Evaluating the character and preservation of DNA within allophane clusters in buried soils on Holocene tephras, northern New Zealand

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Introduction

Clay minerals possess sorptive capacities for organic and inorganic matter, including DNA (Lorenz and Wackernagel, 1994), and hence reduce the utilization and degradation of organic matter or DNA by microorganisms. Buried allophane-rich soils on tephras (volcanic-ash beds) on the North Island, dated using tephrochronology, provide a valuable paleobiological 'laboratory' for studying the preservation of ancient DNA (aDNA) (Haile et al., 2007). Allophane comprises Al-rich nanocrystalline spherules ~3.5–5 nm in diameter (Fig. 1) with extremely large surface areas (up to 1000 m^2 g^{-1}). Moreover, allophanic soils are strongly associated with organic matter (Parfitt, 2009), and so we hypothesize that allophane also plays an important role for DNA protection within such soils.

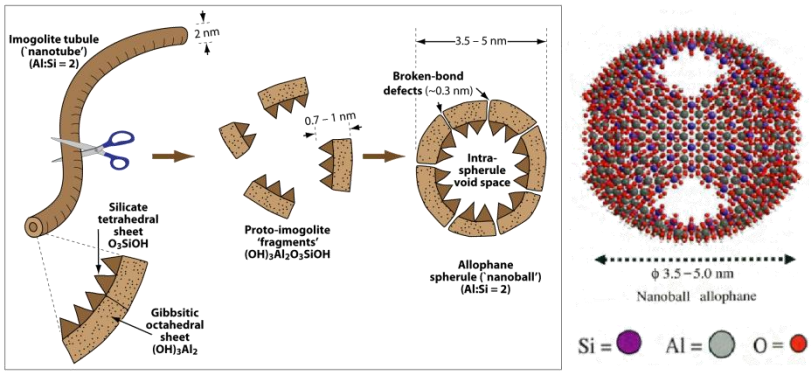

Fig. 1. Nanoscale dimensions and composition of imogolite tubules and allophane spherules (from McDaniel et al., 2012), and (at right) the atomic structure of allophane (from Theng and Yuan, 2008).

Greaves and Wilson (1970) suggested that RNA adsorbed in the central zones of montmorillonites may undergo less attack by microbial enzymes, but the RNA sorbed on surfaces of individual montmorillonite particles is not protected. Their results catalysed our research interest that DNA may be physically protected and preserved in the interspaces between spherules of allophane clustered together as microaggregates as well as being chemically sorbed on the surfaces of allophane spherules. Kahle et al. (2003) showed that coarse and fine clay fractions of illitic soils had different capacities for carbon storage, supporting the hypothesis that allophane clusters of different sizes have various capacities to hold DNA. DNA adsorption isotherms on synthetic and natural allophanes have been completed recently (Saeki et al., 2010), but no one has investigated the influence of allophane cluster

size on DNA adsorption and the exact mechanisms provided by such clusters or microaggregates for adsorption of organic matter.

DNA-clay interaction and analysis

The DNA molecule comprises heterocyclic bases and pentose-phopho-diester backbone (Fig. 2). The distribution, content and structure of DNA in cells have been determined using near-edge X-ray absorption fine structure (NEXAFS) (Zhang et al., 1996; Fujii et al., 2003). Other researchers have also investigated the interactions of DNA with clay minerals, including montmorillonite and kaolinite and other soil colloidal particles using Fourier transform infrared (FTIR) spectroscopy (Cai et al., 2006). Using synchrotron-based NEXAFS, we should be able to determine the electron configuration of the specific atoms (C, N, and P) comprising DNA. To ascertain the real interaction between allophane clusters and DNA (and organic carbon), synchrotron-based techniques provide the ideal approach to clarify the geochemistry of DNA as it occurs in allophanic soils.

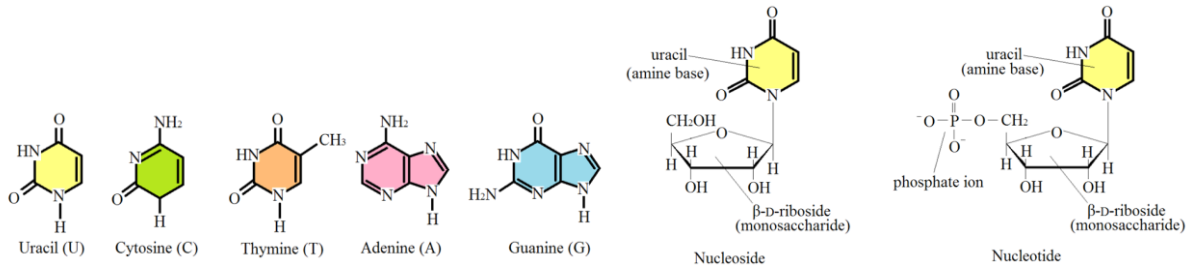

Fig. 2. Five heterocyclic aromatic amine bases most common to nucleic acids and the basic structures of DNA fragments (modified after Brown and Poon, 2005).

Objectives and experimental design

We want to know why and how allophane is able to 'hold' and protect DNA from degradation. The answers are of benefit not only in explaining the development of genetic preservation but also in providing a better understanding of the role of allophanic soils in carbon sequestration. Two objectives of this study are as follows:

1. To evaluate the sorptive capacities for DNA fragments of clusters of allophane (microaggregates) of different sizes and hence to evaluate the physical and chemical adsorption/protection provided by allophane clusters to hold DNA fragments in allophanic soil systems.
2. To determine the fingerprint of DNA bound to allophane clusters using synchrotron-based NEXAFS and to identify ancient DNA within buried soils.

Four sets of allophanic soils from the North Island are to be analysed in this study, three from tephra-derived buried horizons (on rhyolitic Taupo, Whakatane, and Rotoma tephras, deposited c. 1780, 5500, and 9500 cal years ago, respectively) and one from the upper subsoil horizon of the Tirau soil, a Typic Hapludand derived from incremental accumulations of thin, mainly rhyolitic, tephras (Lowe and Palmer, 2005). Clay-size fractions from each sample will be subjected to four physical and chemical

dispersion treatments (hand-inversion, prolonged shaking, zirconium nitrate addition, and pH alteration) to generate allophane clusters/microaggregates with a range of sizes. These allophane clusters will be characterized by measuring their specific surface areas and pore size distributions using nitrogen gas adsorption and laser-sizer analysis. Modern DNA (e.g., salmon-sperm DNA) (Saeki et al., 2010) will be mixed with the allophane cluster fractions of different sizes to determine their DNA sorption capacity. The 'moist' DNA-allophane complexes will be observed using environmental scanning electron microscopy (SEM), because we want to prevent DNA-allophane complexes from transformation during drying. However, dried samples are required for high-vacuum transmission electron microscopy (TEM), and hence air-dried samples without interference will be prepared for TEM analysis. NEXAFS analysis is to be used to examine the air-dried DNA-allophane complexes to obtain the near edge fine structures of C (energy range: 280−300 eV), N (395−425 eV) and P (2140−2180 eV) (1s) transitions comprising the DNA molecules and their surrounding atoms. The X-ray absorption spectra of C, N, and P could be used to enable 'fingerprints' of DNA within allophane clusters, or of DNA fragments in allophanic soil materials, to be recognised. Moreover, speciation of C and N within allophane clusters could be usefully illustrated.

Expected DNA adsorption isotherms and outcomes

We hypothesize that the allophane clusters of different sizes possess differing capacities for DNA adsorption, and that the larger allophane clusters would have more adsorptive sites for DNA (Fig. 3). The small allophane clusters have higher specific surface areas and a higher affinity for DNA adsorption because of chemisorption, and so DNA fragments are easily sorbed on small allophane clusters. Consequently, the adsorptive trend flattens out when available adsorptive sites are saturated (Fig. 3). On the other hand, the large allophane clusters, possessing lower surface areas, have a moderate affinity for DNA as evident in the first part of the adsorption isotherm; nevertheless, large allophane clusters adsorb DNA eventually and have higher sorbed capacity (Fig. 3). We hypothesize that DNA fragments are slowly sorbed into the interspaces (gaps) of the allophane clusters (where they end up being physically 'protected').

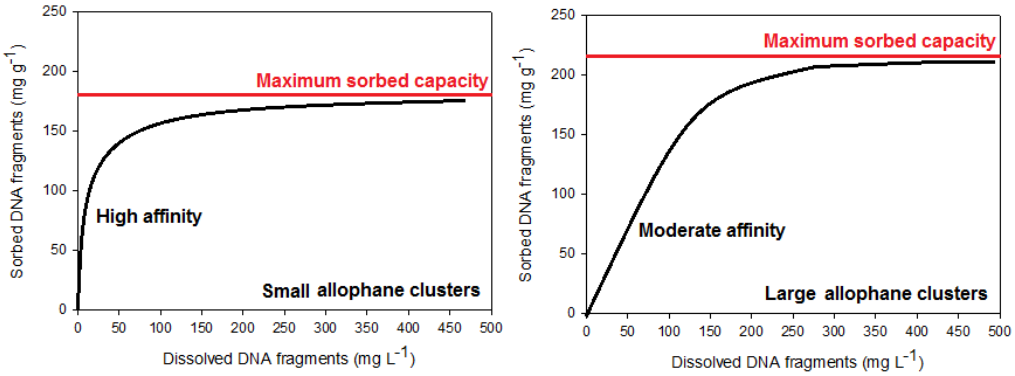

Fig. 3. Predicted DNA adsorption isotherms on small and large allophane clusters (based on findings in Saeki et al., 2010)

Thus, we aim to test this hypothesis to find out if allophane holds DNA (or organic matter) through this 'physical habitation' model – i.e., amidst the interspaces of the allophane clusters – as well as through chemisorption that arises as a consequence of the very large surface area and cation exchange capacity of allophane spherules. It is anticipated that DNA fragments existing in allophanic soils have specific functional groups, and the structures of allophane and the DNA-allophane complex are thus somewhat different. The functional groups and structures of carbonic compounds are able to be identified according to the excited energy (absorption edge) of carbon (Fig. 4). We thus aim to use these differences in functional groups to determine the existence of ancient DNA within buried allophanic soils and to ascertain the DNA-protective ability of such soils.

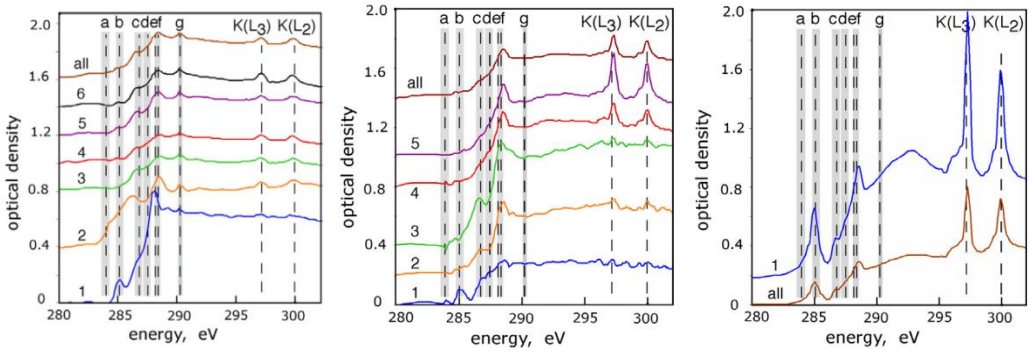

Fig. 4. Carbon NEXAFS spectra obtained within selected areas of microaggregates: (a) quinonic, (b) aromatic, (c) phenolic, (d) aliphatic, (e) peptidic, (f) carboxylic, (g) carbonate/carbonyl functional groups (Wan et al., 2007)

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