Review

Functional Roles of Soil Organic Matter

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Abstract

Soil organic matter (SOM) plays important roles in carbon storage, aggregate formation, plant nutrient supply and retention, and the immobilization and mobilization of metals. These functions are closely related to the dynamics of organic matter (OM) in the soil environment. In this review, the functional roles of SOM are described in relation to the dynamics of SOM. Most OM enters the soil as particulate OM and undergoes microbial decomposition. During decomposition, carbon dioxide and plant nutrients are released, while the decomposition products remain as organo-mineral complexes. Organo-mineral complexes are combined together to form microaggregates by microbial mucilages (extracellular polymeric substances) formed during the decomposition of OM in the soil. The microaggregates are further bound together into macroaggregates by fungal hyphae and microbial metabolites that are produced during the decomposition of SOM. The decomposition products that account for a major part of SOM remain for the long-term as a result of chemical (associated with minerals) and physical protections (aggregate formation), thereby functioning as a C reservoir. Humic substances (HS) that are formed through the decomposition of OM by chemical and biological processes can form soluble and insoluble complexes with metals. The formation of insoluble complexes reduces the mobilization and bioavailability of metals. In contrast, water-soluble HS can form soluble complexes and enhance the mobilization and bioavailability of metals.

Keywords: Aggregates, Carbon sequestration, Complexation, Humic substances, Plant nutrients, Soil organic matter

Introduction

Soil organic matter (SOM) remains in the soil for a prolonged period because of chemical (associated with minerals) and physical protections (aggregate formation) (Six et al. 2004). Thus, SOM functions as an important carbon (C) reservoir. However, SOM is constantly being decomposed by soil microorganisms. Therefore, there has been concern that the recent increase in global warming is accelerating the decomposition of SOM (Jenkinson et al. 1991).

Most of the organic matter (OM) in the soil is present in the form of organo-mineral complexes (Turchenek and Oades 1979). The SOM that is present without forming organo-mineral complexes represents particulate OM (Cambardella and Elliott 1992; Baldock 2002). Organo-mineral complexes are combined together to form microaggregates by organic and inorganic binding agents (Oades and Waters 1991). Microaggregates are further bound together into macroaggregates by fungal hyphae, plant roots, and microbial- and plant-derived OM (Oades and Waters 1991). The formation of aggregates is mainly due to the proliferation of microorganisms during the decomposition of OM in the soil (Golchin et al. 1994; Guggenberger et al. 1999).

In natural ecosystems, readily mineralizable OM that accounts for a relatively small part of the SOM serves as a major source of plant nutrients, especially nitrogen (N), phosphorus (P) and sulfur (S). SOM, in particular humic substances (HS), has net negative charges and retains positively charged nutrients such as calcium, magnesium, potassium, and ammonium ions. Furthermore, HS can form soluble and insoluble complexes with metals, contributing to the mobilization and immobilization of metals in the soil. The formation of insoluble complexes reduces the mobilization and bioavailability of metals (Logan et al. 1997). In contrast, dissolved OM in the soil forms

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soluble complexes with metals and enhances their mobilization and bioavailability (Bolan et al. 2011).

SOM can be regarded as an impermanent material that is originated from plants, animals, and microorganisms and is in the process of decomposition. Therefore, the functions of SOM should be discussed in relation to the decomposition dynamics of OM in the soil environment. In this review, the functional roles of SOM are discussed from this point of view.

Role of SOM as a C reservoir

SOM functions as a C reservoir. The amount of C stored globally in soils is much larger than that in terrestrial biomass. While the terrestrial biomass C pool is approximately 560 Gt of organic C, approximately 2344 Gt of organic C is estimated to be stored in the top three meters of soil, with 1500 Gt of organic C stored in the first meter of soil and about 615 Gt stored in the top 20 cm (Jobbágy and Jackson 2000; Guo and Gifford 2002).

Global warming caused by increasing carbon dioxide (CO_2) in the atmosphere has become a serious environmental threat to humanity. The increase in atmospheric CO_2 is largely due to the cumulative emissions from fossil fuel combustion. Nevertheless, terrestrial ecosystems are closely related to atmospheric CO_2 levels through the photosynthetic fixation of CO_2, incorporation of C into biomass and soil, and subsequent release of CO_2 through respiration and decomposition of OM. Thus, SOM is considered to function as both a C source and a C sink for atmospheric CO_2.

C sequestration is the process of the capture and long-term storage of atmospheric CO_2 (Sedjo and Sohngen 2012). Modification of agricultural practices is a recognized method of C sequestration because soil can act as an effective C sink. The best-known agricultural practice for increasing the sequestration of C in soils is no-tillage or reduced-tillage farming (Paustian et al. 1997). Although no-tillage farming is not widely practiced, other practices, including the application of compost and the use of green manure or multi-cropping, have been used to increase organic C input to soils in Japan. As clearly demonstrated by the long-term experiment at Rothamsted (Jenkinson and Rayner 1977), the annual application of manure increases the level of SOM. There is much evidence in the literature that the repeated application of organic amendments (e.g., compost, manure, and crop residues) can increase SOM levels (Maillard and Angers 2014). Consequently, organic amendments to soils are viewed as a means for enhancing C sequestration in arable lands.

The Ministry of Agriculture, Forestry and Fisheries of Japan calculated the potential C sequestration from compost application. It estimated that about 2.2 Mt of organic C would be accumulated by the application of compost at 10 and 15 t ha^{-1} $year^{-1}$ to all of the paddy soils and upland soils, respectively, in Japan (Yokozawa et al. 2010). However, there are cases in which no significant changes have been observed in SOC stocks following OM application (Angers et al. 2010). Our previous study, conducted on soils from upland fields applied with different amounts of cattle manure for 20 years, indicated that manure significantly increased SOM when applied at rates of 160 and 320 t ha^{-1} $year^{-1}$, but not at a rate of 80 t ha^{-1} $year^{-1}$ (Aoyama and Kumakura 2001). Maillard and Angers (2014) reported, based on a meta-analysis of published studies, that the quality of organic amendments was the dominant factor determining C sequestration efficiencies. There are many issues to be resolved for effective C sequestration (Stockmann et al. 2013).

On the other hand, there has been concern that the increase in global warming is accelerating the decomposition of SOM, releasing CO_2 to the atmosphere, which will further enhance the warming trend. Jenkinson et al. (1991) calculated the amount of CO_2 that would be released from the global stock of SOM. If global temperatures rise by 0.03°C $year^{-1}$, it was estimated that the additional release of CO_2 from SOM over the next 60 years would be 61×10^{15} Gt C. Bellamy et al. (2005) showed, using data from the National Soil Inventory of England and Wales obtained between 1978 and 2003, that C was lost from soils across England and Wales over the survey period at a mean rate of 0.6% $year^{-1}$ relative to the existing soil C content. Taghizadeh-Toosi et al. (2014) analyzed soils collected in 1986, 1997 and 2009 in a Danish nationwide 7-km grid and found an annual loss of 0.2 t C ha^{-1} from the 0–100 cm soil layer between 1986 and 2009. These studies suggest that SOM is more likely to be a C source than a C sink for atmospheric CO_2 with increasing global warming.

Role of SOM in aggregate formation

An adequate supply of water and oxygen to plant roots is essential for the normal growth of plants, and the supply is related to the presence of soil aggregates that contribute to the formation of capillary and non-capillary pores. The aggregate hierarchy concept proposed by Tisdall and Oades (1982) divided soil aggregates into macroaggregates (>250 μm) and microaggregates (<250 μm). The capillary pores that retain water are mainly present in microaggregates, whereas the non-capillary pores that are responsible for aeration and drainage are in macroaggregates (inter-microaggregates).

In the aggregate hierarchy concept, it is postulated that different binding agents act at different hierarchical stages of aggregation (Tisdall and Oades 1982). Free primary particles and silt-sized aggregates (<20 μm) are bound together into microaggregates by organic and inorganic binding agents. Golchin et al. (1994) suggested that when particulate OM enters the soil from small plant fragments and fungal hyphae fragments, it is colonized by bacteria and the bacteria excrete extracellular polymeric substances (EPS) during the decomposition of particulate OM. This particulate OM is encrusted with mineral particles through the binding action of EPS and forms stable microaggregates (Figure 1). In microaggregates, the particulate OM is depleted of its available components, resulting in a cessation of microbial activity and production of EPS. The EPS gradually turns into HS. The micropores formed in microaggregates because of the decomposition of particulate OM retain water and air.

The stable microaggregates, in turn, are bound together into macroaggregates by fungal hyphae, plant roots, and microbial- and plant-derived polysaccharides. Many studies have suggested that fungal hyphae are important for the formation of macroaggregates (Tisdall 1994; Guggenberger et al. 1999). The fungal mycelium in soils has been described as a “sticky string bag”, since it entangles particles within the hyphal network and binds particles together through the production of EPS (Oades and Waters 1991). The enmeshment of particles by the “sticky string bag” is considered a major factor in the formation of macroaggregates (Tisdall et al. 1997; Bossuyt et al. 2001). At the same time, the formation of microaggregates occurs within macroaggregates through the decomposition of small particulate OM (Angers et al. 1997). As macroaggregates become older, they disrupt into microaggregates due to the depletion of the substrates for fungal growth (Angers et al. 1997). The microaggregates released by the disruption of macroaggregates can again be used for the formation of macroaggregates (Six et al. 2000).

As mentioned above, the primary cause of aggregate formation is the decomposition of OM in soils. Thus, the application of OM facilitates the formation of soil aggregates. Our previous study (Aoyama et al. 1999) reported that a long-term (18 years) application of cattle manure favored the formation of macroaggregates resistant to slaking. Although manure application increased the concentration of OM in both macro- and microaggregates, manure-derived OM accumulated preferentially in

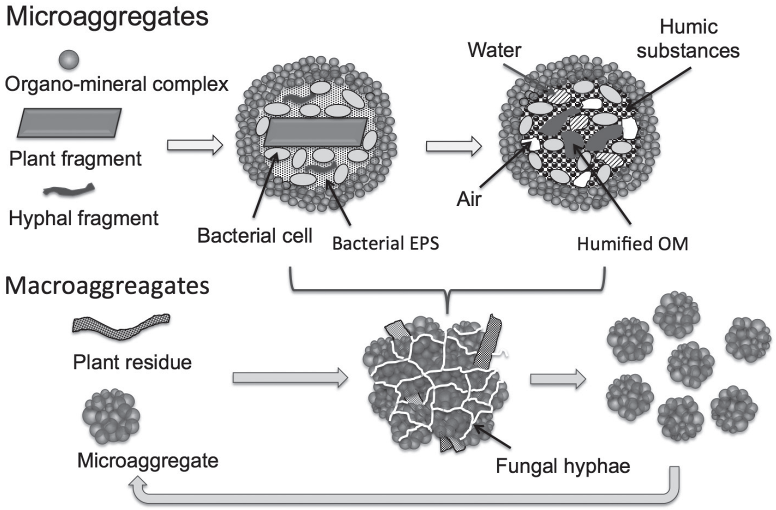

Figure 1. Schematic diagram of the formation of microaggregates and macroaggregates in the soil.

macroaggregates as both mineral-associated and particulate OM (Aoyama et al. 1999). It has been hypothesized that manure-derived OM first enters the soil primarily as particulate material, decomposed within the macroaggregate structure, and is transformed into mineral-associated materials of microbial origin that contribute to the stabilization of the macroaggregates (Aoyama et al. 1999). The positive roles of organic amendments in aggregate formation and stabilization have been reported in many studies (Abiven et al. 2009). The magnitude and longevity of the increased aggregation depend on the nature of the organic input (Baldock 2002). The increase in aggregate stability after organic additions to the soil is related to the decomposition dynamics of the organic amendments (Abiven et al. 2009). Easily decomposable organic amendments have an intense and transient effect on aggregate stability, while ones that are more recalcitrant have a smaller but longer-term effect.

Role of SOM in providing plant nutrients

SOM acts as source and sink for plant nutrients. N, P and S in soils are mostly present in organic forms and are absorbed by plants following mineralization by microbial action.

N is an essential element for plant growth and is frequently the major limiting nutrient in agricultural soils. Thus, the most important role of SOM is to store N in organic forms. In soils, organic N is mineralized to ammonium-N by soil microorganisms and subsequently converted to nitrate-N by nitrifying bacteria. Plants mainly utilize ammonium-N and nitrate-N as nitrogenous nutrients. Readily mineralizable organic N in the soil is accumulated in the clay-sized fraction (Cameron and Posner 1979; Aoyama and Kumada 1982; Aoyama and Taninai 1992). The readily mineralizable organic N in the clay-sized fraction is considered to be microbial biomass and microbial metabolites (Cameron and Posner 1979; Aoyama and Taninai 1992). At the same time, particulate OM is also a source of readily mineralizable organic N in soils accumulated with plant residues (Cameron and Posner 1979) and amended with manure (Aoyama and Kumada 1982; Aoyama and Taninai 1992). Particulate OM mainly consists of decomposing plant residues or manure-derived partially decomposed OM. Mineral N is released during the decomposition of particulate OM.

The N-supplying capacity of soils is assayed by incubating soils under aerobic and anaerobic conditions (Waring and Bremner 1964; Keeney 1982). The incubation method is the most reliable because the amount of mineralized N is closely correlated with the crop yield and crop N uptake. However, the incubation method is time-consuming and labor-intensive, and the results strongly depend on experimental conditions (Keeney 1982). Hence, chemical methods have been proposed as alternatives (Ros et al. 2011). In Japan, the neutral phosphate buffer extraction (Higuchi 1981) and hot water (80°C) extraction (Uezono et al. 2010) methods are used for evaluating the N-supplying capacity of soils.

Aoyama (2006) revealed by high-performance size exclusion chromatography and on-flow measurements of UV absorption spectra and fluorescence emission spectra that the main constituent of the neutral phosphate buffer extract was HS. Furthermore, an incubation experiment with the neutral phosphate buffer extracts and their fractions indicated that readily mineralizable organic N was mainly present in the humic acid and PVP-non-adsorbed fulvic acid fractions. Moriizumi and Matsunaga (2011) applied high-performance size exclusion chromatography with UV, fluorescence, refractive index, and chemiluminescent N detection to the hot-water extracts of soils. They showed that most of the hot-water extractable organic N was HS exhibiting fulvic acid-like fluorescence. At the same time, N-containing substances with poor UV absorption and fluorescence were observed in the large molecular size fraction.

Role of SOM in adsorption and transport of metals

SOM can form insoluble and soluble complexes with metals. The formation of insoluble complexes reduces the mobilization and bioavailability of metals (Logan et al. 1997). In vineyard soils accumulated with Bordeaux mixture-derived copper, organic amendments reduced the mobilization of copper across the soil profiles, and copper was mainly retained in particulate OM derived from the organic amendments (Besnard et al. 2001). Other studies have also reported that the application of organic amendments reduced the mobility of heavy metals in soils (Walker et al. 2003; Janoš et al. 2010).

In contrast, metal ions form soluble complexes with dissolved or water-soluble OM in the soil (Bolan et al. 2011). While dissolved OM and water-extractable OM account for only a small proportion of the SOM, the formation of soluble complexes enhances the movement and bioavailability of metal ions. Evangelou et al. (2004) applied humic acids to a cadmium-contaminated soil at various dosages, and reported that humic acids had a positive effect on the bioavailability of cadmium in the soil and accelerated the phytoextraction efficiency of tobacco plants. They pointed out that plants might take up cadmium complexed with humic acid fragments. Furthermore, dissolved OM contributes to soil genesis, especially to podsolization. In acid forest soils, aluminum and iron can form relatively stable complexes with dissolved OM and can enhance their solubility and transport (Jansen et al. 2005).

The constituents of dissolved or water-soluble OM responsible for the formation of soluble metals are viewed as HS. Aoyama (2002) separated water-soluble OM into humic acid, PVP-adsorbed fulvic acid, and PVP-non-adsorbed fulvic acid fractions, and estimated the amount of HS by size-exclusion chromatography. The results showed that the C present in the HS-rich fractions (humic acid and PVP-adsorbed fulvic acid fractions) accounted for 2.5–33.4% of water-soluble organic C, and most of the HS were present in the PVP-adsorbed fulvic acid fraction (Aoyama 2002). Thus, the fulvic acids in dissolved OM or water-soluble OM are considered to mainly function as complexing agents.

Dissolved OM derived from soils can be transferred to rivers through leaching after percolation into soil layers and by the inflow of surface water. In addition, soil particles may also be carried by the inflow, and some of the OM attached to the soil particles may be extracted by river water (Moroi et al. 2012). The transport route of dissolved OM may vary depending on climatic conditions, vegetation, and soil properties, which may influence the quantity and quality of dissolved OM in rivers (Moroi et al. 2012). Hence, soil-derived dissolved OM is considered to play important roles in transporting metals from the soil to coastal ecosystems through rivers (Watanabe et al. 2009).

Concluding remarks

As described above, the functions of SOM are related to the dynamics of OM in the soil environment. Figure 2 shows the functions of SOM in relation to its dynamics. The OM added to soil as plant residues and organic amendments enters the soil primarily as particulate OM and undergoes microbial decomposition. During the decomposition process, some of the particulate OM is mineralized to CO_2, ammonium ions and other plant nutrients, while the decomposition products remain in the soil mainly as HS and non-HS, which are intimately bound to one another and form organo-mineral complexes with clay- and silt-sized particles. The non-HS are mainly composed of microbial metabolites and function as binding agents to form microaggregates (C in Figure 2). The microaggregates are further combined together by the fungal hyphae and microbial metabolites produced during the decomposition of particulate OM to form macroaggregates (C in Figure 2). Through these processes, SOM serves as a C

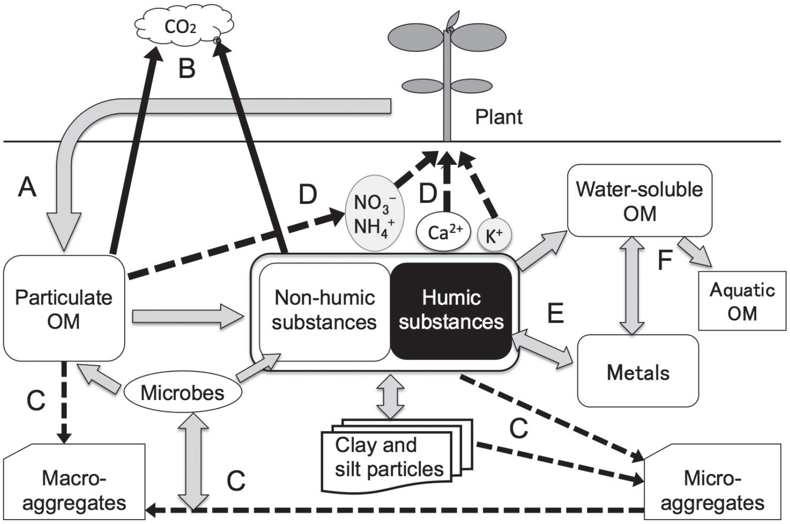

Figure 2. Schematic diagram of the functions of SOM in relation to SOM dynamics. A: carbon sink, B: carbon source, C: aggregate formation, D: plant nutrient supply, E: immobilization of metals, F: mobilization of metals.

source (B in Figure 2) and sink (A in Figure 2) to atmospheric CO_2. Plant nutrients, such as ammonium, nitrate, and phosphate ions, are released during the decomposition of particulate and mineral-associated OM, and positively charged nutrients such as calcium, magnesium, potassium and ammonium ions can be retained by negatively charged HS (D in Figure 2). HS can form soluble and insoluble complexes with metals. The formation of insoluble complexes reduces the mobilization and bioavailability of metals (E in Figure 2), whereas water-soluble OM forms soluble complexes with metals, enhances the mobilization and bioavailability of metals, and is transferred to the aquatic environment (F in Figure 2).

References

Abiven, S., Menasseri, S. and Chenu, C. (2009) The effects of organic inputs over time on soil aggregate stability – A literature analysis. *Soil Biol. Biochem.* **41**, 1-12.

Angers, D. A., Chantigny, M. H., MacDonald, J. D., Rochette, P. and Cote, D. (2010) Differential retention of carbon, nitrogen and phosphorus in grassland soil profiles with long-term manure application. *Nutr. Cycl. Agroecosyst.* **86**, 225-229.

Angers, D. A., Recous, S. and Aita, C. (1997) Fate of carbon and nitrogen in water stable aggregates during decomposition of ^{13}C ^{15}N labelled wheat straw in situ. *Eur. J. Soil Sci.* **48**, 295-300.

Aoyama, M. (2002) Characterization of water-soluble organic matter in soils by size exclusion chromatography and fractionation with polyvinylpyrrolidone. *Soil Sci. Plant Nutr.* **48**, 475-481.

Aoyama, M. (2006) Properties of neutral phosphate buffer extractable organic matter in soils revealed using size exclusion chromatography and fractionation with polyvinylpyrrolidone. *Soil Sci. Plant Nutr.* **52**, 378-386.

Aoyama, M., Angers, D. A. and N'Dayegamiye, A. (1999) Particulate and mineral-associated organic matter in water-stable aggregates as affected by mineral fertilizer and manure applications. *Can. J. Soil Sci.* **79**, 295-302.

Aoyama, M. and Kumada, K. (1982) Characteristics of physical fractionated manured soils, with special reference to the clay fractions. *Jpn. J. Soil Sci. Plant Nutr.* **53**, 425-430. (in Japanese)

Aoyama, M. and Kumakura, N. (2001) Quantitative and qualitative changes of organic matter in an Ando soil induced by mineral fertilizer and cattle manure applications for 20 years. *Soil Sci. Plant Nutr.* **47**, 241-252.

Aoyama, M. and Taninai, Y. (1992) Organic matter and its mineralization in particle size and aggregate size fractions of soils with four-year application of farmyard manure. *Jpn. J. Soil Sci. Plant Nutr.* **63**, 571-580. (in Japanese with English summary)

Bellamy, P. H., Loveland, P. J., Bradley, R. I., Lark, R. M. and Kirk, G. J. (2005). Carbon losses from all soils across England and Wales 1978–2003. *Nature* **437**, 245-248.

Baldock, J. A. (2002) Interactions of organic materials and microorganisms with minerals in the stabilization of structure. In: Interactions between Soil Particles and Microorganisms, Huang, P. M., Bollag, J.-M., Senesi, N., Eds.: John Wiley & Sons, Chichester, West Sussex, UK, pp. 85-131.

Besnard, E., Chenu, C. and Robert, M. (2001) Influence of organic amendments on copper distribution among particle-size and density fractions in Champagne vineyard soils. *Environ. Pollut.* **112**, 329-337.

Bolan, N. S., Adriano, D. C., Kunhikrishnan, A., James, T., McDowell, R. and Senesi, N. (2011) Dissolved organic matter: biogeochemistry, dynamics, and environmental significance in soils. *Adv. Agron.* **110**, 1-75.

Bossuyt, H., Denef, K., Six, J., Frey, S. D., Merckx, R. and Paustian, K. (2001) Influence of microbial populations and residue quality on aggregate stability. *Appl. Soil Ecol.* **16**, 195-208.

Cambardella, C.A. and Elliott, E.T. (1992) Particulate soil organic matter changes across a grassland cultivation sequence. *Soil Sci. Soc. Am. J.* **56**, 777-783.

Cameron, R. S. and Posner, A. M. (1979) Mineralisable organic nitrogen in soil fractionated according to particle size. *J. Soil Sci.* **30**, 565-577.

Evangelou, M. W., Daghan, H. and Schaeffer, A. (2004) The influence of humic acids on the phytoextraction of cadmium from soil. *Chemosphere* **57**, 207-213.

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 ^{13}C

CP/MAS NMR spectroscopy and scanning electron microscopy. *Aust. J. Soil Res.* **32**, 285-309.

Guggenberger, G., Elliott, E. T., Frey, S. D., Six, J. and Paustian, K. (1999) Microbial contributions to the aggregation of a cultivated grassland soil amended with starch. *Soil Biol. Biochem.* **31**, 407-419.

Guo, L. B. and Gifford, R. M. (2002) Soil carbon stocks and land use change: a meta analysis. *Glob. Change Biol.* **8**, 345-360.

Higuchi, M. (1981) Characterization of immobilized nitrogen and organic nitrogen extracted with various buffer solution. *Jpn. J. Soil Sci. Plant Nutr.* **52**, 481-489. (in Japanese)

Janoš, P., Vávrová, J., Herzogová, L. and Pilařová, V. (2010) Effects of inorganic and organic amendments on the mobility (leachability) of heavy metals in contaminated soil: a sequential extraction study. *Geoderma* **159**, 335-341.

Jansen, B., Nierop, K. G. J. and Verstraten, J. M. (2005) Mechanisms controlling the mobility of dissolved organic matter, aluminium and iron in podzol B horizons. *Eur. J. Soil Sci.* **56**, 537-550.

Jenkinson, D. S., Adams, D. E. and Wild, A. (1991) Model estimates of CO_2 emissions from soil in response to global warming. *Nature* **351**, 304-306.

Jenkinson, D. S. and Rayner, J. H. (1977) The turnover of soil organic matter in some of the Rothamsted classical experiments. *Soil Sci.* **123**, 298-305.

Jobbágy, E. G. and Jackson, R. B. (2000) The vertical distribution of soil organic carbon and its relation to climate and vegetation. *Ecol. Appl.* **10**, 423-436.

Keeney, D. R. (1982) Nitrogen-Availability Indices. In: Methods of Soil Analysis, Part 2. Chemical and Microbiological Properties, Page, A. L., Ed.: SSSA, Madison, WI, pp. 711-733.

Logan, E. M., Pulford, I. D., Cook, G. T. and Mackenzie, A. B. (1997) Complexation of Cu^{2+} and Pb^{2+} by peat and humic acid. *Eur. J. Soil Sci.* **48**, 685-696.

Maillard, É. and Angers, D. A. (2014) Animal manure application and soil organic carbon stocks: a meta-analysis. *Glob. Change Biol.* **20**, 666-679.

Moriizumi, M. and Matsunaga, T. (2011) Molecular weight separation of hot-water extractable soil organic matter using high-performance size exclusion chromatography with chemiluminescent nitrogen detection. *Soil Sci. Plant Nutr.* **57**, 185-189.

Moroi, K., Isamoto, N. and Watanabe, A. (2012) Dissolved organic matter-complexed iron in two rivers with different types of soils in watershed area. *Int. J. Geosci.* **3**, 710-718.

Oades, J. M. and Waters, A. G. (1991) Aggregate hierarchy in soils. *Aust. J. Soil Res.* **29**, 815-828.

Paustian, K., Andrén, O., Janzen, H. H., Lal, R., Smith, P., Tian, G., Tiessen, H., Van Noordwijk, M. and Woomer, P. L. (1997) Agricultural soils as a sink to mitigate CO_2 emissions. *Soil Use Manage.* **13**, 230-244.

Ros, G. H., Temminghoff, E. J. M. and Hoffland, E. (2011) Nitrogen mineralization: a review and meta-analysis of the predictive value of soil tests. *Eur. J. Soil Sci.* **62**, 162-173.

Sedjo, R. and Sohngen, B. (2012) Carbon sequestration in forests and soils. *Annu. Rev. Resour. Econ.* **4**, 127-144.

Six, J., Bossuyt, H., Degryze, S. and Denef, K. (2004) A history of research on the link between (micro) aggregates, soil biota, and soil organic matter dynamics. *Soil Till. Res.* **79**, 7-31.

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 Biol. Biochem.* **32**, 2099-2103.

Stockmann, U., Adams, M. A., Crawford, J.W., Field, D.J., Henakaarchchi, N., Jenkins, M., Minasny, B., McBratney, A.X., Courcelles, V.R., Singh, K., Wheeler, I., Abbott, L., Angers, D.A., Baldock, J., Bird, M., Brookes, P.C., Chenu, C., Jastrow, J.D., Lal, R., Lehmann, J., O'Donnell, A.G., Parton, W.J., Whitehead, D. and Zimmermann, M. (2013) The knowns, known unknowns and unknowns of sequestration of soil organic carbon. *Agric. Ecosyst. Environ.* **164**, 80-99.

Taghizadeh-Toosi, A., Olesen, J.E., Kristensen, K., Elsgaard, L., Østergaard, H.S., Lægdsmand, M., Greve, M.H. and Christensen, B.T. (2014) Changes in carbon stocks of Danish agricultural mineral soils between 1986 and 2009. *Eur. J. Soil Sci.* **65**, 730-740.

Tisdall, J. M. (1994) Possible role of Soil microorganisms in aggregation in soils. *Plant Soil* **159**, 115-121.

Tisdall, J. M. and Oades, J. M. (1982) Organic matter and water-stable aggregates in soils. *J. Soil Sci.* **33**, 141-163.

Tisdall, J. M., Smith, S. E. and Rengasamy, P. (1997) Aggregation of soil by fungal hyphae. *Aust. J. Soil Res.* **35**, 54-60.

Turchenek, L. W. and Oades, J. M. (1979) Fractionation of organo-mineral complexes by sedimentation and density techniques. *Geoderma* **21**, 311-343.

Uezono, I., Kato, N. and Moriizuni, M. (2010) Applicability of rapid analysis by 80°C-16 h hot water extraction for estimating available nitrogen in upland soil in Japan. *Jpn. J. Soil Sci. Plant Nutr.* **81**, 39-43. (in Japanese with English summary)

Waring, S. A. and Bremner, J. M. (1964) Ammonium production in soil under waterlogged conditions as an index of nitrogen availability. *Nature* **201**, 951-952.

Walker, D. J., Clemente, R., Roig, A. and Bernal, M. P. (2003) The effects of soil amendments on heavy metal bioavailability in two contaminated Mediterranean soils. *Environ. Pollut.* **122**, 303-312.

Watanabe, A., Asakawa, D., Kawahigashi, M., Ohte, N., Nagao, S., Maie, N., Katoh, H. and Takenaka, M. (2009) Dynamics and function of dissolved organic carbon (DOC) in soil-river-coastal ecosystem. *Jpn. J. Soil Sci. Plant Nutr.* **80**, 89-94. (in Japanese)

Yokozawa, M., Shirato, Y., Sakamoto, T., Yonemura, S., Nakai, M. and Ohkura, T. (2010) Use of the RothC model to estimate the carbon sequestration potential of organic matter application in Japanese arable soils. *Soil Sci. Plant Nutr.* **56**, 168-176.