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Contrast agents for soil investigation with X-ray computed tomography

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ABSTRACT

The majority of microbial mediated soil processes depend on availability of organic matter (OM), water and air. Because of its ability to visualize the 3D architecture of soil non-destructively, X-ray computed tomography (CT) is becoming a widespread tool for studying soil pore network structure. However, phase determination of pore space, soil OM, soil mineral matter (MM) and water is often limited even with the latest technological and software advances, allowing high resolution and better quality imaging. Contrast agents commonly used in histology enable enhancement of X-ray attenuation of targeted structures or compounds. Here we report on the first systematic investigation of the use of such X-ray contrast agents for soil research. An evaluation procedure as well as a method to apply the agents to soil samples was developed and applied on reference soil samples. The effectiveness and selectivity of the contrast agents was evaluated for soil organic matter (SOM), MM and water. Several products were found to selectively increase the attenuation of water or SOM. The four agents with the best OM-staining capabilities (phosphomolybdenic acid (PMA), silver nitrate, lead nitrate and lead acetate) were further tested on an OM-MM mixture and all showed an increased of the SOM attenuation coefficient above the MM values.

1. Introduction

Soil pore size classes vary in scale from several centimeters (macropores) down to the nano-scale level. The smallest pores are inaccessible for microbial life or even hydrolytic enzymes. Soil pore space structure has a profound impact on spatial location and activity of soil microorganisms, either directly, through compartmentalization of organisms and soil organic matter (SOM) (Ekschmitt et al., 2008) or indirectly, through regulation of the water and air distribution (Monga et al., 2008; Nunan et al., 2006; Ruamps et al., 2011; Treves et al., 2003). Several studies have looked into spatial differentiation of soil organisms and C substrates (Poll et al., 2006). However, none closely captured the complexity of the soil pore network. This is despite the fact that, as Herrmann et al. (2007) put it, 'soils function by virtue of their architecture' and in fact pore space is the location of biochemical processes and the habitat space of an overwhelming number of individual organisms belonging to thousands of species. Despite the growing body of evidence showing the importance of pore structure for microbial processes such as decomposition of OM (Sleutel et al., 2012; Strong et al., 2004), sufficient data are lacking to quantify the importance of physical stabilization mechanisms against microbial decomposition. This lack of knowledge is to a large extent due to limitations of current techniques to explore the spatial organization of the soil, and particularly of the spatial location of OM and microorganisms in the soil matrix (Young et al., 2001). Recently, microfocus X-ray computed tomography (μCT) (e.g., De Gryze et al., 2006) has been emerging as a tool to spatially investigate undisturbed soil environments. X-ray CT has already proven to be a valuable tool for three-dimensional analysis of soils for a wide range of samples from several centimeters (Capowiez et al., 2011; Gantzer and Anderson, 2002; Kim et al., 2010; Pierret et al., 2002) in diameter down to the aggregate level (Feeney et al., 2006; Kravchenko et al., 2009; Nunan et al., 2006; Peth et al., 2008; Sleutel et al., 2008). The classification of the pore structure (Anderson et al., 1990; Kravchenko et al., 2011; Perret et al., 1999), water distribution (Mooney, 2002; Tippkotter et al., 2009; Wildenschild et al., 2002), root formation (Heeraman et al., 1997; Kaestner et al., 2006; Seignez et al., 2010; Tracy et al., 2010) and earthworm burrow identification (Bastardie et al., 2005; Capowiez et al., 2011; Jegou et al., 2002) have extensively been studied and related to environmental phenomena (Bastardie et al., 2003; Deurer et al., 2009; Ernst et al., 2009).

However, very little progress has been made in the 3D visualization of soil organic matter (SOM) inside undisturbed soil, although this would be a major advancement in the study of the interaction between soil pore space, the microbial community structure and SOM decomposition. A first attempt to visualize SOM by X-ray CT was made by De Gryze et al. (2006), who identified added particulate OM in μCT data sets of the linear attenuation coefficient μ of finite volume elements (voxels) of the scanned soil macroaggregate samples. A manual

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pinpointing of the particulate OM was necessary as contrast in μ was too limited to allow a (semi)-automated three-phase classification of the CT-data. The μ of each voxel depends on the density and the chemical composition of the material present in the volumes represented by the voxels. Sleutel et al. (2008) studied the ability of different non-synchrotron µCT setups to segment pore space, OM and mineral particles in artificial sand particulate OM mixtures based on μ. Sleutel et al. (2008) also found that the relative overlap in μ-values between the pore space and SOM was more problematic than the overlap between SOM and mineral phase. The μ-value overlap, in combination with CT-related phenomena such as Poisson statistics and partial volume effects (Clausnitzer and Hopmans, 2000; Schluter et al., 2010), complicate the separation of these different soil phases (Taina et al., 2008). Even with the latest technological advances that permit higher resolution and better imaging quality (Dhondt et al., 2010; Terzano et al., 2010), or the latest software developments for more accurate segmentation (Baveye et al., 2010; Brabant et al., 2011; Hapca et al., 2011; Iassonov et al., 2009; Martinez et al., 2010; Pinuela et al., 2010; Schluter et al., 2010; Tarquis et al., 2009; Wang et al., 2011), the (semi-)automated identification of the constituents of soil in CT scans is still problematic. As a possible solution for this, µCT SOM contrast might be enhanced via selective chemical staining of soil phases.

Almost all microscopic techniques, including visible light microscopy, fluorescence microscopy, scanning electron microscopy (SEM) and transmission electron microscopy (TEM), use a variety of staining agents and methods to improve the differentiation of specific features of a studied sample. X-ray contrast agents with heavy elements are used in the medical field since many years to improve diagnostic capabilities and their potential for other applications has proven to be very valuable (Granton et al., 2008; Metscher, 2009; Pauwels et al., 2013). To date, the potential of such staining methods has hardly been investigated in soil science. In one of the only published studies, Chenu and Plante (2006) used staining by U, Pb and Ag in TEM analysis of soil clay fraction ultrathin sections to specifically discriminate SOM from the mineral phase. To the best of our knowledge, there have been no published studies on the use of such staining agents in X-ray CT applications in soil science. This paper reports on the first systematic investigation of the potential of staining agents to enhance either water or SOM contrast relative to air and soil mineral components in X-ray CT analysis of soils. We applied staining agents to either 'pure' OM or mineral samples and investigated the effect on X-ray attenuation. We then applied selected staining agents on artificial mixtures of sand and particulate OM mixtures.

2. Materials and methods

Increasing the µCT attenuation of a specific soil phase relies on the introduction of a chemical staining agent. When this agent contains a significant amount of element(s) with an atomic number higher ($Z > 30$) than otherwise present, the local electron density increases and so does the X-ray photon attenuation. In practice, the staining of pore water thus consists of dissolving a staining agent in the soil water phase, while staining of SOM requires selective binding to the SOM phase. In order to introduce any contrast agent into SOM, a liquid or gaseous medium is required that has sufficient penetration power to allow reaction between the dissolved staining agent and the SOM. A variety of liquid media could be used, but such media should not have a significant impact on the soil morphology and its chemical composition. Non-polar media such as acetone and ethanol are likely to dissolve aliphatic SOM components. Water is therefore the logical choice as a transport medium for the staining agents.

In a first stage, the increase of X-ray attenuation of water amended with a series of contrast agents was assessed. 52 compounds with a molecule bearing a heavy element (Table 1) were dissolved in deionized water. Small plastic vials were filled with these solutions and the X-ray attenuation was measured. In a next stage, these contrast agent solutions were tested for their ability to enhance X-ray attenuation of 'pure' SOM and the possible effects on 'pure' soil mineral matter (MM). In a third stage, a selection of the most promising staining agents were applied to mixtures of SOM and MM particles to assess their ability to improve SOM contrast relative to mineral particles and pore space.

Table 1
Selected heavy element containing compounds with their maximal solubility in water (NA when not available) and their calculated relative effect on the X-ray attenuation of water (C_{water}). For the 20 agents with the highest C_{water}, the ability to relatively increase the X-ray attenuation of SOM (C_{SOM}) was determined, if no adverse effects were observed such as SOM dissolution (superscript d) or shrinkage (superscript s) of the tested SOM samples. For the four agents with the highest C_{SOM}, the relative increase of the X-ray attenuation of MM (C_{MM}) was also measured and calculated.

N°	Agent	max. sol.(g/100 ml)	C_{water}	C_{SOM}	C_{MM}
1	PMA	NA	0.81	0.62	0.11
2	$AgNO_3$	216	0.83	0.6	0.09
3	$Pb(NO_3)_2$	56.5	0.61	0.62	0
4	Pb acetate	44.3	0.55	0.72	0.14
5	OsO_4(l)	6.5	0.64	0.55	
6	$HgCl_2$	7.4	0.58	0.44	
7	$BaCl_2$	35.8	0.57	0.37	
8	$La(NO_3)_3$	136	0.78	0.29	
9	$CdSO_4$	76.4	0.84	0.29	
10	$ZnCl_2$	395	0.78	0.17	
11	KI	140	0.88	0.09	
12	KBr	53.5	0.66	0	
13	Optiray (l)	NA	0.9	0	
14	Visipaque(l)	NA	0.9	0	
15	$Cs_2CO_3^d$	260.5	0.88		
16	$CsCl^s$	162	0.87		
17	PTA^s	200	0.87		
18	Na_2WO_4 ^{d}	74.2	0.79		
19	Na_2MoO_4 ^{d}	84	0.78		
20	$CsNO_3^s$	9.16	0.55		
21	$FeCl_3$(l)	92	0.54		
22	$Co(NO_3)_2$	NA	0.54		
23	$CoCl_2$	52.9	0.52		
24	$Tl(NO_3)$	9.55	0.52		
25	$Ba(ClO_3)_2$	27.5	0.44		
26	BaS	7.68	0.4		
27	$CuSO_4$	32	0.32		
28	Co acetate	NA	0.31		
29	$K_3Fe(CN)_5$	NA	0.31		
30	$(NH_4)_2MoO_4$	38.7	0.27		
31	$(NH_4)_6Mo_7O_{24}$	43	0.27		
32	$VOSO_4$	NA	0.27		
33	KIO_3	4.74	0.26		
34	$Ba(NO_3)_2$	10.5	0.26		
35	$FeSO_4$	25.6	0.24		
36	$NiSO_4$	65	0.21		
37	$ZnSO_4$	22	0.2		
38	$K_2H_2Sb_2O_7$	NA	0.13		
39	$KMnO_4$	6.38	0.11		
40	$TiSO_4$	NA	0.1		
41	PbF_2	0.64	0.06		
42	AgCl	0.5	0.02		
43	MoO_3	0.1	0.02		
44	$Cu(BO_3)_2$	NA	0.02		
45	PbO	NA	0.01		
46	Sr carbonate	0.001	0		
47	I_2	NA	0		
48	HgI_2	0	0		
49	PbI_2	0	0		
50	Pb citrate	NA	0		
51	$NaBiO_3$	NA	0		
52	$Bi_{2}0_{3}$	NA	0		

2.1. Preparation of SOM and MM samples

Two artificial soil phases were prepared: 'pure' SOM, free of mineral particles and 'pure' MM, free of SOM. The SOM sample was isolated through physical fractionation from a sandy soil (sand: 86.3%, silt: 8.1%, clay: 5.6%) collected from the upper soil layer (0–30 cm) of an arable field in Beernem in North Belgium (sandy region). The soil was