Soil. Soil Science Society of America Journal, 59: 816-823

Zhang, H., and Schrader, S., 1993. Earthworm effects on selected physical and chemical properties of soil aggregates. Biology and Fertility of Soils, 15: 229-234

Table 4.1. Total and residue-derived cumulative respiration in soil samples with or without earthworms.

treatment	CO_2-C†	$^{13}CO_2$-C
	mg g^{-1} soil	mg g^{-1} soil
+ earthworms + residue	6.44a	4.37a
- earthworms + residue	6.21a	4.45a
- earthworms - residue	2.49a	

† Values followed by a different lowercase letter are significantly different between sample treatments

Table 4.2. Aggregate-associated carbon (C) and carbon-13(^{13}C) pools of soil samples with or without earthworms

	Total carbon mineralized			^{13}Carbon mineralized	
	+ worms	- worms	- residue	+ worms	- worms
	mg kg^{-1} soil†			mg kg^{-1} soil	
1) Large macroaggregates					
Unprotected C	260.8	n/d*	0	177	n/d
Protected C	6.0	n/d	0	0.0	n/d
2) Small macroaggregates					
Unprotected C	179.5b	283.7a	129.8c	137b	206a
Protected C	1.2a	0.0a	10.7a	1.1a	0.0a
3) Microaggregates					
Unprotected C	168.8b	260.9a	238.4ab	63.3a	72.9a
Protected C	55.8a	72.4a	70.8a	8.8a	6.3a
4) Microaggregate within large macroaggregates					
Protected C	161.7	n/d	0	35.2	n/d
5) Microaggregate within small macroaggregates					
Protected C	62.4a	42.5a	44.1a	0.0a	0.0a†

Values followed by a different lowercase letter are significantly different between sample treatments, n/d: not detectable

List of figures

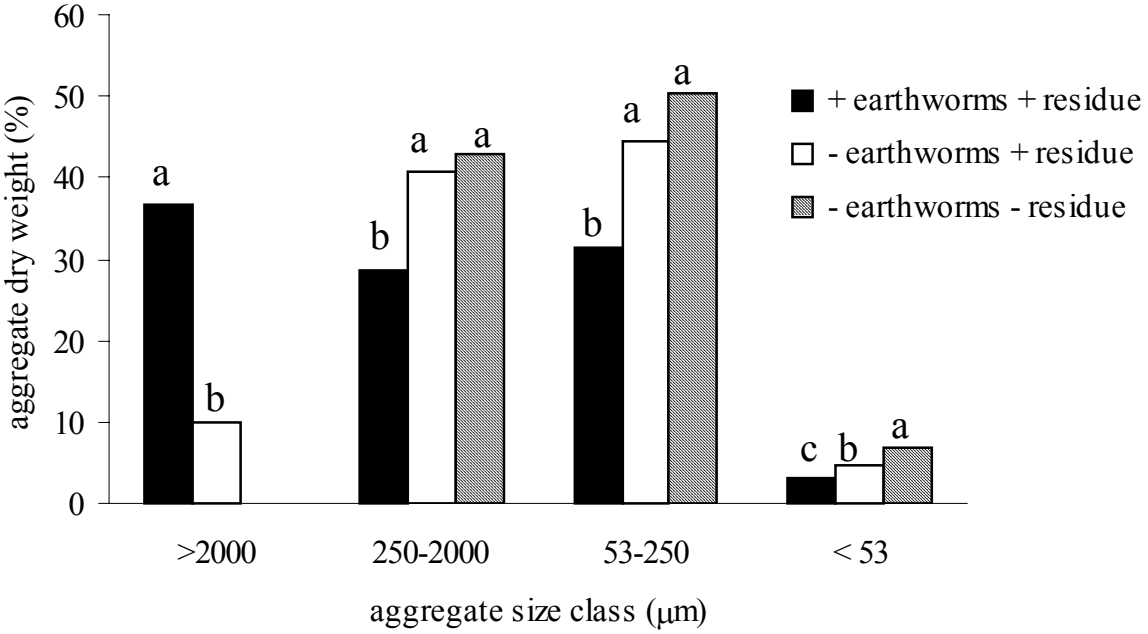
60
50
40
30
20
10
0
aggregate dry weight (%)
a
b
a
a
b
a
b
a
c
b
a
+ earthworms + residue
- earthworms + residue
- earthworms - residue
>2000
250-2000
53-250
< 53
aggregate size class (μm)

Fig. 4.1

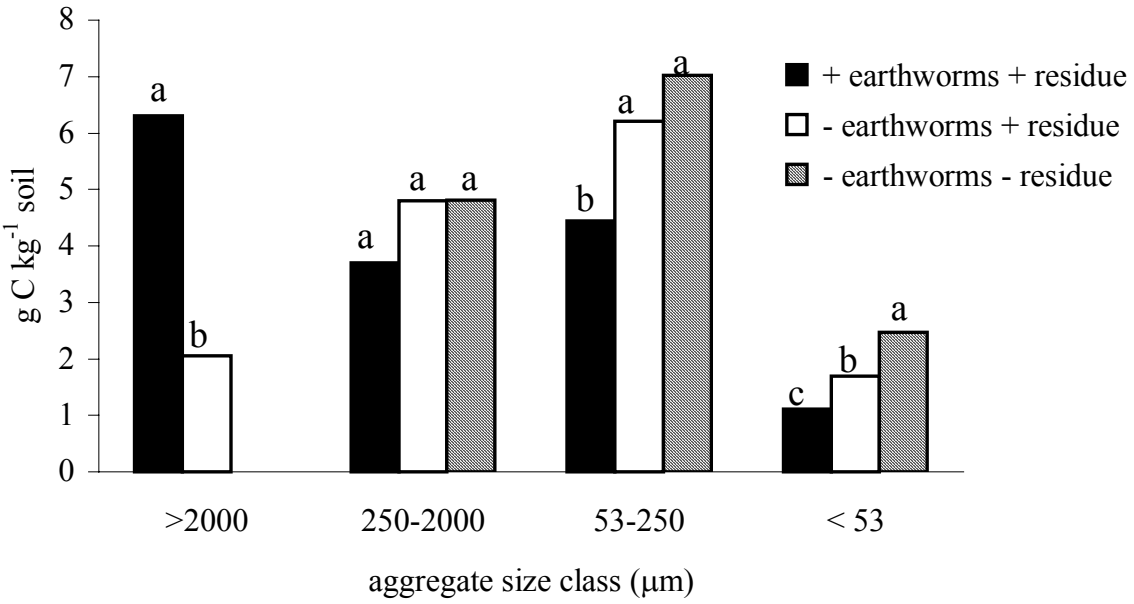
8
7
6
5
4
3
2
1
0
g C kg-1 soil
a
b
a
a
a
b
a
a
c
b
a
>2000
250-2000
53-250
< 53
aggregate size class (μm)
+ earthworms + residue
- earthworms + residue
- earthworms - residue

Fig. 4.2

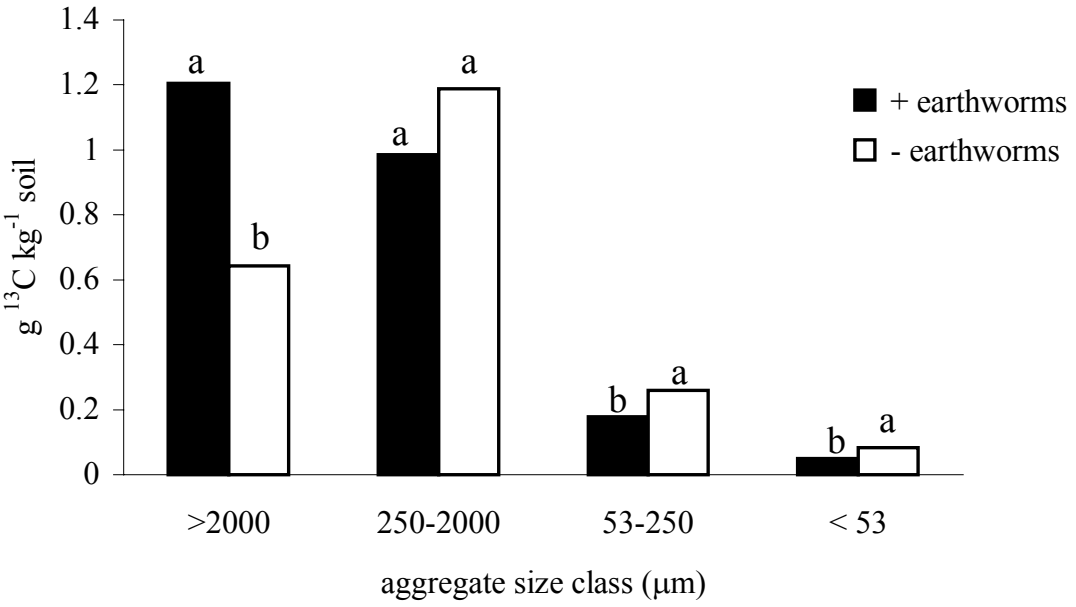
1.4
1.2
1
0.8
0.6
0.4
0.2
0
g ^{13}C kg^{-1} soil
a
b
a
a
b
a
b
a
+ earthworms
- earthworms
>2000
250-2000
53-250
< 53
aggregate size class (μm)

Fig. 4.3

CHAPTER 5

INTERACTIVE EFFECTS OF TWO EARTHWORM SPECIES ON FORMATION OF STABLE MICROAGGREGATES AND INCORPORATION OF FRESH RESIDUE INTO CASTS[1]

[1]Bossuyt, H., J. Six, P.F. Hendrix. To be submitted to *European Journal of Soil Science.*

Abstract

The interactive effects of two earthworm species (*Aporrectodea caliginosa* and *Lumbricus rubellus*) on the incorporation of fresh residue into large macroaggregates and formation of microaggregates within these large macroaggregates were investigated during a short-term laboratory experiment using ^{13}C-labeled sorghum (*Sorghum bicolor (L.) Moench*) residues. Soil was collected from the Horseshoe Bend Research Area in Athens, GA, crushed through a 250-µm sieve and incubated under laboratory conditions. The following treatments were applied: (i) soil + ^{13}C-labeled residue + *Aporrectodea caliginosa*; (ii) soil + ^{13}C-labeled residue + *Lumbricus rubellus*; (iii) soil + ^{13}C-labeled residue + *Aporrectodea caliginosa* + *Lumbricus rubellus* and; (iv) soil + ^{13}C-labeled residue. Residue was mixed in with the soil in half of the samples and placed on the soil surface in the other half. Aggregate size distribution and total C and ^{13}C were measured after 20 days. Microaggregates and fine inter-microaggregate particulate organic matter (inter-POM) were isolated out of macroaggregates; intra-microaggregate POM (intra-POM) was also determined. Formation of microaggregates within large macroaggregates was greatest in the presence of *Aporrectodea caliginosa* and when the residue was mixed in with the soil. When the residue was placed on the surface, residue-derived intra-POM C was highest when *Lumbricus rubellus* was present and residue-derived inter-POM C was highest when a mix of both species was present. These results indicate that interactive effects of earthworm species might have important consequences for the incorporation and protection of C inside of microaggregates within macroaggregates especially when residues are placed on the soil surface.

Introduction

The significant relationship between earthworm activity and soil fertility, soil structure and soil organic matter (SOM) dynamics has long been recognized. Darwin (1881) acknowledged that the activity of earthworms had a positive effect on the soil's physical conditions and plant growth. Hoogerkamp et al. (1983) and Lee (1985) found a positive relationship between soil fertility and the abundance of earthworms. Earthworms are known to play an important role in the incorporation of plant residue into soil aggregates. When they burrow through the soil, they consume and excrete soil and plant residues, greatly affecting SOM dynamics and the formation and stability of soil aggregates.

The protection of SOM is essential because SOM is a key factor in nutrient cycling and an important quality indicator of soil management. There is a two-way relationship between SOM and soil aggregates. While aggregates physically protect SOM, clay particles bind with SOM to form aggregates of different sizes.

Oades (1984) proposed that microaggregates are formed at the center of macroaggregates. Fragmented organic matter originating from roots, fungal hyphae, and fecal matter become incorporated within macroaggregates by, for example, the feeding and casting activities of earthworms and other soil fauna. During the further decomposition of this larger intra-aggregate POM, organic matter fragments become encrusted with microbial byproducts and clay particles, leaving the organic matter occluded and inaccessible to microbial attack. The microaggregates formed in this way

and later released from macroaggregates by various disturbances, are relatively stable and enriched in SOM.

Recent studies have showed the importance of earthworms in the formation of these stable microaggregates within macroaggregates (Jongmans et al., 2001; Bossuyt et al., 2003). Bossuyt et al. (2003a) found that these stable microaggregates are formed after 12 days of incubation in the presence of earthworms. In their study, these microaggregates were also enriched in total C and fresh residue-derived C. Since microaggregates are more stable than macroaggregates, the incorporation of C inside of these microaggregates might be of great importance for the long-term protection of soil C. Bossuyt et al. (2003b), indeed found that a relatively great proportion of newly incorporated C by earthworm activity was only decomposed upon break down of the microaggregate structures.

However, little or nothing is known about the interactive effects of different earthworm species on the formation of microaggregates within macroaggregates and the incorporation of soil C or fresh residue-derived C into these microaggregates. In southeastern US agricultural soils, two earthworm species are very common: *Aporrectodea caliginosa* and *Lumbricus rubellus*. *Lumbricus rubellus* is an epigeic species. Epigeic species are defined as litter dwellers. They live above the mineral soil surface, mainly in the soil litter layer. Their activity is thought to have little effect on soil structure and soil aggregation. *Aporrectodea caliginosa* is an endogeic species. Endogeic species live in mineral soil horizons and feed on soil more or less enriched with organic matter. They are termed “a major agent of soil aggregation and soil organic matter stabilization” (Lavelle and Spain, 2001).

In this study, we used these two earthworm species to investigate their distinct and/or combined effects on the formation of soil aggregates, the incorporation of litter into soil aggregates and the formation of stable microaggregates within macroaggregates. A short term laboratory experiment using ^{13}C-labeled sorghum (*Sorghum bicolor (L.) Moench*) was conducted. The plant residue was mixed in with the soil samples or placed on the soil surface to simulate the different feeding habits from the used earthworm species.

Materials and methods

Site description and soils

Surface no-tillage soil samples were collected with a shovel from the long-term agricultural experimental site (Horseshoe Bend) near Athens, GA. The site is located in the Piedmont of the southern Appalachian Mountains (33° 54'N, 83° 24'W). The soil is a well-drained sandy clay loam (66% sand, 13% silt, 21% clay) in the Hiwassee series (fine kaolinitic thermic typic Kanhapludult). The area receives a mean annual precipitation of 1270 mm. The experimental plots (0.1 ha) were established in 1978 with replicated tillage treatments assigned in a completely randomized design. Details on the treatment histories at the Horseshoe Bend Research Area Site can be found in Beare et al. (1994) and Hendrix (1997).

After collection, the soil was air-dried (moisture content after air-drying 1-2 %) and pushed through a 250 μm sieve. The 250-1000 μm sized sand plus particulate

organic matter (POM) fractions were kept and re-mixed with the soil after sieving. Stones larger than 1000 μm were discarded.

Incubation

From the 250 μm sieved, air-dried soil, 64 subsamples (500 g) were subjected to 8 treatments, each in 4 replicates (n=4): (i) soil + ^{13}C-labeled residue + *Aporrectodea caliginosa* (*A. caliginosa*); (ii) soil + ^{13}C-labeled residue + *Lumbricus rubellus* (*L. rubellus*); (iii) soil + ^{13}C-labeled residue + *Aporrectodea caliginosa* + *Lumbricus rubellus* and; (iv) soil + ^{13}C-labeled residue. Residue was mixed in with the soil in half the samples and placed on the soil surface in the other half Carbon-13 labeled grain sorghum (*Sorghum bicolor (L.) Moench*) leaves ground to a size of 500-1000 μm were used as the labeled plant material and added at a rate of 2070 mg C kg^{-1} soil. The mixture was brought to field capacity (11% water content) and put in glass jars with lids containing septa for gas sampling. The jars were held overnight at 4°C to equilibrate and then incubated at 20°C for 22 days. Earthworms were added after 8 days of incubation. Respiration (total CO_2 and $^{13}CO_2$) was measured at 1, 4, 8, 11 and 14 days after earthworm additions. 14 days after the earthworms were added, they were taken out of the jars and the soil was air-dried for 3 days to allow worm casts to stabilize (Marinissen & Dexter, 1991). After being dried, the soil was slowly rewetted, held overnight at 4°C and aggregate size distribution was determined by wet sieving as described by Elliott (1986). Briefly, a series of three sieves was used to obtain four aggregate size fractions: (i) > 2000 μm (large macroaggregates); (ii) 250-2000 μm (small macroaggregates); (iii) 53-250 μm (microaggregates); (iv) < 53 μm (silt and clay fraction). Following wet

sieving, the aggregate size fractions > 53 μm were dried on the sieves in a dehumidifying chamber (10°C). Particles < 53 μm were collected in a bucket, total volume was measured and a subsample of a known volume was taken for analysis.

Isolation of microaggregates within macroaggregates

Subsamples from the small and large macroaggregate fractions were taken to isolate the microaggegates held within macroaggregates following the method described by Six et al. (2000). A device was used to completely break up macroaggregates while minimizing the break down of the released microaggregates. Macroaggregates (10 g) were immersed in deionized water on top of a 250 μm mesh screen and gently shaken with 50 glass beads (dia. = 4 mm). Continuous and steady water flow through the device ensured that microaggregates were immediately flushed onto a 53 μm sieve and not further disrupted by the glass beads. After all macroaggregates were broken up, the sand and coarse particulate organic matter (coarse POM) retained on the 250 μm sieve were washed off and collected. The material collected on the 53 μm sieve was sieved according to Elliott (1986) to ensure that the isolated microaggregates were water-stable. The proportion of microaggregate weight (53-250 μm) within macroaggregates (250-2000 μm) was calculated according to Six et al. (2000):

$$\frac{\text{(microaggregate weight – weight of 53-250 μm sized sand)}}{\text{(macroaggregate weight – weight of 250-2000 μm sized sand)}}$$

The weights of macro- and microaggregates were corrected for the sand content of the same size as the aggregates because sand of the same size as an aggregate has a higher probability of not being a part of the aggregate and should consequently not be weighed as an aggregate.

Inter-microaggregate particulate organic matter and intra-microaggregate particulate organic matter contents

The inter-microaggregate particulate organic matter (inter-POM) that was retained on the 53 μm sieve together with the microaggregates was isolated by density flotation with 1.85 g cm^{-3} sodium polytungstate (Six et al., 1998). After density flotation, the microaggregates were dispersed by shaking overnight (18 h.) in 5 g L^{-1} hexametaphosphate and intra-microaggregate particulate organic matter (intra-POM) was separated from the clay-and silt fraction (< 53 μm) by sieving. To avoid cross contamination of C, and especially ^{13}C, among samples, sodium polytungstate was recycled according to Six et al. (1999b).

Analyses

Total CO_2 evolved during the incubations was analyzed on a Varian Star 3600CX (Varian Analytical Instruments, Sugar Land, Texas) gas chromatograph, which determined concentration based on thermal conductivity. Variations in ^{13}C of the CO_2 evolved during the incubations were determined using a micromass VG optima mass spectrometer (Micromass UK Ltd., Manchester, UK). Results are expressed as:

$$^{13}C‰ = [(\frac{^{13}R_{sample}}{^{13}R_{standard}}) - 1] \times 1000, \quad \text{where } ^{13}R = {}^{13}C/{}^{12}C$$

and the standard is the international Pee Dee Belemnite (PDB).

The amount of CO_2-C derived from the sorghum residue (Q_w) is calculated using the following mass balance :

$$Q_t \times \delta_t = Q_w \times \delta_w + Q_s \times \delta_s + Q_b \times \delta_b$$

where Q_t = the total amount of CO_2-C; δ_t = its isotopic composition; Q_w = the amount of CO_2-C derived from the wheat; δ_w = its isotopic composition (296 ± 2.7‰); Q_s = the amount of CO_2-C derived from the soil; δ_s = its isotopic composition (-24.76 ± 0.18‰); Q_b = blank CO_2-C amount; δ_b = its isotopic composition (-7.5 ± 0.59‰). The control samples (no sorghum added) are used to measure Q_s and δ_s ; with the assumption of no priming effect.

Total C and ^{13}C from the aggregate size fractions was determined using a Finnigan Delta C Mass Spectrometer coupled to a Carlo Erba, NA 1500, CHN Combustion Analyzer via Finnigan's Conflo II Interface.

Statistical analysis

The data were analyzed, using the SAS statistical package for analysis of variance (ANOVA-PROC GLM, SAS Institute, 1990). Separation of means was tested with the DIFF option of the LSMEANS statement with a significance level of $P < 0.05$.

Results

Respiration

Cumulative respiration is shown in Table 5.1. Cumulative respiration was significantly influenced by earthworm activity and residue application. After 14 days of incubation with or without earthworms, total and residue-derived respiration was significantly higher in all samples with residue on the surface compared to those with residue mixed in the soil. Within the mixed residue treatment, total respiration was lower when no earthworms were present than in the presence of any earthworm species. Residue-derived respiration was highest when *A. caliginosa* was present and lowest when a mix of both species was present. Within the surface residue treatment, total respiration was highest when *L. rubellus* was present and lowest when no earthworms were present. No significant differences were detected between earthworm treatments for the residue-derived respiration.

Water-stable aggregates

There was a significant influence of earthworm activity and residue application on the formation of water-stable aggregates (Fig. 5.1). There were significantly more large macroaggregates (> 2000 μm) when residue was mixed in with the soil than when residue was added on the surface for all earthworm treatments. The proportion of small macroaggregates (250-2000 μm) was significantly larger in the surface residue treatment except when no earthworms were added. The amount of microaggregates (53-250 μm) did not differ between the mixed residue treatment and the surface residue treatment,

except when a mix of both species was present where the amount of microaggregates was significantly larger when residue was added on the surface. For all earthworm treatments, there was a smaller proportion of clay- and silt particles (< 53 μm) when residue was mixed in with the soil.

Within the mixed residue treatment, the greatest amount of large macroaggregates was found in samples with only *A. caliginosa* (~30%). A smaller amount was found in the presence of *L. rubellus* or a mix of both species (~ 25%) and even less large macroaggregates were found when no earthworms were present (~ 7%). Within the surface residue treatment, the highest amount of large macroaggregates was found in samples with *A. caliginosa* or a mix of both species (~15%). Significantly less large macroaggregates were found in the presence of only *L. rubellus* and only ~5% of the soil consisted of large macroaggregates when no earthworms were present.

Residue-derived C concentrations in aggregates

Residue-derived C concentrations in the aggregate size classes were significantly impacted by earthworm activity and residue application (Fig. 5.2). Within the mixed residue treatment, residue-derived C concentration in large macroaggregates was higher in the presence of *A. caliginosa* than when a mix of species was present and all the samples with earthworms had higher C concentrations than the samples without earthworms. In small macroaggregates, residue-derived-C concentrations were higher when both species were together or without earthworms than with *A. caliginosa* or *L. rubellus*. No significant differences between earthworm treatments were detected for the microaggregates. For the silt-and clay fraction, the samples without earthworms had a

higher residue-derived C concentration than the other earthworm treatments. Within the surface residue treatment, residue-derived C concentrations were larger in *A. caliginosa* and *L. rubellus* than with both species or no earthworms for the large macroaggregates. There were no significant differences between earthworm treatments for the small macroaggregates. For the microaggregates and the silt-and clay fraction, residue-derived C concentration was higher in samples with *A. caliginosa* than in the other earthworm treatments.

There were no significant differences in residue-derived C concentrations between the mixed and the surface residue treatment for the large macroaggregates. In the small macroaggregates, residue-derived C concentrations were higher when residue was on the surface for all treatments except when both species were present. Residue-derived C concentrations were higher when residue was mixed in for all earthworm treatments in the microaggregates.

Proportion of microaggregates within macroaggregates

The proportion of microaggregates within macroaggregates was significantly influenced by earthworm treatment, residue application (Fig. 5.3). The formation of stable microaggregates within large macroaggregates (> 2000 μm) was significantly higher for all earthworm treatments when residue was mixed in with the soil than when residue was placed on the surface.

The amount of microaggregates within large macroaggregates was highest in the presence of *A. caliginosa*, was significantly lower in the presence of *L. rubellus* or a mix of both species and was even lower when no earthworms were present for both residue

treatments. The proportion of stable microaggregates within small macroaggregates (250-2000 μm) was significantly higher when no earthworms were present than in the presence of earthworms for both residue treatments.

Within the mixed residue treatment, the proportion of microaggregates within macroaggregates was significantly higher in large macroaggregates than in small macroaggregates in the presence of *A. caliginosa* and significantly lower when no earthworms were present. Within the surface residue treatment, the amount of microaggregates was significantly higher in small macroaggregates than in large macroaggregates for all earthworm treatments.

Residue-derived C distribution in inter- en intra-POM fractions

Residue-derived C concentrations in intra-POM fractions within large macroaggregates were not significantly different between residue treatments, except in the presence of *A. caliginosa* where the concentration was larger when residue was mixed in with the soil. Within the mixed residue treatment, residue-derived C concentrations in intra-POM fractions within large macroaggregates were significantly larger in treatments with earthworms compared to no earthworms (Fig. 5.4).

Within the surface residue treatment, residue-derived C concentrations were significantly lower when no earthworms were present or in the presence of *A. caliginosa* than in the presence of *L. rubellus* or a mix of both species. There were no significant differences between earthworm treatments for the residue-derived intra-POM-C concentrations in small macroaggregates.

Residue-derived inter-POM C concentrations did not show any significant differences between earthworm treatments for both large and small macroaggregates in both residue treatments (Fig. 5.5), except for the residue-derived inter-POM C concentration in large macroaggregates when both earthworm species were present, which was significantly higher than for the other earthworm treatments.

Discussion

Respiration

At the end of the incubation, total respiration was significantly higher in all soils with earthworms than in those without earthworms when the residue was mixed with the soil. When residue was placed on the soil surface, total respiration was highest when only *L. rubellus* was present and lowest when no earthworms were present. Residue-derived respiration was highest in the presence of *A. caliginosa* and lowest when both earthworm species were present when the residue was mixed with the soil. Earthworms are known to have direct and indirect effects on decomposition and mineralization of SOM. They may stimulate decomposition directly, by aeration, fragmentation of litter (Edwards and Lofty, 1977; Lee, 1985) and indirectly by mixing organic matter into the soil and by stimulating microbial activity in their guts (Shaw and Pawluk, 1986). Zhang and Hendrix (1995) found an increase in respiration rates for both *Aporrectodea caliginosa* and *Lumbricus rubellus* with residues placed on the soil surface. In contrast, Bossuyt et al. (2003b) found no increase in total or residue-derived respiration with the addition of earthworms.

At the end of the incubation, in the present study, cumulative total and residue-derived respiration was higher when residue was placed on the surface compared to when residue was mixed with the soil. This is in contrast with several other studies where decomposition rates were significantly higher when plant residue was mixed with the soil (Curtin et al., 1998; Stemmer et al., 1999; Wang et al., 2002). These studies, however, did not look at decomposition rates when earthworms were added. When residues are placed on the surface, they are less associated with mineral soil and therefore possibly less protected from microbial attack. The increase of microbial activity caused by the presence of earthworms is likely to increase decomposition of surface earthworm casts which also contain less mineral soil than below surface castings.

Water-stable aggregates

The proportion of large macroaggregates was significantly highest in the presence of *A. caliginosa* and significantly lowest when no earthworms were present. *A. caliginosa* is an endogeic earthworm species while *L. rubellus* is an epigeic species. Epigeic species live mainly in the upper layers of the soils and may have less effect on soil aggregation than endogeic species which live in deeper soil layers and are known to feed on soil more or less enriched with organic matter. Endogeic species have a major influence on soil aggregation and soil organic matter stabilization (Lavelle and Spain, 2001). The quantity of large macroaggregates was significantly higher for all earthworm treatments when residue was mixed with the soil compared to when residue was placed on the surface. This is consistent with the respiration data. Microbial activity was

significantly higher when residues were placed on the surface, resulting in a lower rate of aggregate formation and a faster rate of aggregate and/or earthworm cast breakdown.

Residue-derived C concentrations in aggregates

When residues were mixed with the soil, residue-derived C concentrations in large macroaggregates (> 2000 μm) were significantly lower when both earthworm species were present compared to when only *A. caliginosa* was present. When residue was placed on the surface, residue-derived C concentrations in large macroaggregates was significantly lower when both earthworm species were present than when only one species was present. It is not totally clear why these C concentrations were lower in the presence of both earthworm species. It is apparently due to some interactions between the two species. When the residue was mixed with the soil, residue-derived C concentrations in large macroaggregates were generally higher in the presence of earthworms. This has been reported before by several other researchers (Shipitalo and Protz, 1988; Mulongoy and Bedoret, 1989; Daniel and Anderson, 1992; Barois et al., 1993; Buck et al., 1999). Residue-derived C concentrations in small macroaggregates (250-2000 μm) were significantly higher when no earthworms were present. This is possibly due to the fact that the residue added was of a size 500-1000 μm. Undecomposed residue-C would be measured in this fraction.

Proportion of microaggregates within macroaggregates

The proportion of microaggregates within large macroaggregates was highest in the presence of *A. caliginosa* and lowest when no earthworms were present. This effect

was opposite for the formation of microaggregates within small macroaggregates where the proportion of microaggregates was highest when no earthworms were present. The general effect of earthworm activity on microaggregate formation within macroaggregates or earthworm casts has been reported by several other researchers (Jongmans et al., 2001; Pulleman et al., submitted; Bossuyt et al., 2003a). Barois et al. (1993) found that the soil microstructure was completely destroyed in the earthworm's gut and that during gut transit old microaggregates were destroyed and new ones formed. The higher proportion of microaggregates in the presence of *A. caliginosa* is consistent with the higher activity of this endogeic species in the formation of casts and larger aggregates.

Residue-derived C distribution in inter- and intra-POM fractions

When the residue was mixed with the soil, residue-derived intra-POM-C concentrations in large macroaggregates were higher in the presence of earthworms than without earthworms. Bossuyt et al. (2003a) found similar results in the presence of *A. caliginosa.* There were no significant differences between earthworm species in the incorporation of residue-derived C into microaggregates within large macroaggregates. When the residue was mixed with the soil, the food supply (mainly associated with the freshly added residues) was evenly distributed throughout the soil. The two species together exploited the total soil volume with the epigeic species on the surface and the endogeic species feeding deeper in the soil. This would enable them to incorporate the same amount of OM into microaggregates within large macroaggregates.

An important difference between earthworm species in residue incorporation was found when residue was placed on the surface. Because *L. rubellus* are known to feed on the soil surface, they could incorporate more surface litter into aggregates than A. *caliginosa*. Because *A. caliginosa* live in deeper layers of the soil, this species seemed to feed less on the surface residue and more on the older C in the deeper in the soil.

Residue-derived inter-POM-C concentrations were significantly higher in the presence of both species of earthworms when residue was placed on the surface. In the conceptual model described by Six et al. (1999), stable microaggregates are formed within macroaggregates after ageing and microbial activity. Bossuyt et al. (2003) found, however, that these microaggregates were formed very rapidly in the presence of earthworms without the requirement of ageing. In this study, a significantly greater amount of inter-POM-C in large macroaggregates was found when both earthworm species were present. We speculate that casts formed by *L. rubellus* were re-ingested by *A. caliginosa* leading to a partial release of intra-POM-C. The new formation of microaggregates and stabilized C into these microaggregates within macroaggregates might be in a second "cycle" where the organic matter is still incorporated in the large macroaggregates or casts, but not yet incorporated into a newly formed microaggregate within this large macroaggregate. Marinissen and Dexter (1990) suggested that different cycles of re-ingestion of soil material by earthworm species can possibly lead to the formation of new microaggregates, while leaving the most stable microaggregates intact.

Martin et al. (1991) found an increased mineralization in earthworm casts compared to control soil in the short term (24 hrs.). In a longer incubation of 420 days, they found a decrease in C mineralization rates compared to control soil. In this study,

we found a higher cumulative mineralization after 14 days of incubation, but we also found more C incorporated into microaggregates within earthworm casts. These microaggregates are known to be relatively stable and may be important in protecting organic matter and fresh residue-derived carbon in the longer-term.

Conclusions

Based on the results of this study, we can conclude that important interactive effects on soil aggregation and accumulation of new C inputs take place in the presence of two earthworm species with different feeding strategies. In general, earthworms had a significant impact on the fast formation of stable microaggregates within large macroaggregates and on the incorporation of fresh residue into these microaggregates. Interactive effects between the epigeic and the endogeic species occurred mostly when residue was placed on the surface. While the epigeic species induced a larger incorporation of fresh residue into microaggregates within large macroaggregates, the combination of both species caused a much higher incorporation of fresh residue between microaggregates within macroaggregates. These interactive effects might have important consequences for the incorporation and long-term stabilization of soil C, particularly in conservation tillage and forest soils where plant residues remain on the soil surface.

References

Barois, I., Villemin, G., and Lavelle, P., 1993. Transformation of the Soil Structure through *Pontoscolex corethrurus* (Oligochaeta) intestinal tract. Geoderma, 56: 57-66.

Beare, M.H., Hendrix, P.F., and Coleman, D.C., 1994. Water-stable aggregates and organic matter fractions in conventional – and no-tillage soils. Soil Science Society of America Journal, 58: 777-786.

Bossuyt, H., Six, J., and Hendrix, P.F., 2003a. Rapid incorporation of fresh residue-derived carbon into newly formed microaggregates within earthworm casts. European Journal of Soil Science, submitted.

Bossuyt, H., Six, J., and Hendrix, P.F., 2003b. Soil carbon protection at the microaggregate level within earthworm casts. Soil Biology and Biochemistry, submitted.

Buck, C., Langmaack, M., and Schrader, S., 1999. Nutrient content of earthworm casts influenced by different mulch types. European Journal of Soil Biology, 35: 23-30.

Curtin, D., Selles, F., Wang, H., Campbell, C.A., and Biederbeck, V.O., 1998. Carbon dioxide emissions and transformation of soil carbon and nitrogen during wheat straw decomposition. Soil Science Society of America Journal, 62: 1035-1041.

Daniel, O., and Anderson, J.M., 1992. Microbial biomass and activity in contrasting soil materials after passage through the gut of the earthworm *Lumbricus rubellus*. Soil Biology and Biochemistry, 24: 465-470.

Darwin, C., 1881. The Formation of Vegetable Mould, through the Action of Worms with Observations on Their Habits. Murray, London, 298 pp.

Edwards, C.A. and Lofty, J.R., 1977. Biology of Earthworms. Chapman and Hall, London. 33 pp.

Elliott, E.T., 1986. Aggregate structure and carbon, nitrogen, and phosphorus in native and cultivated soils. Soil Science Society of America Journal, 50: 627-633.

Gale, W.J., Cambardella, C.A., and Bailey, T.B., 2000. Surface residue- and root-derived carbon in stable and unstable aggregates. Soil Science Society of America Journal, 64: 196-201.

Hendrix, P.F., 1997. Long-term patterns of plant production and soil carbon dynamics in a Georgia Piedmont agroecosystem. pp. 235-245. *In*: Soil Organic Matter in Temperate Agroecosystems; Long-term experiments in North America. E.A. Paul, K. Paustian, E.T. Elliott, and C.V. Cole (ed.). CRC Press, Ind., Boca Raton, FL.

Hoogerkamp, M., Rogaar, H., and Eijsackers, H.J.P., 1983. Effect of earthworms on grassland on recently reclaimed polder soils in the Netherlands. *In*: Earthworm Ecology. Satchell, J.E. (ed.). Chapman and Hall, London, pp 88-105.

Jongmans, A.G., Pulleman, M.M., and Marinissen, J.C.Y., 2001. Soil structure and earthworm activity in a marine silt loam under pasture versus arable land. Biology and Fertility of Soils, 33: 279-285.

Lavelle,P., and Spain, A.V., 2001. Soil Ecology. Kluwer Academic Publishers, 654 pp.

Lee, K.E., 1985. Earthworms, Their Ecology and Relationships with Soils and Land Use. Academic Press, Sydney.

Marinissen, J.C.Y., and Dexter, A.R., 1990. Mechanisms of stabilisation of earthworm casts and artificial casts. Biology and Fertility of Soils, 9: 163-167.

Martin, A., 1991. Short- and long-term effects of the endogeic earthworm *Millsonia anomala* (Omodeo) (Megascolecidae, Oligochaeta) of tropical savannas on soil organic matter. Biology and Fertility of Soils, 11: 234-238.

Mulongoy K., and Bedoret, A., 1989. Properties of worm casts and surface soils under various plant covers in the humid tropics. Soil Biology and Biochemistry, 21: 197-203.

Oades, J.M., 1984. Soil organic matter and structural stability, mechanisms and implications for management. Plant and Soil, 76: 319-337.

Pulleman, M.M., Marinissen, J.C.Y., Jongmans, A.G., Six, J., Uijl, A., 2003. Submitted to Biology and Fertility of Soils.

SAS Institute, 1990. SAS/STAT User's guide. Vol. 2. Version 6 ed. SAS Inst., Cary, NC.

Shaw, C. and Pawluk, S., 1986. Faecal microbiology of *Octolasion tyrtaeum*, *Aporrectodea turgida* and *Lumbricus terrestris* and its relation to the carbon budgets of three artificial soils. Pedobiologia, 29: 377-389.

Shipitalo, M.J., and Protz, R., 1988. Factors influencing the dispersibility of clay in worm casts. Soil Science Society of America Journal, 52: 764-769.

Six, J., Elliott, E.T., and Paustian, K., 1998. Aggregate and SOM dynamics under conventional and no-tillage systems. Soil Science Society of America Journal, 63: 1350-1358.

Six, J., Elliott, E.T., Paustian, K., and Doran, J.W., 1999a. Aggregation and soil organic matter accumulation in cultivated and native grassland soils. Soil Science Society of America Journal, 62: 1367-1377.

Six, J., Schultz P.A., Jastrow J.D., and Merckx R., 1999b. Recycling of sodium polytungstate used in soil organic matter studies. Soil Biology and Biochemistry, 31: 1193-1196.

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: 2099-2013.

Stemmer, M., Von Lutzow, M., Kandeler, E., Pichlmayer, F., and Gerzabek, M.H., 1999. The effect of maize straw placement on mineralization of C and N in soil particle size fractions. European Journal of Soil Science, 50: 73-85.

Tisdall, J.M., and Oades, J.M., 1982. Organic matter and water-stable aggregates in soils. Journal of Soil Science, 33: 141-161.

Wang, H., Curtin, D., Jame, Y.W., McConkey, B.G., and Zhou, H.F., 2002. Simulation of carbon dioxide flux during plant residue decomposition. Soil Science Society of America Journal, 66: 1304-1310.

Zhang, Q.L. and Hendrix, P.F., 1995. Earthworm (*Lumbricus rubellus* and *Aporrectodea caliginosa)* effects on carbon flux in soil. Soil Science Society of America Journal, 59: 816-823.

Table 5.1. Total and residue-derived cumulative respiration in soil samples with or without earthworms.

treatment	CO_2-C		$^{13}CO_2$-C	
	μg g^{-1}soil		μg g^{-1}soil	
	mixed	surface	mixed	surface
+ A. *caliginosa* + residue	304a*	366b	169a*	230a
+ L. *rubellus* + residue	329a*	431a	157ab*	231a
+ A. *caliginosa* + L. *rubellus* + residue	333a*	387ab	147b*	221a
+ residue	263b*	321b	151ab*	212a

Values followed by a different lowercase letter among earthworm treatments within the same residue treatment, are significantly different.

*Values within the same earthworm treatment between residue treatments are significantly different.

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followed by a different lowercase letter among earthworm treatments within the same residue treatment, are significantly different.

*Values within the same earthworm treatment between residue treatments are significantly different.

Fig. 5.5: Effect of two earthworm species on ^{13}C in inter- microaggregate particulate organic matter in small (250-2000 μm) and large (> 2000 μm) macroaggregates. Values followed by a different lowercase letter among earthworm treatments within the same residue treatment, are significantly different.

*Values within the same earthworm treatment between residue treatments are significantly different.

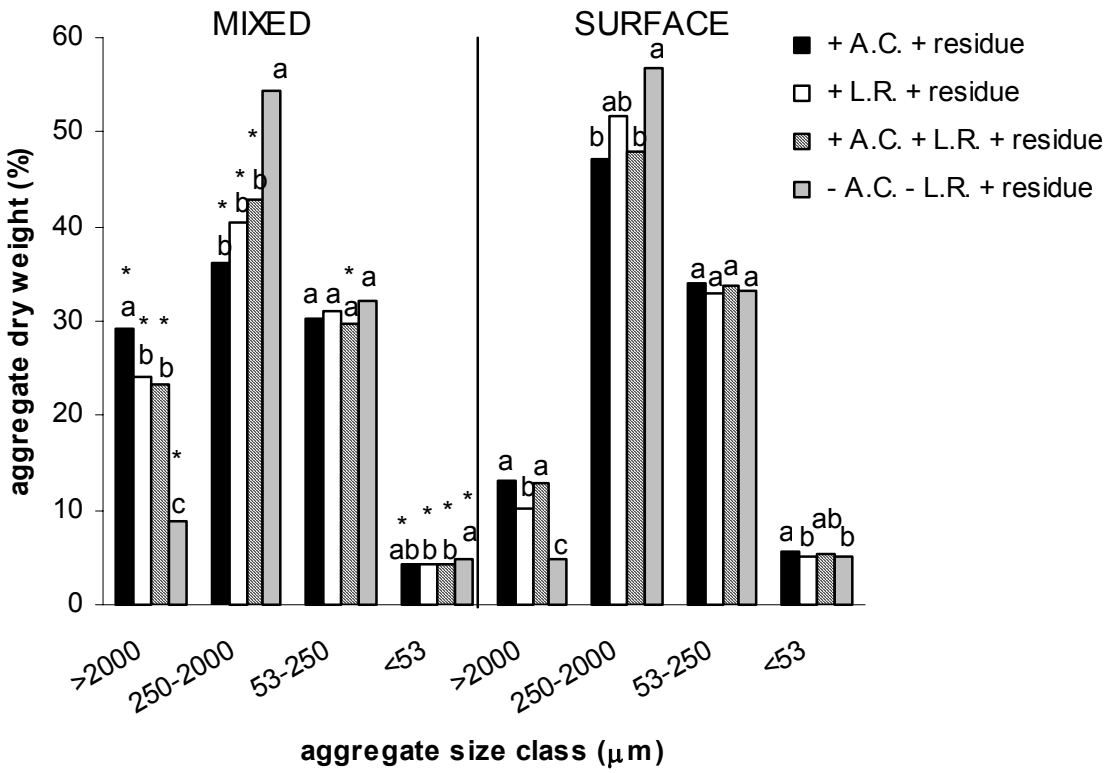
MIXED
SURFACE
+ A.C. + residue
+ L.R. + residue
+ A.C. + L.R. + residue
- A.C. - L.R. + residue
aggregate dry weight (%)
60
50
40
30
20
10
0
>2000
250-2000
53-250
<53
aggregate size class (μm)

Fig. 5.1

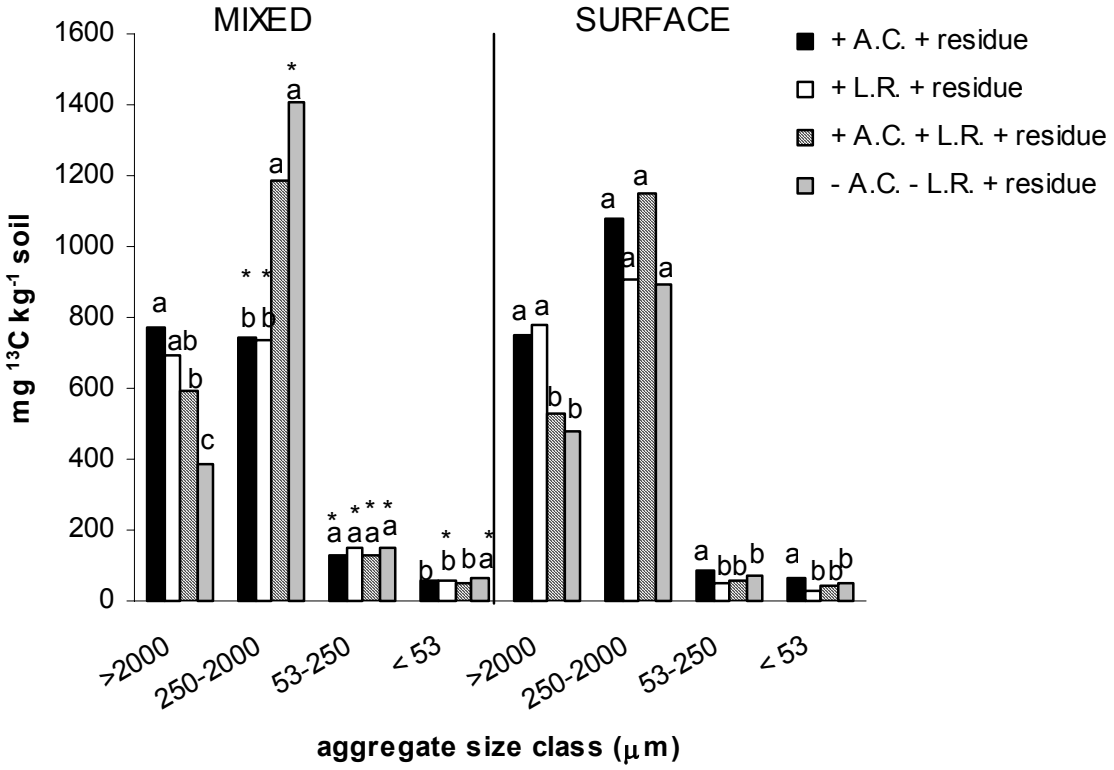
MIXED
SURFACE
+ A.C. + residue
+ L.R. + residue
+ A.C. + L.R. + residue
- A.C. - L.R. + residue
mg 13C kg-1 soil
1600
1400
1200
1000
800
600
400
200
0
>2000
250-2000
53-250
< 53
aggregate size class (μm)

Fig. 5.2

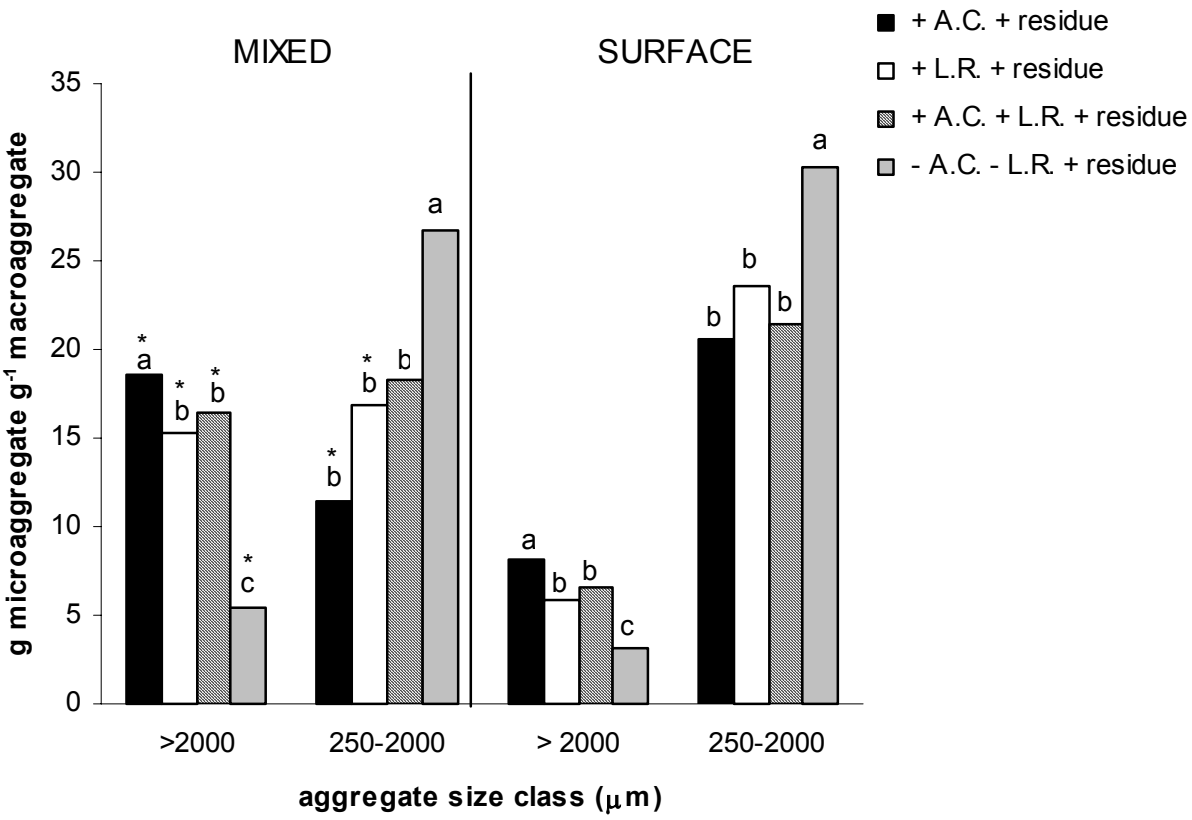
MIXED
SURFACE
+ A.C. + residue
+ L.R. + residue
+ A.C. + L.R. + residue
- A.C. - L.R. + residue
g microaggregate g-1 macroaggregate
35
30
25
20
15
10
5
0
>2000
250-2000
> 2000
250-2000
aggregate size class (μm)

Fig. 5.3

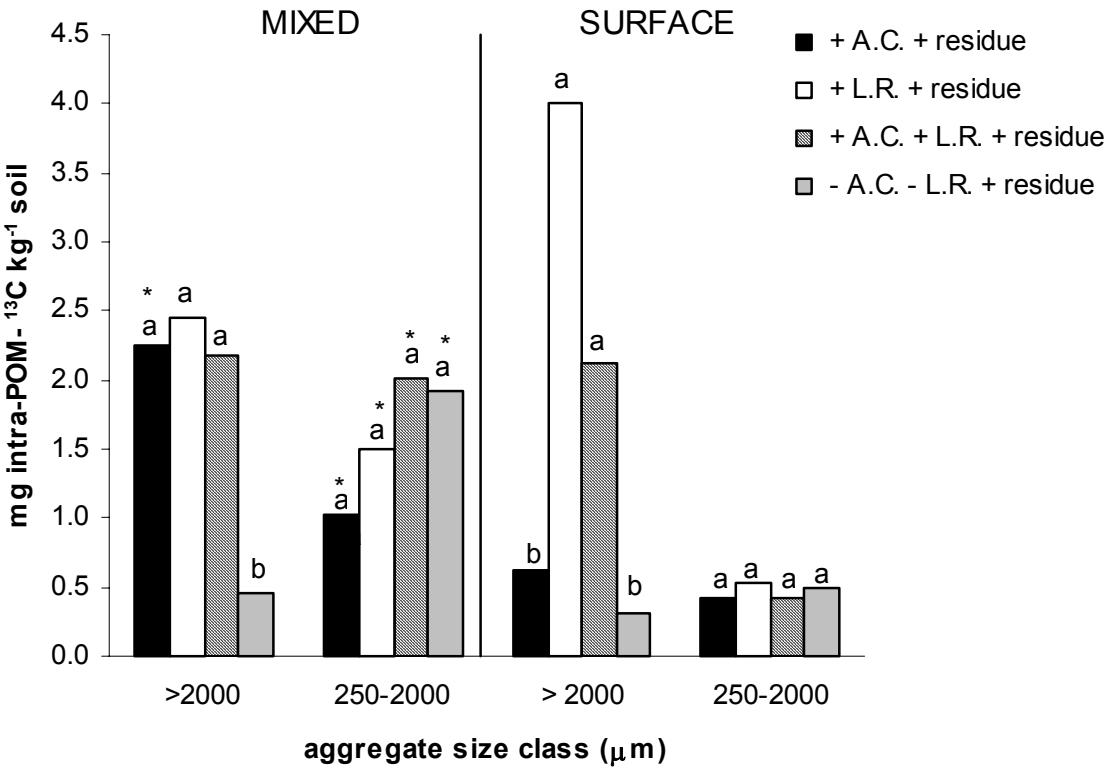
MIXED
SURFACE
+ A.C. + residue
+ L.R. + residue
+ A.C. + L.R. + residue
- A.C. - L.R. + residue
mg intra-POM- 13C kg-1 soil
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
>2000
250-2000
> 2000
250-2000
aggregate size class (μm)

Fig. 5.4

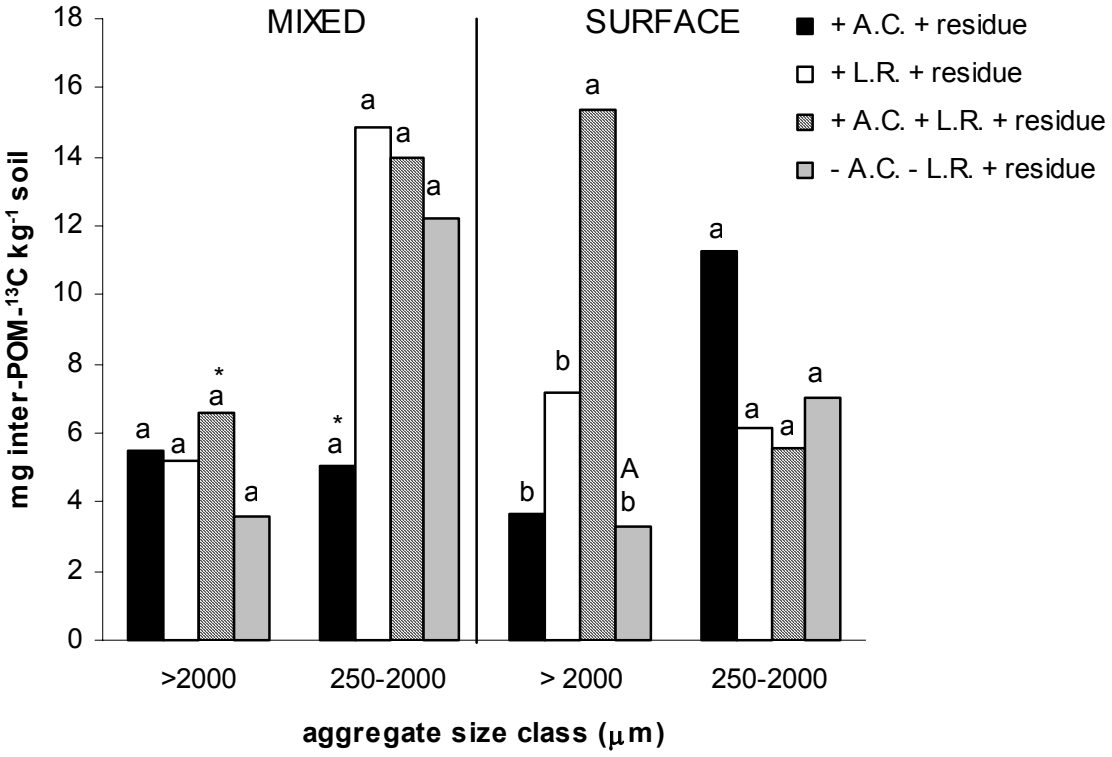
MIXED
SURFACE
+ A.C. + residue
+ L.R. + residue
+ A.C. + L.R. + residue
- A.C. - L.R. + residue
mg inter-POM-13C kg-1 soil
18
16
14
12
10
8
6
4
2
0
a
a
a*
a
a*
a
a
a
b
b
a
A
b
a
a
a
a
>2000
250-2000
> 2000
250-2000
aggregate size class (μm)

Fig. 5.5

CHAPTER 6

CONCLUSIONS

With the increasing concern over global change and the rise in atmospheric CO_2, the implementation of agricultural management practices that lead to an increase of the soil C stock are receiving more and more attention. In addition to the soil C sequestration issue, soil organic matter (SOM) is widely recognized as a key component in the cycling of nutrients. Maintenance of SOM is desirable in agroecosystems because of its beneficial effects on nutrient dynamics and soil structure. Soil organic matter improves soil structural stability, reduces surface crusting and compaction, and increases infiltration, percolation, and water holding capacity of the soil. Soil organic matter dynamics and soil aggregation are closely linked. Aggregates are thought to play an important role in the physical protection of SOM and at the same time, SOM binds with mineral particles to form aggregates of different sizes.

This study aimed to improve understanding of underlying physical mechanisms that may play a crucial role in the protection of C in agricultural ecosystems. The focus was on management practices and earthworm activity as controlling factors. The general basis of this study's hypotheses is the conceptual model of the 'life cycle' of an aggregate as described in Fig. 1.1. The formation of stable microaggregates within

macroaggregates and the stabilization and protection of SOM inside of these microaggregates might be of great importance for the long-term protection of soil C.

Chapter 2 described the effect of tillage practices on aggregate size distribution, total and young carbon concentrations, and decomposition and stabilization of aggregate-associated C fractions. This experiment showed that the impact of tillage practices on aggregate size distribution, C content and organic matter protection was most pronounced in the surface layers of the soil. In these layers, soils that were not tilled contained more large macroaggregates and more total and young C. Organic matter was more protected at the surface under NT than under CT and this protection occurred more at the microaggregate level than at the macroaggregate level. At lower depths, more young C was accumulated in CT soils than in NT soils, but this carbon was not stabilized in the long term.

While the conceptual model suggests the requirement of ageing and microbial activity for the formation of these stable microaggregates, this study showed that earthworms induce a very fast formation of stable microaggregates within their casts and a subsequent sequestration of C.

Chapters 3 and 4 described the effects on an endogeic species of earthworms (*Aporrectodea caliginosa*) on soil aggregate formation, incorporation of fresh residue-derived C into soil aggregates, the formation of stable microaggregates within earthworm casts and the protection of C inside their casts at a microaggregate level. We concluded that earthworms have a significant impact on the formation of large macroaggregates and they induced a very rapid formation of stable microaggregates within their casts. These microaggregates were also enriched in total and residue-derived C compared to bulk soil.

Furthermore, earthworms were responsible for the formation of a significant pool of microaggregate within macroaggregate-protected C after 12 days of incubation and the fast protection of C at this microaggregate level was much larger than at the macroaggregate level.

Finally, chapter 5 described the interactive effects of an endogeic species (*Aporrectodea caliginosa*) and an epigeic species (*Lumbricus rubellus*) on soil aggregate formation, incorporation of fresh residue and formation of stable microaggregates within earthworm casts. This study showed that important interactions take place affecting the incorporation of residue-derived C and the formation of stable microaggregates. Most interactive effects occurred when fresh residue was placed on the soil surface.

Generally, we can conclude that management practices and earthworm activity, which are closely linked, have significant impacts on the incorporation and possible long-term stabilization and sequestration of soil C.