Adsorptions of DNA molecules by soils and variable-charged soil constituents

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Understanding the adsorption of extracellular DNA on soil particles is essential to assess the risk of release of genetically modified organisms and to develop efficient methods of DNA extraction from soils in the analysis of soil microbial communities using culture-independent methods. Much research has used 2:1 type layer phyllosilicates (e.g., montmorillonite) as adsorptive particles to understand DNA adsorption by soils. However, the results of DNA adsorption by phyllosilicates were not enough to study DNA molecule behaviors in variable-charge soils such as andosol. Therefore, this mini-review summarized the adsorption of DNA molecules by natural soils and variable-charged soil constituents such as goethite, allophane, and humic acids.

Keywords: DNA adsorption; soil; variable-charged soil constituents; allophane; humic acids

1. Significance of studies on DNA adsorption by soil constituents

Three topics require knowledge of DNA adsorption by soils and soil components: the ecosystem influences of genetically modified organisms (GMOs); the analytical problems of DNA extraction from soils; and the involvement of clays in the chemical evolution of life.

1.1 Ecosystem influences of GMOs

For the last few decades, it has been believed that nucleases rapidly degrade DNA released from dead or metabolizing microorganisms [1]. However, it has recently been observed that the adsorption of nucleic acids released from microorganisms and plant tissue on soil particles alters the reactivity and susceptibility of the nucleic acids to nucleases and makes them resistant to biodegradation (Fig. 1). Moreover, DNA molecules have a persistent ability to transform competent cells when bound to clay minerals and other particles [2-5]. Hoffmann et al. [6] reported that transformations by extracellular genes from GMOs occur between crops and microorganisms as well as among microorganisms. With rapid developments in the commercial production of genetically modified microorganisms and plants, great emphasis has been placed on the security of GMOs. Soil DNA plays an important role in biological activity and diversity in soil as well as in the transfer of genetic information among bacteria [2, 5]. Therefore, knowledge of the binding of extracellular DNA to soil constituents is essential to understand the horizontal transfer and transformation of extracellular DNA in soil environments. Understanding the adsorption of extracellular DNA by soil particles is also helpful for the study of soil biodiversity and in assessing the risk of the release of GMOs into the soil.

1.2. Analytical problem of DNA extraction from soils

A billion microorganisms exist per gram of soil and the number of the species therein reaches several tens of thousands [7]. Most bacteria in soil cannot be cultivated in artificial media. Therefore, for the past few decades, the presence of these nonculturable bacteria has been detected by methods based on hybridization or polymerase chain reaction amplification of DNA sequences extracted directly from soil. However, it is more difficult to extract DNA from volcanic ash soils (andosol), which are found abundantly in Japan, than from other types of soil [8]. This has been a major problem in the analysis of soil microbial communities using culture-independent methods. Therefore, it is important to understand the mechanism of DNA adsorption by soil particles in order to develop efficient extraction methods.

1.3. Involvement of clays in the chemical evolution of life

J. D. Bernal suggested in 1951 that clay minerals play an important role in the prebiotic formations of biomolecules that are basic to life, which has paved the way for a series of studies on the adsorption of various organic molecules, including nucleic acids [9, 10]. This motivation might stimulate researchers to perform further studies on the adsorption of nucleic acids by phyllosilicates such as montmorillonite. These studies indicate the possible involvement of clay minerals in the chemical evolution of life [11, 12].

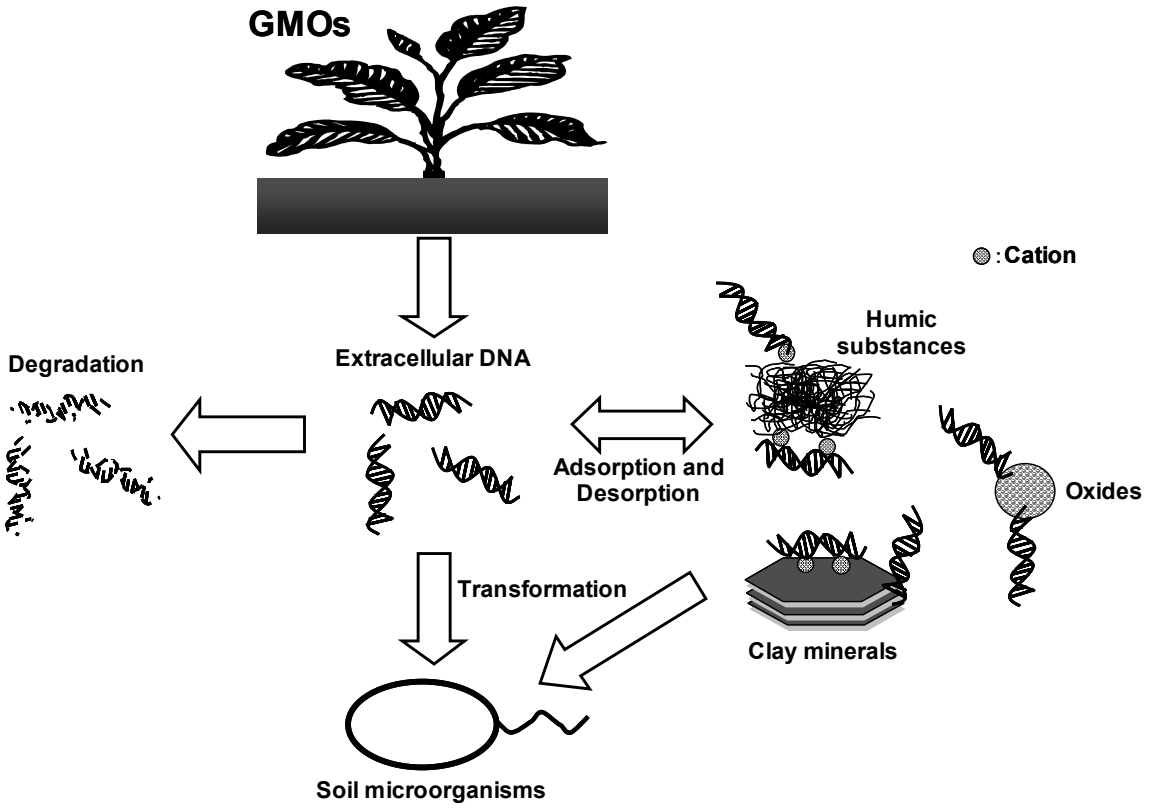

Figure 1. The behaviour of extracellular DNA in soil

2. Models of DNA adsorption by soil and soil constituents

Many researchers have used 2:1 layer phyllosilicates such as montmorillonite as adsorptive particles to study DNA adsorption in soils [2, 9, 13-15]. However, the results of DNA adsorption by phyllosilicates only are not enough to study the DNA molecule behaviors in variable-charge soils. Therefore, this review summarized the adsorption of DNA molecules by soils such as andosol and variable-charged soil constituents such as allophane and humic acids.

In order to help readers understand the contents described below, several conceptual mechanisms of extracellular naked DNA adsorption by soil particles are shown in Fig. 2. In the first mechanism, (Fig. 2(a) and 2(b)), the phosphate group at the end of the DNA molecule binds directly to the OH groups on the surface of oxide particles such as allophane and to the OH groups present on the edges of phyllosilicates such as montmorillonite [14, 16]. In the second mechanism (Fig 2(c)), DNA molecules associate with the surface of negatively charged particles such as humic substances via a bridging of inorganic cations. In the third mechanism (Fig. 2(d)), DNA molecules may be bound directly to soil organic matter or precipitated on soil particles.

3. Adsorption of DNA molecules by variable-charged minerals

To better understand the contribution of soil components to DNA adsorption by andosol particles, the effects of hydrogen peroxide and oxalate treatments on DNA adsorption were examined [17, 18]. These studies indicated that oxide minerals in soils are among the most important adsorbents of DNA molecules. Here we review the literature on adsorption of DNA molecules by a variety of variable-charged minerals except for pure montmorillonite and kaolinite.

3.1. Hydroxyaluminum-montmorillonite complexes

It was reported that the binding affinity of DNA to the hydroxyaluminum-montmorillonite complex Al(OH)x-M was higher than that to montmorillonite, but the amount of DNA adsorbed was less for Al(OH)x-M than for montmorillonite [19], suggesting strong bonds such as coordination linkage between DNA and Al(OH)x-M based on the measurements of adsorption enthalpy and other analyses.

3.2. Goethite

Adsorption maximums were found at pH 3.0 in goethite, kaolinite, and montmorillonite [20], suggesting that DNA denaturation occurred at this pH and led to aggregation or precipitation on the mineral surfaces. Saeki et al. [21] compared DNA adsorption by a synthetic goethite with that by allophane. The DNA adsorption capacity of goethite was much higher than that of allophane minerals, although the specific external surface area of goethite was smaller than of allophane.

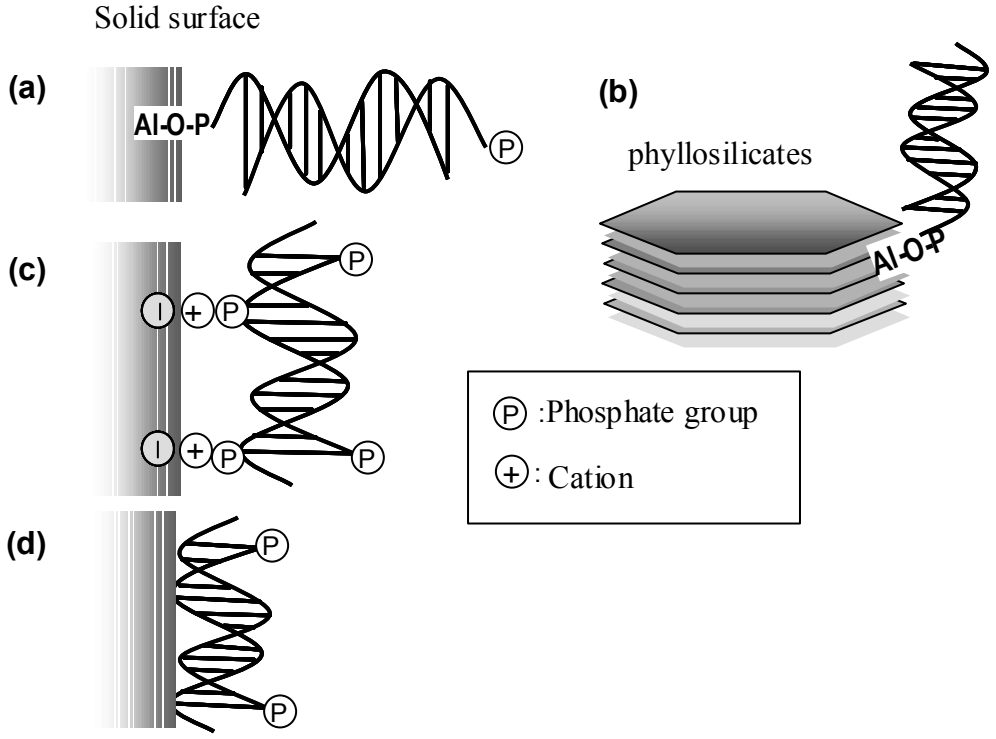

Figure 2. Conceptual figures of DNA adsorptions, quoted from Saeki and Sakai (2009) [18]

3.3. Silica

DNA adsorption by silica was controlled by shielded intermolecular electrostatic forces, dehydration, and hydrogen bond formation [22]. At pH 6 and 8, both DNA and silica surfaces are negatively charged and electrostatic repulsion occurs. The presence of a monovalent cation such as Na^+ screens the negative charges on the phosphate backbone of DNA, reducing the electrostatic energy barrier between DNA and silica to increase DNA adsorption [23]. A divalent cation such as Ca^{2+} binds to the DNA phosphate backbone and effectively neutralizes it [23]. Ca^{2+} ions also bind to Si-OH groups of the silica and reduce electrostatic repulsion between the DNA and the silica surface, resulting in increased DNA adsorption. Furthermore, DNA adsorption by silica is enhanced by the presence of divalent cations because the binding of the cations depresses intramolecular electrostatic repulsion among DNA molecule subunits, which leads to a higher diffusion coefficient of DNA due to its more compact formation [23].

3.4. Allophane

The adsorption of adenine, adenosine, ribose and adenosine-5′-phosphate (5′-AMP) by allophane extracted from a soil was investigated at pH 4, 6, and 8 [24]. Allophane adsorbed larger amounts of adenine, adenosine, and ribose at higher pH than at pH 4. This was caused by that the electrostatic attraction between these solutes and allophane should increase with solution pH due to pH-dependent charge characteristics. Allophane had a greater affinity for 5′-AMP than for adenine, adenosine, or ribose. The adsorption of 5′-AMP at pH 4 and pH 6 was about 60 times greater than that at pH 8. It was subsequently proposed that 5′-AMP is adsorbed through ligand exchange reactions between the 5′-AMP phosphate group and the allophane hydroxyls associated with the $(HO)Al(OH_2)$ groups that are exposed at perforations on the spherule surface [24].

Saeki et al. [21] investigated the adsorption of DNA by natural and synthetic allophane samples, which are a primary clay mineral of andosols, with respect to DNA concentration, pH, ionic strength, and competition with phosphate ions to understand the behavior of extracellular DNA molecules in andosols. In both the natural and the synthetic allophane

samples, the relationships between DNA adsorption and the final concentrations were significantly fitted to a simple linear Langmuir equation, suggesting that the adsorbed DNA molecules were likely to form monomolecular layers on the allophane particle surfaces. The adsorption decreased with increasing suspension pH, which is similar to those observed in inorganic anion adsorption by minerals such as goethite and alumina [25-28]. Adsorption by the allophane samples began to decline when the solution pH was >5. The isoelectric point of DNA is around pH 5 [5], whereas the point of zero net charge (pznc) of allophanes is close to pH 6 [29]. When the solution pH is increased to >6, AlOH groups on the surface of allophane particles are deprotonated and converted to AlO^- with a negative charge. Concomitantly, the negative charges of DNA molecules, which may be those of the phosphate functional groups, also increase with pH. Accordingly, it can be assumed that most DNA molecules are hardly adsorbed on allophane surfaces. The addition of phosphate reduced the adsorption by allophane s , indicating that there was competition between DNA molecules and phosphate ions for adsorption. This suggests that the phosphate group at the end of the DNA molecule would bind directly to the OH groups of the AlOH layer on the allophane particles. These results imply that allophane plays a significant role in DNA adsorption by andosols among the constituents. Allophane consists of hollow, irregularly spherical particles with diameters of 3.5–5 nm. The spherical particles consist of an $Al_2O_3 \cdot nH_2O$ layer with attached monosilicates on the internal surface [30, 31]. Theoretically, DNA molecules cannot enter into the small pores because the diameter of double-stranded DNA molecules is 2 nm. DNA adsorption was considerably less on the silica sample than on gibbsite, probably suggesting a low affinity of DNA molecules to silica [21]. Any contribution of the SiOH groups on the internal surface of allophane particles to DNA adsorption would be negligible.

3.5. Sand

In the presence of Na^+, the amount of DNA adsorbed on sand was large at low pH, whereas Mg^{2+} addition caused minimum adsorption at neutral pH range and increased adsorption at acidic and especially alkaline conditions [32]. The reason for high DNA adsorption at high pH remains unclear.

3.6. Mica

Analysis with atomic force microscopy showed that DNA was strongly adsorbed on mica in the presence of a divalent cation (Ni^{2+}, Co^{2+}, or Zn^{2+}) with an ionic radius of 0.069–0.074 nm, but cations with larger ionic radii (Ca^{2+}, Cd^{2+}, or Hg^{2+}) did not facilitate the adsorption of DNA by mica [33]. Perhaps the smaller divalent cations are likely to play a role in bridging between the negatively charged DNA to mica, when the cations can enter into the hexagonal cavities of the mica surface. The cations, which are too large to fit into the mica cavities, could not cause substantial adsorption of DNA by mica despite their small ionic radius. However, the presence of Mg^{2+} did not result in DNA adsorption by mica [33].

3.7. DNA adsorption sequences among various minerals

Cai et al. [34] reported that the DNA adsorption capacity decreased in the order of montmorillonite > kaolinite > goethite, although the specific external surface area increased in the order of kaolinite < montmorillonite < goethite. DNA adsorption was compared between synthetic minerals (allophane and goethite) and commercial minerals (montmorillonite, silica, kaolinite, and gibbsite) under similar pH and ionic strength conditions (Table 1) [21]. The adsorption capacity decreased in the order of goethite > gibbsite > kaolinite > allophane > silica > montmorillonite. In general, the adsorption of solutes on solid surfaces is dependent on many factors, such as surface areas and surface charge conditions of the solids. DNA adsorption is not likely to depend on the N_2-BET surface areas of the minerals because the surface areas (>300 $m^2\ g^{-1}$) of the allophane samples [21] are much greater than those of goethite (131 $m^2\ g^{-1}$) and gibbsite (5.0 $m^2\ g^{-1}$) [26].

3.8. An analytical problem in measurement of DNA for the adsorption experiment

In most of the studies reporting DNA adsorption by minerals, the amount of DNA adsorbed was estimated by spectrophotometric determination (i.e., absorbance at 260 nm) of unbound DNA in the supernatant after centrifugation. However, it is clarified that Fe^{3+}-containing clays such as montmorillonite do not completely sediment, even after centrifugation at 40,000 × *g*, and that they absorb light around the 260-nm wavelength [35], indicating that the amount of DNA adsorbed on clays might have been underestimated in several previously reported studies.

4. Humic substances

Humic substances, which are abundant in soils, especially in andosols, should have an impact on DNA adsorption and stability [5]. Much (70–80%) of the DNA adsorbed in the presence of Na^+ by purified humic acid (HA) from andosol in Italy was not desorbed by 0.1 M NaCl or DNA buffer (10 mM Tris, 0.1 mM EDTA, and 4 mM NaCl (pH 7.5)), suggesting the stable binding of DNA [4]. DNA adsorption increased with increased HA at constant DNA concentration

(50 μg DNA in 1 mL DNA buffer), reaching a plateau at 2 and 3 mg HA in pH 3 and 4, respectively. At a constant HA concentration (2 mg HA in 1 mL DNA buffer), DNA adsorption increased with DNA concentrations up to 300 μg [4]. The adsorption mechanism has not yet been elucidated in detail. Pietramellara et al. [36], without unambiguous evidence, suggested that at low pH, DNA may be adsorbed on humic substances by a hydrophobic bond. DNA molecules interact with the surface of negatively charged soil particles via bridging of inorganic cations (Fig. 2(c)) [37]. The mechanisms involved in DNA adsorption on humic substances are poorly understood; therefore, they require further clarification [36].

The influence of soil organic matter on DNA adsorption on andosols was investigated using various types of andosol samples, including a hydrogen peroxide (H_2O_2)-treated soil, a heated (400 °C) soil, and a slurry-added soil [18]. DNA adsorption by the slurry-added soil was remarkably lower than that of the original soil (without slurry). The increase in soil organic matter owing to the addition of slurry to the soil resulted in an obvious negative influence on DNA adsorption. The decrease in organic matter content of several andosols by H_2O_2 treatment slightly raised DNA adsorption per unit weight on soil particles. The DNA adsorption maximum estimated from a simple Langmuir equation was greatly raised in organic matter-free andosol samples after H_2O_2 treatment and heat (400 °C) treatment, as compared with that in untreated soil, although the surface area was greatly decreased by both treatments. Statistical analysis of the data showed no correlation between total carbon content and estimated DNA adsorption maximum of all of the soil samples used in the study. These results suggested that soil organic matter contributes little to DNA adsorption on the andosols.

Table 1 Comparison of DNA adsorptions by various minerals [21]

	DNA adsorption			pH	Ionic strength
	average (%)	SD			(mol L^{-1})
Montmorillonite K-10	6.1	2.2	n=3	6.9	0.16-0.18
Kaolinite	36.7	4.1	n=3	6.8	0.16-0.17
Silica	16.6	2.1	n=4	6.7-7.1	0.16-0.17
Goethite	86.1	1.9	n=4	6.9	0.17-0.18
Gibbsite	54.0	3.1	n=3	6.9	0.17-0.18
Synthetic allophane	30.5	1.4	n=3	6.8	0.14-0.18
Natural allophane	49.5	2.9	n=3	6.8-7.0	0.17-0.18

[Condition] solid volume: 10 mg, solution volume: 1.0 mL; initial DNA concentration: 100 μg mL^{-1}; background : 0.1 M NaCl; reaction time: 2 hours.

5. Organo-mineral complexes

Synthesized or purified clays were used in most previous studies for DNA adsorption. However, most of the clay surface is expected to be covered with humic substances in soils. It is therefore important to understand the DNA adsorption on organo-mineral complexes in relation to the mechanism of DNA stability in the actual soils. Few studies have dealt with organo-mineral complexes for DNA adsorption compared with purified clays. Nguyen and Elimelech [38] examined DNA adsorption on natural organic matter (NOM)-coated silica in the presence of Na^+. Linear DNA adsorbed more on the coated silica than did supercoiled DNA at low Na^+ concentration (i.e., low ionic strength), whereas both forms of DNA significantly adsorbed on it at comparable rates at high Na^+ concentration (i.e., high ionic strength), with linear DNA having greater thickness. The greater adsorption of the linear DNA at low ionic strength on the NOM-coated silica was attributed to effective binding of the ends of the linear molecule, which forms an extended conformation due to intramolecular electrostatic repulsion [38]. For both forms of DNA, adsorption in the presence of Ca^{2+} was greater than that observed for Mg^{2+} [39]. Unlike the other alkaline earth metal ions, Ca^{2+} can form inner-sphere complexes with both the phosphate groups of DNA and the carboxyl groups of NOM. Thus, Ca^{2+} can form bridges between DNA and NOM [39], whereas Mg^{2+} can only neutralize the charges of DNA and NOM.

DNA adsorption by complexes of Al or Fe hydroxypolymers–montmorillonite–humic acids (extracted from Italian andosol) (Al-M-HA or Fe-M-HA) depended on both the composition and the preparation of the complexes, suggesting several mechanisms of adsorption [13]. The amount of adsorbed DNA decreased with increases in organic C content in

the Al-M-HA and Fe-M-HA. For both complexes, the DNA adsorbed tended to increase as pH decreased. It was also found that only 1% of DNA adsorbed on Fe-M-HA and Al-M-HA was desorbed by washing with distilled water, 0.1 M NaCl, or 0.1 M $Na_4P_2O_7$ [13].

6. Adsorption of DNA molecules by soils

DNA adsorption on an andosol was much larger than those on a fluvisol and an acrisol [17]. Adsorption of DNA molecules by soils is dependent on the content and species of clay minerals in soils, soil solution composition, and soil organic matter content [40]. Clay content is especially likely to greatly affect the DNA adsorption capacity of soils [37]. Our study reported only on the effects of H_2O_2 treatment and oxalate treatment of the soil samples on DNA adsorption [17, 18] to understand contributions of various soil components, and it indicated that oxide minerals in the soils are among the most important adsorbents for DNA molecules.

6.1. pH effect

DNA adsorption by 2 andosols was investigated as a function of solution pH so as to understand the behaviour of extracellular DNA molecules in andosols [41]. DNA adsorption greatly decreased as the suspension pH increased in the range of pH 3–9. The mechanism of DNA adsorption by soil depends on solution pH as well as clay minerals [37]. The surface functional groups of soil particles are protonated or the surface negative charges are decreased as pH decreases. Concomitantly, the negative charges, perhaps of the phosphate functional groups, on the DNA molecules also lowered as pH became <5 since the isoelectric point of DNA is around pH 5 [5]. In other words, negatively charged DNA molecules at pH >5 associate with the surface of negatively charged soil particles via a bridging of inorganic cations (Fig 2(c)), whereas at pH <5, the positively charged phosphate groups at the end of the DNA molecule bind directly to the OH groups of the Al- or Fe-oxides (Fig. 2(a) and 2(b)).

6.2. Effects of cations and ionic strength

We investigated the effect of ionic strength (NaCl solution) in bulk solutions on DNA adsorption by andosols [41]. It was found that the amounts of DNA adsorbed by the andosols did not fluctuate remarkably in the range of ionic strength (0.02–0.9 mol L^{-1}) of NaCl solution as a background salt. Lorenz and Wackernagel [32] found, however, that raising the ionic strength (NaCl concentration, from 0.1 to 4 mol L^{-1}) in the solutions increased DNA retention on a sand. In pH >5, coexistence of inorganic cations such as Na^+ and Mg^{2+} enhance DNA adsorption by soil particles. The effect of divalent cations on DNA adsorption by soil is stronger than that of monovalent cations. At least 70–100×higher concentrations of monovalent cations than divalent cations were required to bind the same amount of plasmid DNA to sand [42]. The contribution of divalent cations to DNA retention by soil was evident because the effect occurred even when their concentrations were low in the soil solutions [42]. Because the DNA adsorption varied with both solution pH and with coexistence of salts, attention should be paid to experimental conditions such as buffer components when comparing these data with previous data on DNA retention by soils.

6.3. Effects of moisture and pores

The observation that shorter fragments of DNA were adsorbed to a greater extent than longer fragments by most soils may be explained by the fact that larger fragments are excluded from some pores in the soil particles [43]. Additionally, it was reported that adsorption of long fragments was greater than that of shorter fragments on the relatively nonporous surface of sand [43, 44]. It was summarized by Nielsen et al. [45] that the presence of DNA molecules inside soil microaggregates or micropores, which are inaccessible to microorganisms or extracellular nucleases, could lead to the long-term physical stability of DNA. In general, it has been thought that released extracellular DNA can diffuse only in a very limited distance. However, Ceccherini et al. [46] observed that DNA molecules adhering loosely to soil particles could be desorbed and transferred via water vertical moving by capillarity. Extracellular DNA molecules might move *in situ* more than expected.

6.4. DNA adsorption by clay fractions extracted from soils

Cai et al. [34] investigated the adsorption of salmon sperm DNA by various types of clay fractions extracted from alfisol and ultisol in China. In comparison with those by the phyllosilicates, adsorption capacity decreased in the order of montmorillonite > inorganic fine clay fraction > organic fine clay fraction > kaolinite > silica > inorganic coarse clay fraction > organic coarse clay fraction, suggesting that the high capacities of fine clay fractions attributed to the large contents of 2:1 type phyllosilicates in the fraction [47]. Cai et al. [48] also found that DNA adsorption by goethite, phyllosilicates, and clay fractions were decreased by the limited presence of citrate and tartrate possibly released from the plant root. However, DNA adsorption by the phyllosilicates (montmorillonite and kaolinite) increased with organic

acid concentration (>5 mM), implying an important role of montmorillonite and kaolinite in DNA behaviour within the rhizosphere.

DNA adsorption by organic matter-eliminated soil fractions was similar to that by the original soils, indicating that soil organic matters may not contribute significantly to the DNA adsorption [18]. The DNA adsorption capacities of oxide mineral-free fractions of the organic matter-eliminated soil were much lower than that of the original soils [17]. The contribution of allophane and oxide minerals to DNA adsorption would be great among various andosol constituents.

7. Conclusion

It is difficult to distinguish adsorption of DNA molecules from precipitation on soil particle surfaces. In previous studies [17, 18], the isotherms of DNA adsorption by andosols were categorized into *L*- or *H*-types, which meant that the adsorbed DNA molecules were likely to form mono-molecular layers on the particle surfaces rather than precipitate on the surfaces.

In order to understand the mechanism of DNA adsorption by andosols, it is essential to know which components can adsorb DNA. Saeki et al. [17] showed that the oxide minerals are the most important adsorbents of DNA molecules in soils. We have shown that the adsorption of DNA molecules on allophanes was relatively higher than that on montmorillonite and silica, but it was relatively lower than that on gibbsite or goethite [21]. Therefore, DNA adsorption is likely to be dependent on the qualitative properties of the soil particle surfaces rather than simply on the N_2-BET-specific surface areas, because the surface areas (>300 $m^2\ g^{-1}$) of allophane samples are much greater than those of goethite (131 $m^2\ g^{-1}$) and gibbsite (5.0 $m^2\ g^{-1}$) [26, 49].

We suggest 3 mechanisms for DNA molecular adsorption by soil particles:

(i) phosphate groups on the edge of the DNA molecule's backbone directly bind by way of a ligand exchange reaction to the OH groups of Al or Fe oxide minerals in soils (Fig. 2(a) and 2(b)), as already suggested for the binding of DNA molecules to the OH groups on the edge of phyllosilicates such as montmorillonite [2, 32, 36, 50, 51]. The adsorption of DNA by allophane, which is the primary clay mineral of andosols, declined through increases in suspension pH values [21], probably because the AlOH groups of the allophane particles were deprotonated to AlO^- and the negative charges of phosphate functional groups of DNA molecules were concomitantly increased. Inorganic anions such as phosphate, selenite, and arsenate can be adsorbed by oxide minerals and soil particles by ligand exchange reactions [26-28, 51, 52]. We cannot exclude that the ligand exchange reactions occurred between the phosphate groups of the DNA molecule and the OH groups of the Al oxide layer of the allophane particles in andosols. Hashizume and Theng [24] proposed that 5′-AMP molecules are adsorbed through ligand exchange reactions on the surface of allophane particles and that this may occur for the phosphate groups on the DNA molecules.

(ii) DNA molecules interact with the surface of negatively charged soil particles via bridging of inorganic cations (Fig. 2(C)). We have found that a small number of DNA molecules can be adsorbed by soils around pH 8.0 and that not every DNA adsorption was necessarily interfered with by the addition of a high concentration of phosphate [41]. A small number of DNA molecules can be adsorbed by silica, on which few inorganic anions adsorb due to strong negative charges [21]. These observations are hardly explained only by the binding reaction between phosphate groups at the ends of DNA molecules and OH groups on surfaces of minerals. As already shown by Khanna and Stotzky [2] and Paget et al. [50], certain DNA retention can occur on negatively charged phyllosilicates through the formation of bridges by cations. Ca^{2+} binds to the SiOH groups of minerals such as silica and depresses the negative charges on the mineral surfaces, resulting in increased DNA adsorption [23]. Moreover, charge neutralization upon Ca^{2+} binding to DNA phosphate groups increases adsorption [23]. This mechanism may be dominant in DNA adsorption in the high pH range rather than the direct bindings. This adsorption may be enhanced by increasing cation concentrations (ionic strength) of the solutions, as shown by Lorenz and Wackernagel [32]. However, in our study, the adsorption of DNA by andosols did not change by increasing ionic strength until 0.5 mol L^{-1} [41]. The effect of Mg^{2+} on this mechanism is much greater than Na^+ [36]. These results imply that the DNA adsorption capacity of soils depends on both the quality and the quantity of exchangeable cations on soil particles, soil solution cations as well as the contents of the clay and oxide minerals in the soil.

(iii) DNA molecules may be bound directly to soil organic matter, but we have shown that this adsorption was not important because it increased upon removal of the organic matter from the soil and decreased upon addition of organic materials to the soil [17, 18].

It is unclear which andosol components adsorb DNA molecules. We consider that DNA molecules are predominantly adsorbed by various inorganic minerals but are not fixed by humic substances in soils. However, it has been concluded from both other studies and our results that humic substances have a non-neglectable role in fixation of DNA in high pH range in soil. As the substances are negatively charged in neutral and alkaline conditions, the main DNA adsorption mechanism by humic substances is binding on the particle surfaces via bridging of inorganic cations (Fig. 2(c)).

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