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DNA adsorption by nanocrystalline allophane spherules and nanoaggregates, and implications for carbon sequestration in Andisols

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ABSTRACT

This study provides fundamental knowledge about the interaction of allophane, deoxyribonucleic acid (DNA), and organic matter in soils, and how allophane sequesters DNA. The adsorption capacities of salmon-sperm DNA on pure synthetic allophane (characterised morphologically and chemically) and on humic-acid-rich synthetic allophane were determined, and the resultant DNA–allophane complexes were characterised using synchrotron-radiation-derived P X-ray absorption near-edge fine structure (XANES) spectroscopy and infrared (IR) spectroscopy. The synthetic allophane adsorbed up to 34 $\mu g\ mg^{-1}$ of salmon-sperm DNA. However, the presence of humic acid significantly lowered the DNA uptake on the synthetic allophane to 3.5 $\mu g\ mg^{-1}$ by occupying the active sites on allophane so that DNA was repulsed. Both allophane and humic acid adsorbed DNA chemically through its phosphate groups. IR spectra for the allophane–DNA complex showed a chemical change of the Si–O–Al stretching of allophane after DNA adsorption, possibly because of the alteration of the steric distance of the allophane outer wall, or because of the precipitation of aluminium phosphate on allophane after DNA adsorption on it, or both. The aluminol groups of synthetic allophane almost completely reacted with additions of small amounts of DNA (~2–6 $\mu g\ mg^{-1}$), but the chemical adsorption of DNA on allophane simultaneously led to the formation of very porous allophane aggregates up to ~500 μm in diameter. The formation of the allophane nano- and microaggregates enabled up to 28 $\mu g\ mg^{-1}$ of DNA to be adsorbed (~80% of total) within spaces (pores) between allophane spherules and allophane nanoaggregates (as "physical adsorption"), giving a total of 34 $\mu g\ mg^{-1}$ of DNA adsorbed by the allophane. The stability of the allophane–DNA nano- and microaggregates likely prevents encapsulated DNA from exposure to oxidants, and DNA within small pores between allophane spherules and nanoaggregates may not be accessible to enzymes or microbes, hence enabling DNA protection and preservation in such materials. By implication, substantial organic carbon is therefore likely to be sequestered and protected in allophanic soils (Andisols) in the same way as demonstrated here for DNA, that is, predominantly by encapsulation within a tortuous network of nanopores and submicropores amidst stable nanoaggregates and microaggregates, rather than by chemisorption alone.

1. Introduction

Andisols cover only approximately 0.8% of the ice-free surface in the world (Soil Survey Staff, 1999; McDaniel et al., 2012) but sequester upwards of ~1.8% of the total global soil carbon stocks (Matus et al., 2014; Takahashi and Dahlgren, 2016). Many Andisols are dominated by allophane and usually contain relatively large amounts of organic matter, up to ~8–12% organic carbon (McDaniel et al., 2012). Allophane is a nanocrystalline aluminosilicate with a formula $(1–2)\mathrm{SiO_2}\cdot\mathrm{Al_2O_3}\cdot(2–3)\mathrm{H_2O}$ (Abidin et al., 2007; McDaniel et al., 2012) and it comprises hollow spherules ~3.5 to 5 nm in diameter with high specific surface areas (SSAs)[2] — from about 250 $m^2\ g^{-1}$ to as much as 1125 $m^2\ g^{-1}$ (e.g. Maeda et al., 1977; Parfitt et al., 1980; Wada, 1980; Allbrook, 1985; Parfitt, 1990; Ohashi et al., 2002; Iyoda et al., 2012). The high organic carbon content generally is significantly correlated with allophane content and SSA (Chevallier et al., 2010; Parfitt and Yuan, 2012), and carbon turnover in Andisols is slower than in other soils (Torn et al., 1997; Parfitt, 2009). Hence it is commonly acknowledged that Andisols can

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[2] All abbreviations are defined together at the end of the text.

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not only adsorb substantial organic carbon but also protect it from degradation (Dahlgren et al., 2004; Goh, 2004; Calabi-Floody et al., 2014). Allophane spherules are reportedly crucial in enabling Andisols firstly to store organic carbon and secondly to strongly adsorb phosphate (Parfitt, 1989, 2009; Calabi-Floody et al., 2011; Yuan and Wada, 2012).

Adsorption of organic matter by these Al-rich nanocrystalline minerals governs the mobility of organic matter in the Andisols (Harsh, 2012), and the chemical bonding between the active aluminol groups on allophane spherules and organic matter is then considered to allow carbon to be adsorbed and stored (Yuan et al., 2000; Buurman et al., 2007; Parfitt, 2009; McDaniel et al., 2012; Takahashi and Dahlgren, 2016). Moreover, allophane spherules tend to form clusters of subrounded "nanoaggregates" about 100 nm in diameter (Calabi-Floody et al., 2011), which could also stabilize organic carbon within aggregates and allow long-term carbon sequestration in allophane-rich soils (Six et al., 2000a; Blanco-Canqui and Lal, 2004; Lehmann et al., 2007; Chevallier et al., 2010).

Nanocrystalline aluminosilicates show a phosphate adsorption capacity up to two orders of magnitude greater than that of long-range-order phyllosilicates and Fe- and Al-oxides/hydroxides (Hesterberg, 2010). Andisols thus have a high affinity for deoxyribonucleic acid (DNA) (Hashizume and Theng, 2007; Saeki et al., 2010a, 2010b) as well as phosphate (Allbrook, 1983, 1985; Parfitt, 1989). The adsorption of DNA on clay minerals is one of the most important mechanisms of DNA retention in soils (Ogram et al., 1988; Paget et al., 1992), and allophane, along with organic matter, presumably could facilitate the preservation of environmental DNA in soils hence could, if extractable, enable reconstruction of past environments via the DNA preserved (Huang et al., 2012; Rawlence et al., 2014). Furthermore, Matsuura et al. (2014) have hypothesised that allophane is able to protect DNA and ribonucleic acid (RNA) from ultraviolet light and, using computer modelling, simulated the interaction between DNA and allophane. Their simulations illustrated that the DNA strands underwent elongation and the phosphate backbone of DNA altered after bonding to allophane (Matsuura et al., 2013), possibly as a result of chemical adsorption of DNA through its phosphate groups to aluminol groups at the wall perforations of allophane (Huang et al., 2014). However, a more detailed understanding of the adsorption mechanism of DNA on allophane has not been developed, and the driving factor allowing allophane to adsorb more DNA than other clay minerals has remained vague, thus providing impetus for the studies reported here.

In natural allophanic soil systems, the humic substances associated with allophane strongly bind to DNA and immobilize it (Saeki et al., 2011). However, some studies have contrarily shown that clay from which organic matter has been removed adsorbs more DNA than organic-matter-rich clay (Cai et al., 2006, 2007). Hence the level of influence of humic substances and organo-minerals on DNA adsorption in allophanic soils has been controversial and requires further examination.

1.1. Hypothesis

In this study it is therefore hypothesised that DNA is adsorbed by allophane both chemically and physically, and "physical adsorption" of DNA within pores amidst allophane spherules and nanoaggregates (defined here as aggregated clusters of allophane spherules up to ~100 nm in size) or microaggregates (defined here as aggregated clusters of allophane nanoaggregates up to several hundred micrometres in size, after Elliot, 1986) brings about the exceptionally high DNA adsorption capacity of allophane and enables DNA to be preserved. To test this hypothesis, the interactions between well-characterised synthetic allophane, salmon-sperm DNA, and humic acids are examined using P X-ray absorption near-edge structure (XANES) spectroscopy. XANES spectroscopy has been widely used in soil science to analyse the species of molecules, the oxidation state of a targeted atom, and the binding geometry of this atom with surrounding atoms in a molecule (e.g. Hesterberg, 2010; Lehmann and Solomon, 2010; Terzano et al., 2010). As well, the structural alterations of DNA and allophane spherules after they bind to each other are determined using infrared (IR) spectroscopy, which has been extremely useful to characterise chemicals and to describe structural or molecular alteration of chemicals adsorbed on clays (e.g. Farmer, 1968; Parfitt, 1989; Shin et al., 2004; Tahoun, 2014). Finally, the degree of aggregation/complexation of DNA and allophane are examined using high-resolution laser sizing.

The findings with respect to DNA provide a detailed mechanism to help explain carbon sequestration and its unique longevity in Andisols.

2. Materials and methods

2.1. Synthetic allophane

2.1.1. Allophane synthesis

Pure allophane was synthesised according to Ohashi et al. (2002) with two minor modifications as follows. The Si source, Na_2SiO_4, for synthetic allophane was replaced with Na_2SiO_3, and the alkalinity of the Si solution was adjusted by adding NaOH, given that the alkalinity of Si solution should be triple the Al molarity. The allophane precursor was made with a mixture of Si and Al solutions (at the same concentration but with an atomic ratio of Si/Al of 0.75) together with the addition of NaOH, and the precursor was incubated for 48 h. In the current study, two synthetic allophane products with 50 mmol L^{-1} and 100 mmol L^{-1} of initial solutions were prepared, and the products were labelled as *50-allophane* and *100-allophane*, respectively. After hydrothermal incubation for 48 h, the synthetic products were washed with deionised (DI) water until the pH became neutral. For the DNA adsorption experiments, the dispersed allophane in DI water was adjusted accurately to pH 6.5 and stored without drying.

2.1.2. Allophane analysis

Transmission electron microscopy (TEM) was employed to observe the morphology of the synthetic allophane. For TEM images of the synthetic allophane, 10 μL of diluted allophane dispersion was spread on a lacey formvar/carbon copper grid (200 mesh) followed by drying at 40 °C for moisture removal. Two TEM facilities were used, a Philips CM30 TEM and a JEOL TEM-3010 scanning TEM for images with varied magnifications. The synthetic allophane was damaged and melted in a fairly short time under the X-ray beam because allophane is heat-sensitive, and so the synthetic allophane was photographed as quickly as possible to avoid over-exposure of the samples to X-rays.

As well as TEM-based morphological observation, the allophane content of the synthesised product was determined. To accurately estimate its Si/Al ratio, the synthetic allophane was frozen rapidly with liquid nitrogen followed by freeze-drying. The allophane content was estimated by oxalate extraction of Al and Si and pyrophosphate extraction of Al (following Parfitt and Wilson, 1985; Blakemore et al., 1987). The results showed that the Al/Si ratio was 1.2 and the allophane yield was 95% of the total product. The SSA of the synthetic allophane was analysed via the Brunauer–Emmett–Teller (BET) method with nitrogen gas adsorption at −195 °C (Brunauer et al., 1938), and pore volume and pore size were evaluated via the Barrett–Joyner–Halenda (BJH) method (Barrett et al., 1951).

2.1.3. Preparation of humic-acid-laden synthetic allophane

To understand the influence of humic substances on the adsorption of DNA on allophane, the dispersed 50-allophane in DI water was incubated overnight with humic acid (Pahokee peat humic acid standard, which was obtained from the International Humic Substances Society) to allow humic acid (HA) adsorption on synthetic allophane. Prior to incubation, the dried HA powder was dissolved in diluted NaOH and then adjusted to pH 6.5 using 0.1 M HCl, and the resulting solution was then centrifuged at 8000 rpm (11,325 *g*) to remove insoluble HA. After incubation, the allophane–HA complex was repeatedly washed with DI