How do microaggregates stabilize soil organic matter?

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Abstract

Microaggregates play a key role to protect soil organic matter (SOM) from microbial decomposition. Several physical, physico-chemical and biochemical mechanisms have been proposed to describe the SOM stabilization in soil. However, no scientific consensus exists about a range of hypotheses. The aim of this review is to consolidate common notions and hypotheses on physical and physico-chemical protection mechanisms. The key notion of physical protection is exclusion of microbes and enzymes from microaggregate pores. Recent investigations showed higher microbial diversity and presence of accessible pore networks in microaggregates. The physico-chemical protection mechanism is more robust but monolayer or patchy adsorption of SOM onto clay surfaces requires further detailed research. The adsorption of SOM and exo-enzymes on pore walls and clay surfaces has been identified as a plausible concept of SOM stabilization.

Key Words

Microaggregation, physical protection, physico-chemical protection, intercalation.

Introduction

Stabilization of soil organic matter (SOM) in soil aggregates is on the global climate change agenda and viewed as an antidote to rapid carbon dioxide release from soil by human induced management practices. Scientific discussion over the past decade emphasizes the potential of soil to sequester atmospheric carbon dioxide through several stabilization mechanisms. Oades (1988) and Sollins *et al.* (1996) published comprehensive reviews of organic matter retention and stabilization in soil. The pivotal role of soil structure and texture was discussed in detail by Van Veen and Kuikman (1990). The authors presented experimental evidence of the effect of soil architecture on organic matter decomposition. In recent years a number of reviews were published to crystallize the stabilization mechanisms of SOM (Baldock and Skjemstad 2000; Kaiser *et al.* 2002; Krull *et al.* 2003; Von Lutzow *et al.* 2006; Kögel-Knabner *et al.* 2008a,b). However, the hypotheses put for some protection mechanisms such as physical and physico-chemical processes still lack a substantial body of experimental evidence and explanation. Advances in instrumentation for directly viewing inside microaggregates (<250 µm) would offer one such opportunity for further detailed research on SOM protection mechanisms. This review will attempt to consolidate the current understanding on physical and physico-chemical protection mechanisms of SOM in microaggregates.

Stabilization of SOM in microaggregates

Physical protection

Occlusion into microaggregates during its formation is one of the most important processes by which SOM can circumvent decomposition. The exclusion of microbes and enzymes from pores is the key protection mechanism for occluded SOM in microaggregates (Sollins *et al.* 1996). There is a sharp increase of pore volume that has diameters between 0.1 µm and 1 µm within microaggregates. The pore volume at diameters <0.1 µm is about 21% of the total pore volume present in microaggregates (McCarthy *et al.* 2008). The accessibility of bacteria and fungi to the occluded SOM is restricted due to the ratio between dimensions of the microhabitat and the size of the organisms. However, the diversity of microbial population is reportedly higher in silt and clay fractions, which may be due to higher nutritional availability and exclusion of protozoan grazing (Sessitsch *et al.* 2001). The methodological discrepancies regarding separation of aggregates between the investigations by McCarthy *et al.* (2008) and Sessitch *et al.* (2001) were considered not ‘out of the range’ for comparison by the current authors. If microaggregates are a protective space for SOM then these two findings must be complementary. Looking at the different approaches we can assume that the microbial community in microaggregates is less active due to inactivation or inaccessibility of extracellular enzymes in pore networks. Small mineral mesopores (0.002-0.01 µm) are not accessible to exo-enzymes, and organic matter in mesopores is protected against mineralization or decomposition (McCarthy *et al.* 2008; Zimmerman *et al.* 2004a). However, Mayer *et al.* (2004) and McCarthy *et al.* (2008) proposed that the arrangement of pore networks in microaggregates, rather than simple exclusion of exo-enzymes, is

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responsible for protection of SOM. Although some pores in microaggregates are habitable for small bacteria and accessible to exo-enzymes, the production of enzymes to decompose SOM might not be 'physiologically profitable' due to tortuosity of the pore network. The development of computerized microtomography has allowed us to look inside the microaggregates by non-destructive means. Recent studies based on synchrotron microtomographic imaging showed highly interconnecting micropore networks in soil aggregates (Lindquist 2002; Peth *et al.* 2008). The interconnected pore networks enable the transport of oxygen and soil solution to the center of aggregates, where SOM can be stabilized by organo-mineral complexes (Park *et al.* 2007; Peth *et al.* 2008) or by interacting with the pore walls (Zimmerman *et al.* 2004b). Further research is required to explore the relationship between microbial diversity, exclusion and/or activity of exo-enzymes, and the connectivity of pore networks to resolve the nature of physical protection of microaggregates to SOM.

Among the proposed SOM protection mechanisms the intercalation of SOM between silicate clay layers has received less attention than other mechanisms such as soil wettability. The principal reason behind this is likely related to uncertainty of intercalation of SOM. SOM is generally composed of complex macromolecules and structurally not fitted in interlayer space of clays. However, solubility and flexibility of the organic molecules and the presence of efficient metal complexers has been found to determine the extent of SOM intercalation (Schnitzer *et al.* 1988). Organic macromolecules such as enzymes, proteins, fatty acids or organic acids can be intercalated into 2:1 expandable phyllosilicates at pH <5 (Violante and Gianfreda 2000). Moreover, the penetration of small organic molecules into the smectite interlayer with conformational change and their subsequent polymerization into larger molecules are catalyzed by Al^{3+} or Fe^{3+} (Schulten *et al.*1996; Kennedy *et al.* 2002). Generally, the intercalated materials are depleted in lignin-derived aromatic compounds (Schulten *et al.* 1996). The stability of interlayer complexes depends on the isomorphic substitution in tetrahedral sheets of phyllosilicates due to the localization of a negative layer charge near the reaction sites. This statement leads to the conclusion that minerals such as vermiculite and mica, having isomorphous substitution in the outer silicate sheet, are able to form the most stable interlayer complexes (Tunega *et al.* 2007). Some researchers consider that there exist no specific methods to determine intercalated organic carbon, and hypothesize that chemical characterization and quantification of intercalated SOM are highly unreliable (Eusterhues *et al.* 2003). Clearly, the contribution of intercalation in SOM stabilization requires further detailed scientific dialogue and research.

Physico-chemical protection

Physico-chemical protection of soil organic matter arises from the interaction of different types of organic carbon with clay particles. The types of organic matter that are adsorbed on the clay particles can range from simple organic acids to complex bio-macromolecules, such as extracellular enzymes, suberins, DNA etc. The main causes of such interaction are large surface area and charged surfaces on clay particles. The surface area of the minerals is not a good predictor of organic matter and mineral interaction in soil because of its discontinuous coverage by SOM (Kögel-Knabner *et al.* 2008a). A term 'monolayer equivalent' is proposed by Mayer (1994) with the caution that organic matter should be distributed across the whole clay surface in a single layer. The author held the view that the surface area of clay controls the organic matter preservation. However, Ransom *et al.* (1997) rejected this hypothesis and presented evidence of patchy distribution of organic matter on clay. In accordance with Ransom *et al.* (1997) several other investigators proposed patchy surface adsorption of organic matter (Kaiser and Guggenberger, 2003; Curry *et al.* 2007). However, in recent work of Wagai *et al.* (2009) it was shown that the surface area of clay of most of the studied soil surface horizons developed on acidic metasedimentary and ultrabasic igneous rocks is completely covered by organic matter, except of a few with 'painted' clay surfaces.

The interaction of organic matter and clay particles is further determined by the architecture of clay minerals. The neutral siloxane surfaces or tetrahedral sheets of 2:1 and 1:1 phyllosilicates, where no isomorphous substitution has occurred, do not strongly participate in reactions, whereas the permanent charge sites within the tetrahedral sheets are very active. The reactivity of the siloxane surface increases if the major isomorphism substitution occurs at the octahedral sheets of phyllosilicates (Schoonheydt and Johnston, 2006). The hydroxyl surfaces where octahedral sheets are exposed also act as reaction sites for SOM interaction. It is clear from the above that physico-chemical protection of SOM needs to take into account the distribution of charge in clay mineral surfaces and its origin. Since the presence of the neutral siloxane surface can impair continuous strong organic matter coating on clay surfaces, the straightforward measurement of protection capacity of soil by relating it with percent clay content may not be judicious.

Moreover, if clay adsorbs SOM in a 'monolayer', the question of its maximum thickness and hence protection capacity will need to be worked out. Organic matter protection can be explained as either (i) physical protection due to adsorption on clay surfaces or (ii) protection due to adsorption of enzymes on clay (Theng 1979). Experiments on nuclease activity on DNA in the presence of kaolinite, illite and montmorilllonite showed that adsorption on clay surfaces provided physical protection against decomposition. The adsorption of nuclease on clay surfaces separates the enzyme from the substrate, and the conformation of the enzyme and adsorption strength also plays a significant role. However, part of the adsorbed DNA remained accessible to bacteria (Demaneche *et al.* 2001). The nucleic acid molecule adsorbed on the external surface of montmorillonite is subject to more rapid bacterial decomposition compared to that adsorbed inside the interlayer (Khanna *et al.* 1998). Fiorito *et al.* (2008) observed less bioavailability of clay bound proteins. The conformational change of protein molecules inhibits the protease-substrate reaction. However, there is a report of higher activity of clay bound enzymes due to exposure of more active sites of enzymes by 'allosteric regulation' as in biological systems (Fiorito *et al.* 2008). The diversity of results regarding clay-enzyme interaction clearly indicates the necessity of more detailed investigation rather than generalization.

Conclusion

The understanding of protection mechanisms of SOM in microaggregates with its protective capacity is crucial for adopting better soil management practices and to inform soil policy makers. Undoubtedly, microaggregates have the capacity to significantly slow SOM decomposition. However, recent findings on pore connectivity and microbial diversity in microaggregates indicate simple physical exclusion of exo-enzymes and microbes may not solely be responsible for SOM protection. The protection of SOM by occlusion in microaggregate pores, in an absolute sense, is related to the adsorption of SOM on the pore walls or on exposed facets of clays. Moreover, interlayers of clay minerals have the capacity to stabilize SOM by adsorption and complexation. The adsorption of SOM and exo-enzymes on clays with conformational change may be a better explanation of its protection against decomposition because conformational change interferes with enzyme-substrate recognition. The capacity of clays to protect SOM depends on its adsorption coverage and thickness of monolayers. An integrative research approach is required linking microbiology, clay mineralogy, chemistry and physics to further advance our understanding on protection mechanisms of SOM in microaggregates.

References

Baldock JA, Skjemstad JO (2000) Role of the soil matrix and minerals in protecting natural organic materials against biological attack. *Organic Geochemistry* **31**, 697-710.

Curry KJ, R.H. Bennett, Mayer LM, Curry A, Abril M, Biesiot PM, Hulbert MH (2007) Direct visualization of clay microfabric signatures driving organnic matter preservation in fine grained sediment. *Geochimica et Cosmochemica Acta* **71**, 1709-1720.

Demaneche SL, Jocteur-Monrozier, Quiquampoix H, Simonet P (2001) Evaluation of Biological and Physical Protection against Nuclease Degradation of Clay-Bound Plasmid DNA. *Applied and Environmental Microbiology* **67**, 293-299.

Eusterhues K, Rumpel C, Kleber M, Kögel-Knabner, I (2003) Stabilization of soil organic matter by interactions with minerals as revealed by mineral dissolution and oxidative degradation. *Organic Geochemistry* **34**, 1591-1600.

Fiorito TM, Icoz I, G. Stotzky (2008) Adsorption and binding of the transgenic plant proteins, human serum albumin, b-glucuronidase and Cry3Bb1, on montmorillonite and kaolinite: Microbial utilization and enzymatic activity of free and clay bound proteins. *Applied Clay Science* **39**, 142-150.

Kaiser K, Eusterhues K, Rumpel C, Guggenberger G, Kögel-Knabner I (2002) Stabilization of organic matter by soil minerals - investigations of density and particle-size fractions from two acid forest soils. *Journal of Plant Nutrition and Soil Science* **165**, 451-459.

Kaiser K, Guggenberger G (2003) Mineral surfaces and soil organic matter. *European Journal of Soil Science* **54**, 219-236.

Kennedy MJ, Pevear DR, Hill RJ (2002) Mineral surface control of organic carbon in Black Shale. *Science* **295**, 657-660.

Khanna M, Yoder M, Calamai L, Stotzky G (1998) X-ray diffractometry and electron microscopy of DNA from *Bacillus subtilis* bound on clay minerals. *Science of Soils* **3**, 1-10.

Kögel -Knabner I, Ekschmitt K, Flessa H, Guggenberger G, Matzner E, Marschner B, M. Von Lutzow (2008b) An intergrative approach of organic matter stabilization in temperate soils: Linking chemistry, physics and biology. *Journal of Plant Nutrition and Soil Science* **171**, 5-13.

Kögel -Knabner I, Guggenberger G, Kleber M, Kandeler E, Kalbitz K, Scheu S, Eusterhues K, Leinweber P (2008a) Organo-mineral associations in temperate soils: Integrating biology, mineralogy and organic matter chemistry. *Journal of Plant Nutrition and Soil Science* **171**, 61-82.

Krull ES, Baldock JA, Skjemstad JO (2003) Importance of mechanisms and processes of the stabilisation of soil orgnaic matter modelling carbon turnover. *Functional Plant Biology* **30**, 207-222.

Lagaly G, Ogawa M, Dekany I (2006). Clay mineral organic interactions. In 'Handbook Clay Science.' Vol. 1 (Eds Bergaya F, Theng BKG, Lagaly G) pp. 309-377. (Elsevier Ltd.: Amsterdam).

Lindquist WB (2002). Quantitative analysis of three dimensional X-ray tomographic images. Proc. SPIE **4503**, 103–115.

Mayer LM, Schick LL, Hardy KR, Wagai R, McCarthy J (2004) Organic matter in small mesopores in sediments and sois. *Geochimica Cosmochemica Acta* **68**, 3863-3872.

McCarthy JF, Ilavsky J, Jastrow JD, Mayer LM, Perfect E, Zhuang J (2008) Protection of organic carbon in soil microaggregates occur via restructuring of aggregate porosity and filling pores with accumulating organic matter. *Geochimica Cosmochemica Acta* **72**, 4725-4744.

Oades JM (1988) The retention of organic matter in soil. *Biogeochemistry* **5**, 35-70.

Park EJ, Sul WJ, Smucker AJM (2007) Glucose additions to aggregates subjected to drying/wetting cycles promote carbon sequestration and aggregate stability. *Soil Biology and Biochemistry* **39**, 2758-2768.

Peth S, Horn R, Beckmann F, Donath T, Fischer J, Smucker AJM (2008) Three-dimensional quantification of intra-aggregate pore-space features using synchrotron-radiation-based microtomography. *Soil Science Society of America Journal* **72**, 897-907.

Ransom B, Bennett RH, Baerwald R, Shea K (1997) TEM study of in situ organic matter on continental margins: occurrence and the monolayer hypothesis. *Marine Geology* **138**, 1-9.

Schnitzer M, Ripmeester JA, Kodama, H (1988) characterization of the organic-matter associated with a soil clay. *Soil Science* **145**, 448-454.

Schoonheydt RA, Johnston CT (2006) Surface and Interface Chemistry of Clay Minerals. In 'Handbook Clay Science.' Vol. 1 (Eds Bergaya F, Theng BKG, Lagaly G) pp. 87-113. (Elsevier Ltd.: Amsterdam).

Schulten HR, Leinweber P, Theng BKG (1996) Characterization of organic matter in an interlayer clay-organic complex from soil by pyrolysis methylation-mass spectrometry. *Geoderma* **69**, 105-118.

Servagent-Noinville S, Revault M, Quiquampoix H, Baron MH (2000) Conformational changes of bovine serum albumin induced by adsorption on different clay surfaces: FTIR analysis. *Journal of Colloid and Interface Science* **221**, 273–283.

Sessitsch A, Weilharter A, Gerzabek MH, Kirchmann H, Kandeler E (2001) Microbial population structures in soil particle size fractions of a long-term fertilizer field experiment. *Applied and Environmental Microbiology* **67**, 4215-4224.

Sollins P, Homann P, Caidwell BA (1996) Stabilization and destabilization of soil organic matter: mechanisms and controls. *Geoderma* **74**, 65-105.

Soma Y, Soma M (1989) Chemical Reactions of Organic Compounds on Clay Surfaces. *Environmental Health Perspectives* **83**, 205-214.

Theng BKG (1979). 'Formation and properties of clay-polymer complexes' (Elsevier Science Publishing Co.: Amsterdam).

Tunega D, Gerzabek MH, Haberhauer G, Lischka H (2007) Formation of 2,4-D complexes on montmorillonites an *ab initio* molecular study. *European Journal of Soil Science* **58**, 680-691.

Van Veen JA, Kuikman PJ (1990) Soil structural aspects of decomposition of organic matter by microorganisms. *Biogeochemistry* **11**, 213–233.

Violante A, Gianfreda L (2000) Role of biomolecules in the formation and reactivity toward nutrients and organics of variable charge minerals and organomineral complexes in soil environments. In 'Soil Biochemistry.' (Eds Bollag, JM, G Stotzky) pp. 207–270. (Marcel Dekker: New York).

Von Lutzow M, Kögel-Knabner I, Ekschmitt K, Matzner E, Guggenberger G, Marschner B, Flessa H (2006) Stabilization of organic matter in temperate soils: mechanisms and their relevance under different soil conditions-a review. *European Journal of Soil Science* **57**, 426-445.

Wagai R, Mayer LM, Kitayama K (2009) Extent and nature of organic coverage of soil mineral surfaces assessed by gas sorption approach. *Geoderma* **149**, 152-160.

Zimmerman AR, Chorover J, Goyne KW, Brantley SL (2004b) Protection of mesopore-adsorbed organic matter from enzymatic degradation. *Environmental Science and Technology* **38**, 4542-4548.

Zimmerman AR, Goyne KW, Chorover J, Komarneni S, Brantley SL (2004a) Mineral mesopore effects on nitrogen organic matter adsorption. *Organic Geochemistry* **35**, 355-375.