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Interactive adsorption of phenolic acids and amino acids on soil minerals

Implications for the formation and properties of soil mineral–organic associations

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Chapter 5

Organic matter coatings of soil minerals affect adsorptive interactions with phenolic and amino acids

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Abstract

Phenolic acids (PAs) and amino acids (AAs) potentially contribute to the formation of mineral-organic associations (MOAs) resulting in the stabilization of organic matter (OM) in soils. However, little is known about how natural OM coating affects the subsequent adsorption of these compounds and their mutual interaction upon adsorption. Therefore, batch adsorption experiments were conducted to study how the coating of mineral surfaces with dissolved OM (DOM; derived from forest leaf litter (L-DOM) and O horizon (O-DOM)) affects adsorptive interactions of PAs (salicylic, syringic, ferulic and vanillic acid) and AAs (lysine, Lys; glutamic acid, Glu; leucine; phenylalanine) on goethite, kaolinite, and montmorillonite at pH 6. Adsorption of DOM depended on the mineral (goethite >> kaolinite > montmorillonite) and DOM sources (O-DOM > L-DOM for goethite). Coating of all minerals with both DOM sources reduced the adsorption of PAs and acidic AA (Glu), but enhanced the adsorption of basic AA (Lys). The effect of organic coatings was strongly dependent on the amount of adsorbed OM. The strong bonds between AAs and OM-coated minerals resulted in generally enhanced adsorption of PAs with surface conditioning by AAs, which possibly indirectly evidences a multilayer model of MOAs. However, adsorption of AAs was rarely influenced by surface conditioning with PAs. Competition between AAs and PAs generally suppressed the adsorption of AAs on coated minerals, whereas it had little influence on the adsorption of PAs. These results emphasize that the amount and composition of OM coatings affect the adsorption of PAs and AAs.

5.1 Introduction

Substantial efforts have been dedicated to understanding the formation of mineral-organic associations (MOAs) by adsorptive interaction between soil minerals and natural organic compounds (Schmidt *et al.*, 2011; Lehmann & Kleber, 2015). These interactions are decisive for the protection of organic matter in soils against microbial decay (Schneider *et al.*, 2010; Mikutta *et al.*, 2007). At new mineral surfaces and low C loading organic matter (OM) is very strongly adsorbed through multidentate bonding, while at larger C loading less functional groups of the organic molecules are involved (Kaiser & Guggenberger, 2007), thus rendering OM more susceptible to desorption and vulnerable to microbial decomposition.

Currently, there is no consensus on the processes and involved organic substances in the formation of MOAs. The classical view of a preferential bonding of lignin-derived components such as phenolic acids (PAs) to many soil minerals (Kaiser & Guggenberger, 2000; Kalbitz *et al.*, 2005; Kothawala *et al.*, 2012; Kramer *et al.*, 2012) is in contrast to the "onion" or "multilayer" model (Sollins *et al.*, 2006; Kleber *et al.*, 2007) assuming that amphiphilic, N-rich molecules (e.g. amino acids; AAs) build up an inner layer directly linked to the charged aluminosilicates surfaces and an outer layer of more loosely bound organic substances, probably including PAs. Thus, exploring competitive adsorption processes of AAs versus PAs could advance our current understanding which class of compounds might be more relevant in the formation of MOAs.

As degradation products of lignin and proteins, respectively, AAs and PAs are very active components in the cycling of N and C in soils. So far, the knowledge about the adsorption patterns of nitrogenous and aromatic components including AAs and PAs to soils or soil minerals has been obtained either in mono-component systems or by the changes in the composition of dissolved organic matter (DOM) following adsorption (Kaiser *et al.*, 1997; Kalbitz *et al.*, 2005; Chefetz *et al.*, 2011; Yeasmin *et al.*, 2014). However, consequences of interactions between AAs and PAs during and upon adsorption for the formation of MOAs were rarely studied, yet are crucial to assess the validity of conceptual adsorption models. It can be expected that these interactions between AAs and PAs might not only be affected by the nature of the mineral surface (e.g. variable charged oxides vs. uncharged and hydrophobic 1:1 clay minerals vs. permanently charged 2:1 clay minerals) but also by the coverage of these surfaces by adsorbed OM. Adsorption of DOM depends on its composition, with usually higher adsorption of DOM derived from decomposed OM than from

fresh litter (Schneider *et al.*, 2010), and modifies the surface properties of soil minerals, such as specific surface area (SSA), surface charge and hydrophilicity. Such changes in surface properties are important for DOM cycling as they govern the remaining adsorption and stabilization capacity of the mineral phase (Sanderman *et al.*, 2008; Kaiser & Kalbitz, 2012). However, effects of these changes in surface properties on the adsorption of AAs and PAs are not well understood, besides the above mentioned decrease in chemical bonds of organic compounds on mineral surfaces with increasing C loading.

Recently we have shown that a preceding adsorption of both AAs and PAs on soil minerals enhanced the adsorption of the other group of compounds (Gao *et al.*, 2017). In other studies, the retention of non-polar organic compounds was greatly enhanced on minerals after coating with OM (Murphy *et al.*, 1990; Wang *et al.*, 2008; Polubesova *et al.*, 2009). However, whether that holds true for polar compounds such as PAs and AAs is unknown.

Therefore, the main objective of our study was to clarify how the coating of mineral surfaces with DOM affects adsorptive interactions between AAs and PAs. Goethite (α -FeOOH), kaolinite, and montmorillonite were chosen as test minerals, representing the most important minerals in many soils (Fe oxides, 1:1 and 2:1 clay minerals) and exhibiting different surface properties (goethite: variable charge, kaolinite: little isomorphous substitution and wide hydrophobic region, montmorillonite: rich isomorphous substitution and permanent negative charge; Sposito, 2008). We studied (i) the adsorption behavior of different AAs (glutamic acid, leucine, lysine and phenylalanine; representing acidic, neutral, basic and aromatic AAs) and PAs (salicylic, syringic, ferulic and vanillic acid; representing lignin degradation products) to these minerals coated with DOM; (ii) changes in the adsorption behavior of PAs on DOM-coated minerals after surface conditioning by AAs; (iii) changes in the adsorption behavior of AAs on DOM-coated minerals after surface conditioning by PAs, and (iv) competition effects between PAs and AAs on their adsorption on DOM-coated minerals. Herein, “coating” and its derivatives refer to the adsorption of DOM, “conditioning” and its derivatives refer to the adsorption of PAs and AAs.

5.2 Material and methods

5.2.1 Minerals

Goethite was synthesized according to the method of Atkinson *et al.* (1967) by

alkaline hydrolysis of 0.5 M FeCl₃ and freeze-dried at the end. Montmorillonite was obtained by separating the < 2- μ m fraction via sedimentation from Morocco bentonite (Ddani *et al.*, 2005). Exchange sites were saturated with Ca²⁺ by suspending the clay twice in 1 M CaCl₂ (1:10; w:v). Kaolinite (KGa-2) was purchased from the Clay Mineral Society (Warren County, Georgia, USA). A suspension of kaolinite in deionised H₂O (1:15; w:v) was sonicated for 8 minutes (120 W) to break the aggregates. Afterwards, exchange sites were also saturated with Ca²⁺ by suspending the clay thrice in 1 M CaCl₂ (1:5; w:v). Finally the montmorillonite and kaolinite were washed until salt-free, freeze-dried, and passed through a 200- μ m sieve. The X-ray diffraction patterns (Siemens, D500, Cu-K α radiation, Karlsruhe, Germany) indicated the presence of well-crystalline goethite and a high purity of the < 2- μ m fraction of the Morocco bentonite and kaolinite (Figures B1 and C1, Appendix). The specific surface area (SSA) and total pore volume (TPV) of minerals were derived from duplicate N₂ gas adsorption isotherms recorded with a Quantachrome Autosorb1-MP analyzer (Quantachrome, Boynton Beach, FL, USA). For this purpose, 20–200 mg of each sample was weighed into the sample cell and degassed at 40 °C until the pressure was < 0.12 Pa. The SSA was calculated from the linear Brunauer–Emmett–Teller (BET) plot (Brunauer *et al.*, 1938) by using up to 10 adsorption points. The TPV was taken at P/P₀ ca. 0.995, where P and P₀ are the equilibrium and the saturation pressure, respectively of N₂. The results and other properties are listed in Table 5.1.

5.2.2 Amino acids and phenolic acids

L-leucine (Leu), L-glutamic acid (Glu), L-lysine (Lys), DL-phenylalanine (Phe), DL-norvaline (Nva), salicylic acid (Sal), syringic acid (Syr), ferulic acid (Fer), vanillic acid (Van) and ethylvanillin (Eva) were purchased from Sigma-Aldrich (Zwijndrecht, The Netherlands). The purity was > 98%, except for Syr (\geq 95%) and Van (\geq 97%). The chemical structure and properties of these compounds are listed in Table 4.1 (**chapter 4**). Nva and Eva were used as the internal standards (ISs) for the measurement of AAs and PAs, respectively.

5.2.3 Preparation of PA and AA solutions

Stock standard solutions of each AA (2000 mg l⁻¹) were prepared in 0.1 M HCl while those of PAs were prepared in water at concentration of 1000 mg l⁻¹ for

Sal, Syr and Van and 500 mg l^{-1} for Fer, respectively. A lower concentration was used for Fer due to its low solubility in water (Table 4.1, **chapter 4**). The stock solutions were stored at $4 \text{ }^{\circ}\text{C}$ for not longer than one month. Working mixture solutions with equal concentration for individual PAs and AAs were freshly prepared by mixing and diluting the stock standard solutions.

5.2.4 Dissolved organic matter

Dissolved OM was extracted from litter (Oi horizon) and the Oe+Oa horizon of a beech forest (Grinderwald, Germany; Leinemann *et al.*, 2016) to represent DOM from the two most important sources percolating into the mineral soil. The leaf litter from Oi horizon was dried and ground into small pieces. The field moist samples from Oe+Oa horizon was sieved to $< 4.8 \text{ mm}$ and stored at $4 \text{ }^{\circ}\text{C}$. Suspensions of leaf litter in deionized H_2O (1:10; w/v) or of Oe+Oa horizon sample (1:5; w:v) were stirred with a glass rod first and left at $20 \text{ }^{\circ}\text{C}$ for 16 hours. The supernatant of the suspension was centrifuged (3500 g , 20 minutes) and filtered under vacuum through $0.45\text{-}\mu\text{m}$ mixed cellulose ester membrane (ME25, GE Healthcare Life Sciences, Whatman, Little Chalfont, UK). The DOM stock solution was stored at $4 \text{ }^{\circ}\text{C}$ before use. The dissolved organic carbon (DOC) concentration and pH of the extracts derived from leaf litter (L-DOM) and Oe+Oa horizon (O-DOM) were 1107 and 138 mg l^{-1} , 5.1 and 4.4 , respectively. More properties of the DOM were measured and showed in another study (Kalbitz *et al.*, 2003).

5.2.5 Preparation of OM-coated minerals

In order to verify a similar coating efficiency for all minerals, adsorption isotherms for the two DOM types (L-DOM and O-DOM) and three minerals were first constructed in a batch adsorption experiment in duplicate at pH 6 (Schneider *et al.*, 2010). To inhibit microbial activity and assure a constant electrolyte background, NaN_3 was added to the solutions with a final concentration of 10 mM and the pH was adjusted to 6 before mixing with minerals by dropwise addition of 0.1 M NaOH or HCl . Based on the adsorption isotherms, we selected the solid-to-solution conditions under which 50% of the maximum DOM adsorption capacity of the minerals was achieved. Fifty percent coverage was selected to ensure that the unsaturated minerals had sufficient remaining adsorption sites to interact with PAs and AAs. Briefly, goethite (20–

Table 5.1 Cation exchange capacity (CEC), specific surface area (SSA), total pore volume (TPV), average pore radius (APR), C content, OC loading, and ζ potential of pure and OM-coated minerals^a.

Mineral	CEC /mmol _c kg ⁻¹	SSA /m ² g ⁻¹	TPV /mm ³ g ⁻¹	APR /nm	C content /mg g ⁻¹	OC loading ^b /%	ζ potential measurement		
							ζ potential /mV	EC ^c /μS cm ⁻¹	pH
G ^d	13 ^m	69.7 (1.1)	730 (3)	21.0 (0.2)	1.4 (0.1)		40.4 (1.7)	4.0 (0.0)	6.2 (0.1)
L-G ^e	-	63.0 (2.4)	647 (36)	20.5 (0.4)	9.1 (0.0)	54	-6.8 (0.5)	5.3 (0.6)	6.0 (0.1)
O-G ^f	-	69.3 (3.5)	702 (66)	20.2 (0.9)	17.9 (0.0)	53	-27.5 (2.1)	7.3 (0.6)	6.3 (0.1)
K ^g	37 ⁿ	25.0 (0.9)	315 (51)	25.2 (3.2)	0.5 (0.0)		-23.2 (1.3)	4.0 (0.0)	6.0 (0.1)
L-K ^h	-	23.4 (1.1)	379 (6)	32.4 (1.0)	1.5 (0.0)	59	-37.9 (1.8)	4.0 (0.0)	6.1 (0.0)
O-K ⁱ	-	23.6 (0.5)	403 (84)	34.1 (6.4)	1.6 (0.0)	54	-42.7 (0.3)	4.0 (0.0)	6.0 (0.1)
M ^l	888 ^o	94.7 (6.0)	187 (6)	4.4 (1.0)	1.1 (0.0)		-29.5 (3.5)	5.0 (0.0)	6.3 (0.1)
L-M ^k	-	51.8 (0.2)	112 (4)	4.3 (0.2)	2.0 (0.0)	59	-49.3 (1.6)	6.0 (0.0)	6.2 (0.1)
O-M ^l	-	48.6 (0.1)	110 (2)	4.5 (0.1)	2.2 (0.0)	74	-54.1 (2.8)	5.0 (0.0)	6.2 (0.1)

^a the results are presented as mean values with standard deviation except for the OC loading. All the measurement had three replicates except for the C content, which had two replicates; ^b proportion of the maximum adsorption capacity for the respective DOM; ^c electrical conductivity; ^d goethite; ^e goethite coated with L-DOM; ^f goethite coated with O-DOM; ^g kaolinite; ^h kaolinite coated with L-DOM; ⁱ kaolinite coated with O-DOM; ^j montmorillonite; ^k montmorillonite coated with L-DOM; ^l montmorillonite coated with O-DOM; ^m calculated from the negative surface charge density at pH 6 in NaCl background electrolyte Walsch & Dultz (2010); ⁿ Borden & Giese (2001); ^o Daou et al. (2015).

700 mg), kaolinite (50–1200 mg), or montmorillonite (40–1500 mg) were suspended in 40 ml L-DOM or O-DOM solution adjusted to pH 6 (ca. 120 mg OC l⁻¹ for goethite and ca. 50 mg OC l⁻¹ for kaolinite and montmorillonite) and shaken horizontally (95 rpm) for 16 h in the dark at 4 °C to achieve equilibrium, then centrifuged (3500 g, 20 min), and filtrated through 0.45-µm mixed cellulose ester membranes. The UV absorbance of the filtrates at 280 nm was measured using a Spectroquant Pharo 300 spectrometer (Merck KGaA, darmstadt, Germany). The pH of the filtrate was measured using a multi-channel analyser (CONSORT C831, Abcoude, The Netherlands). The DOC concentrations of the filtrates were measured by a TOC analyser (TOC-V CPH Shimadzu, Kyoto, Japan). Adsorbed OC was calculated as the difference between the TOC in the initial and equilibrium solutions.

The OM-coated minerals (L-DOM-coated goethite, L-goethite; L-DOM-coated kaolinite, L-kaolinite; L-DOM-coated montmorillonite, L-montmorillonite; O-DOM-coated goethite, O-goethite; O-DOM-coated kaolinite, O-kaolinite; O-DOM-coated montmorillonite, O-montmorillonite) were freeze-dried, ground with agate mortar and pestle, sieved to < 200 µm. The C contents (Table 5.1) were measured by a CN analyser (Elementar Vario EL, Hanau, Germany). The C loadings of the three minerals were between 45–67%, which is close to the desired 50% of the maximum adsorption capacity.

5.2.6 Adsorption experiments with PAs and AAs

Adsorption of PAs (mixed solution of Sal, Syr, Fer and Van) and AAs (mixed solution of Leu, Glu, Lys and Phe) were conducted with pure goethite, kaolinite, and montmorillonite and minerals coated with L-DOM and O-DOM. Experiments were conducted in triplicate and at three different initial concentrations of the individual adsorbates: 0.01 (small), 0.05 (intermediate), and 0.1 mM (large). The solutions were added to each OM-coated mineral separately (control experiment), together (competition experiment), and after pre-conditioning of the other mix (conditioning experiment). For the latter, two conditioning experiments were conducted: PAs adsorption on OM-coated minerals after adsorption of AAs (conditioning experiment I) and AAs adsorption on the OM-coated minerals after adsorption of PAs (conditioning experiment II). The initial pH conditions were set at 6 for all the experiments and they rose to 6.2–6.7 after adsorption. Details of the adsorption procedure and results of PAs and AAs adsorption on pure goethite and montmorillonite are given in Gao *et al.*

(2017). Adsorption results of PAs and AAs on pure kaolinite are presented in the Appendix (Figures C2 and C3).

5.2.7 Measurement of PAs and AAs

The PAs in the filtrates were analysed directly by a Prominence high performance liquid chromatography (HPLC) system equipped with a diode array detector (Shimadzu, Kyoto, Japan). The details are given in Appendix (C) and Table C1. Prior to measurement, the AAs in the filtrates were diluted 10 or 20 times in organic solvent (90% acetonitrile, 10% H₂O, 8 mM ammonium formate, 0.12% formic acid, v/v%) and spiked with Nva (IS) at a final concentration of 0.1 mg l⁻¹. Hydrophilic interaction LC (Shimadzu, Kyoto, Japan) coupled with a 4000 QTRAP LC-MS/MS System (AB SCIEX, MA, USA) was used to analyse the AAs (Gao *et al.*, 2016; Gao *et al.*, 2017). High accuracies of the analytical methods of PAs and AAs were obtained by comparing the measured concentrations of the mixture with the calculated values (Table C2, Appendix). These results indicated the measurement of PAs and AAs were not affected by each other. The typical chromatograms of PAs and AAs were showed in Figures C4 and C5 (Appendix).

5.2.8 Desorption experiments and potential flocculation of PAs or AAs with desorbed OM

The adsorption reversibility of OM and the potential contribution of DOM-derived PAs and AAs was assessed by a desorption experiment using 10 mM NaN₃ (pH 6) as the same electrolyte background in the adsorption experiment. Suspensions of OM-coated minerals in 40 ml NaN₃ solution (200 mg goethite, 600 mg kaolinite or montmorillonite) were shaken horizontally (95 rpm) for 2 hours in the dark at 4 °C, then centrifuged (3500 g, 20 minutes) and filtered through 0.45-µm mixed cellulose ester membranes. The minerals were re-suspended by vortex before carrying out a second cycle. The DOC concentrations in the filtrates were analysed by a TOC analyser (TOC-V CPH Shimadzu, Kyoto, Japan). Desorption was generally small for kaolinite and goethite (Table C3, Appendix). Strong mechanical force was needed to grind the OM-coated montmorillonite, this may result in the relatively higher desorption of OM (22.2–26.7%). The PAs and AAs in the solutions of the first desorption cycle were generally undetectable except for little AAs for the L-

goethite (Table C4, Appendix). This indicates that the PAs and AAs measured in the adsorption experiments almost exclusively derive from the added compounds.

The filtrate after the first desorption cycle was further used to test potential flocculation of PAs or AAs by possible desorbed OM, as flocculation would result in decreasing concentrations of PAs and AAs in filtrates after adsorption and therefore in overestimation of their adsorption. Therefore, 5 ml desorption solution was spiked with mixture solution of PAs or AAs with final concentration of 1, 4, 10 and 20 mg l⁻¹ (the concentration was the same for individual compound in each mixture). The high recoveries of the spiked PAs and AAs (91–93% and 86–101%, respectively; Table C5, Appendix) implies the negligible effects of flocculation.

5.2.9 Potential exchange of PAs or AAs with adsorbed OM

The PAs and AAs solutions (intermediate initial concentration) in the control experiments were analysed for TOC concentration as well. The differences between the measured TOC concentration and the calculated results from HPLC analysis were similar to the desorbed OC that obtained from the desorption experiments (data not shown). In addition, the desorption results showed that the majority of the adsorbed OM was irreversible. Thus, the exchange of PAs or AAs with adsorbed OM was supposed to be negligible in this study.

5.2.10 Zeta potential measurement of OM-coated minerals

Zeta (ζ) potential measurements were carried out using phase-analysis light scattering (ZetaPALS, Brookhaven Instruments Corp., Holtsville, NY, USA). Mineral suspensions of 100 mg l⁻¹ were prepared in ultrapure water in 25-ml flasks without further pH adjustment. After gentle shaking, 1.6 ml was sampled with a pipette, transferred into a cuvette and measured at 20 °C. Each sample was measured five times and each of the five replicates consisted of 20 single measurements. The ζ potential was calculated from electrophoretic mobility using the Smoluchowski equation (Hunter, 1981). The electrical conductivity of the suspensions was determined simultaneously and used to reflect electrolyte concentration. The pH was determined in the cuvette using a miniaturized electrode (BlueLine 16pH, Si Analytics, Mainz, Germany).

5.2.11 Statistics

Statistical analysis was performed with SigmaPlot (version 13.0). Two way ANOVA was used to evaluate the influences of mineral types, OM coating, adsorbate concentration, competition and surface conditioning on the adsorption of PAs and AAs. Data were transformed if necessary to meet both the normality (Shapiro-Wilk) and homogeneity of variance (Brown-Forsythe) tests using appropriate methods. Fisher's least significant difference (LSD) was used for multiple comparisons of means. The significant level of difference was set at $P < 0.05$.

We introduced the relative adsorbed amount because this approach more clearly showed the effects of OM coating, competition and surface conditioning on the adsorption of PAs and AAs. The references referred to the adsorption results obtained from control experiment and the same reference mean values were used for each of the three replicates in calculating the relative values of adsorbed amounts.

5.3 Results

5.3.1 Adsorption of natural DOM on pure minerals

The adsorption of L-DOM and O-DOM on soil minerals followed the same order of goethite >> kaolinite > montmorillonite on both mass and surface area basis under nearly neutral conditions (Figure 5.1 and Table C6). Goethite retained more O-DOM than L-DOM, while a similar adsorption of both DOM solutions was observed for kaolinite and montmorillonite. The experiments with L-DOM showed a preferential adsorption of aromatic compounds to all minerals revealed by decreasing specific UV absorbance at 280 nm (Figure C6, Appendix). For O-DOM, such a preferential adsorption could only be observed to montmorillonite. The SSA of different minerals followed the order of montmorillonite > goethite > kaolinite, while a different order of goethite > kaolinite > montmorillonite was observed for the TPV (Table 5.1) and this was in line with the adsorption results. The SSA of montmorillonite was remarkably reduced by both types of DOM, while the effects on goethite and kaolinite were small (Table 5.1). In addition, the equilibrium pH after adsorption increased with the reduction of OC in solution (Figure C7, Appendix), which depended on the mineral and the type of DOM. The ζ potential of goethite was reversed to negative by adsorption of DOM with a stronger effect for O-DOM (Table 5.1).

Organic matter coatings led to more negative ζ potentials of kaolinite and montmorillonite with similar effect for L-DOM and O-DOM. The increase in negative surface charge upon DOM coating increased with the amount of C adsorbed (Figure C8, Appendix). These changes were largest for goethite and differed between the two types of DOM, as more O-DOM was adsorbed on goethite than L-DOM (Figure 5.1).

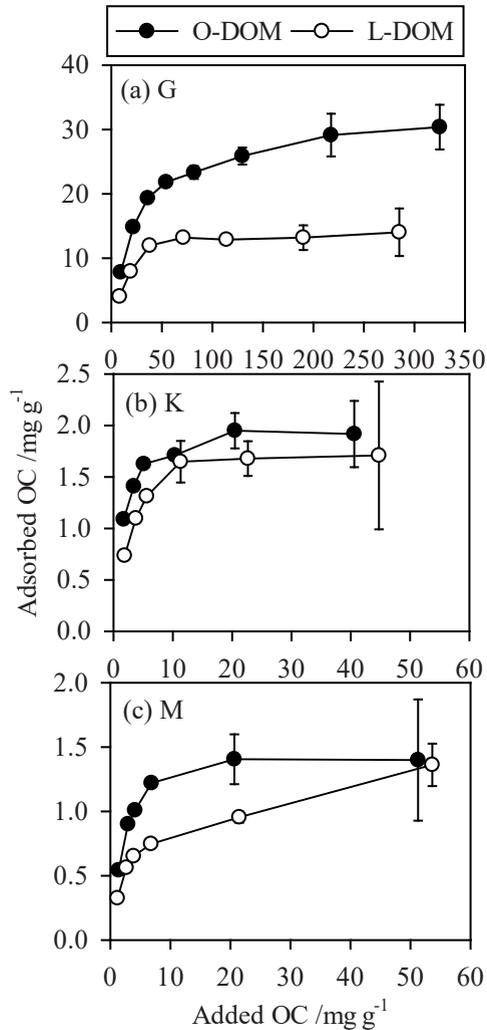


Figure 5.1 Adsorption isotherms of O-DOM and L-DOM on different minerals. The results are presented as mean values with standard deviation ($n = 2$). G, goethite; K, kaolinite; M, montmorillonite.

5.3.2 Control experiment – adsorption of PAs or AAs on OM-coated minerals

5.3.2.1 Phenolic acids

The adsorption of PAs on pure minerals followed the order: goethite >> kaolinite > montmorillonite under slightly acidic conditions (Figure C2a, Appendix) and was suppressed by the OM coating irrespective of the mineral and the type of DOM (Figure 5.2a and b). For both DOM types, the suppressing effect increased with increasing adsorption of DOM. The reduction of PAs adsorption on the OM-coated mineral as compared to the pure mineral was largest for goethite, which had the highest OM loading (Figure 5.2a and b). As a result of this effect, the final adsorption sequence of PAs on the O-DOM-coated minerals was in the following order: montmorillonite > kaolinite > goethite (Figure C2c, Appendix). That means the adsorption order of PAs was completely reversed by O-DOM coating compared to pure minerals. When differentiating between L-DOM and O-DOM, the O-DOM-coated minerals suppressed the adsorption of PAs to a larger extent, except for coated montmorillonite where both types of DOM had a similar effect. Coating of goethite with O-DOM was so large that it completely suppressed adsorption of all PAs. For the other minerals, Sal was the only adsorbed PA on their O-DOM coated forms, while adsorption of the other PAs (Syr, Fer and Van) was completely blocked by the O-DOM coating. Desorption of PAs from OM-coated minerals was larger than that from pure minerals (Gao *et al.*, 2017) and larger from minerals coated with L-DOM than O-DOM (Table C4, Appendix).

5.3.2.2 Amino acids

On pure minerals, adsorption of AAs followed the order: montmorillonite \geq kaolinite > goethite under slight acidic conditions (Figure C3, Appendix). Among the different AAs, goethite adsorbed only Glu, whereas montmorillonite adsorbed only Lys (Gao *et al.*, 2017). Both Glu and Lys were adsorbed on kaolinite with a dominating role for Lys (Figure C9, Appendix). No adsorption of Leu and Phe was observed on kaolinite either. The adsorption of AAs responded very differently to the OM coating compared to PAs. If effects occurred they were mostly positive, i.e. increasing adsorption of AAs by OM coating (Figure 5.2c and d). Adsorption of AAs on L-goethite and L-montmorillonite increased by 56–145% and 24–40%, respectively, for different initial concentrations. All the differences were significant ($P < 0.05$) except for goethite at the small initial concentration (Figure 5.2c). Specifically, Lys, Leu

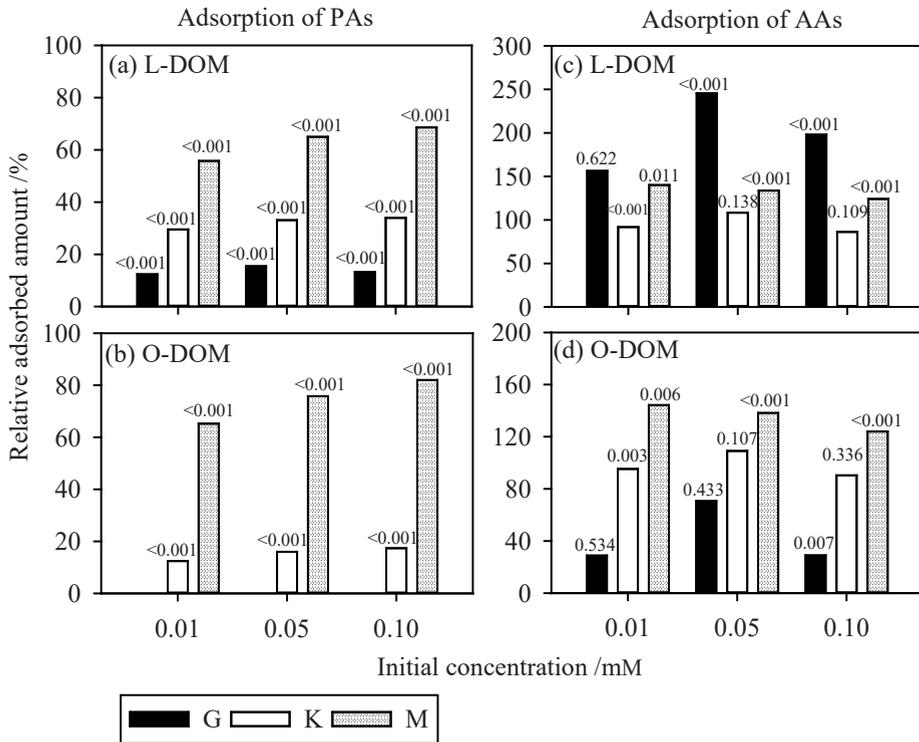


Figure 5.2 Relative adsorption of: (a) PAs on coated minerals with L-DOM, (b) PAs on coated minerals with O-DOM, (c) AAs on coated minerals with L-DOM and (d) AAs on coated minerals with O-DOM in the control experiment. Dotted lines refer to the reference values (absolute adsorption results on pure minerals). The results are presented as mean values ($n = 3$). P value above each column is from the analysis of variance of the absolute adsorption results and it indicates the significance of the difference compared with the corresponding reference. G, goethite; K, kaolinite; M, montmorillonite.

and Phe, which did not show adsorption on pure goethite, were adsorbed on L-goethite besides Glu (Figure C10a, Appendix). There were no significant differences between the adsorption of Glu on pure goethite and L-goethite (Figure C11a, Appendix). The adsorption of Lys on L-kaolinite significantly increased by 18–36% for different initial concentrations ($P < 0.05$; Figure C12a, Appendix), although the sum of various AAs did not change. Conversely, the adsorption of Glu on L-kaolinite significantly decreased by 74% or 60% for the small or intermediate initial concentration ($P < 0.05$) and it was entirely suppressed for the large initial concentration (Figure C11b, Appendix).

Coating of goethite with O-DOM did reduce the adsorption of AAs (Figure 5.2d) and changed the type of adsorbed AA, i.e. Glu replaced by Lys. Nevertheless, the adsorption of Lys on O-goethite ($0.10\text{--}0.96 \mu\text{mol g}^{-1}$) was much lower than that on O-kaolinite ($0.57\text{--}3.29 \mu\text{mol g}^{-1}$) and O-montmorillonite ($0.71\text{--}5.74 \mu\text{mol g}^{-1}$). Similar to goethite, coating of kaolinite with O-DOM resulted in entirely suppressed adsorption of Glu and increased adsorption of Lys (increase by 24–54%), thus resulting in no changes of total adsorption of AAs to O-kaolinite (Figure 5.2d). Moreover, the adsorption of Lys, the only adsorbed AA on both pure montmorillonite and O-montmorillonite, was significantly increased by 24–44% on O-montmorillonite ($P < 0.05$; Figure C12b, Appendix).

In contrast to PAs, the presence of OM coatings reduced desorption of AAs from minerals. Percentages of desorbed AAs from OM-coated minerals were even lower than those of PAs (Table C4, Appendix).

5.3.3 Competition experiment – adsorption of PAs and AAs on OM-coated minerals

5.3.3.1 Phenolic acids

Competition with AAs was minor and resulted mostly in a slightly decreased PAs adsorption to OM-coated minerals ($< 0.32 \mu\text{mol g}^{-1}$; Figure 5.3a and b). Sal was the only adsorbed PA. There was a tendency of increasing adsorption of PAs on O-kaolinite (significant for the intermediate and large initial concentrations) as compared to the situation without AAs (Figure 5.3b).

5.3.3.2 Amino acids

The presence of PAs resulted in significantly decreased adsorption of AAs on all L-DOM-coated minerals ($P < 0.05$; Figure 5.3c). Glu was the only adsorbed AA on L-goethite, and Lys on L-kaolinite and L-montmorillonite under competition with PAs. The strongest reduction in adsorption was for L-goethite, with a decrease by 66–76% ($0.40\text{--}2.24 \mu\text{mol g}^{-1}$; Figure 5.3c).

The adsorption of AAs on O-goethite was significantly increased by 130% ($0.95 \mu\text{mol g}^{-1}$) or 556% ($2.58 \mu\text{mol g}^{-1}$) for the intermediate or large initial concentration ($P < 0.05$; Figure 5.3d). Little change ($0.02 \mu\text{mol g}^{-1}$) was observed for the small initial concentration. Both Glu and Lys were adsorbed on

O-goethite and O-kaolinite, while only Lys was adsorbed on O-montmorillonite under competition with PAs. There were no consistent effects of competition on the adsorption of AAs on O-kaolinite. However, adsorption of Glu became detectable ($0.02\text{--}0.41\ \mu\text{mol g}^{-1}$) besides Lys. Competition resulted in reduced adsorption of AAs on O-montmorillonite by 9–30% ($0.21\text{--}0.54\ \mu\text{mol g}^{-1}$) for different initial concentrations ($P < 0.05$, Figure 5.3d).

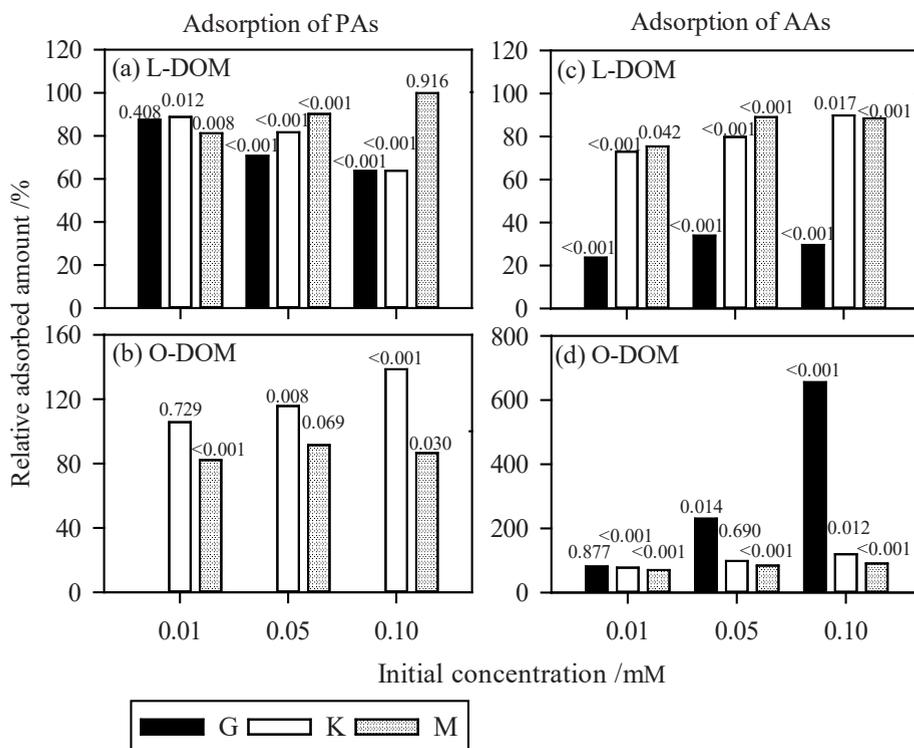


Figure 5.3 Effects of competition on the adsorption of: (a) PAs on coated minerals with L-DOM, (b) PAs on coated minerals with O-DOM, (c) AAs on coated minerals with L-DOM and (d) AAs on coated minerals with O-DOM. Dotted lines refer to the reference values (control experiments). The results are presented as mean values ($n = 3$). P value above each column is from the analysis of variance of the absolute adsorption results and it indicates the significance of the difference compared with the corresponding reference. The adsorption of PAs on O-goethite (b) is not displayed because the values of the reference are zero. G, goethite; K, kaolinite; M, montmorillonite.

5.3.4 Conditioning experiment I – adsorption of PAs on OM-coated minerals after adsorption of AAs

Preceding adsorption of AAs on OM-coated minerals resulted mostly in significantly increased adsorption of PAs (Figure 5.4a and b). The largest effect was observed for L-montmorillonite (increase by 25–86%) but little changes were observed for both L-goethite and L-kaolinite ($< 0.13 \mu\text{mol g}^{-1}$). Surface conditioning by AAs on O-goethite resulted in an increased adsorption of PAs from 0 to $0.20\text{--}1.22 \mu\text{mol g}^{-1}$ at different initial concentrations (Figure C13, Appendix), whereas for both O-montmorillonite and O-kaolinite PAs increased by 24–70% ($P < 0.05$). Sal was the only adsorbed PA on L-goethite, L-kaolinite, and O-kaolinite with or without surface conditioning of AAs. Despite little adsorption of Syr, Fer and Van was observed for L- and O-montmorillonite following surface conditioning, Sal remained the predominantly adsorbed PA.

5.3.5 Conditioning experiment II – adsorption of AAs on OM-coated minerals after adsorption of PAs

In general, preceding adsorption of PAs on OM-coated minerals did not result in significant changes in the adsorption of AAs (Figure 5.4c and d). The large relative increase of adsorbed AAs on O-goethite at the small initial concentration was very small in absolute numbers ($0.2 \mu\text{mol g}^{-1}$). Lys remained the predominantly adsorbed AA on OM-coated kaolinite and montmorillonite after adsorption of PAs.

5.4 Discussion

5.4.1 Adsorption of natural DOM on pure minerals

The observed stronger adsorption capacity of goethite for the two types of DOM relative to kaolinite and montmorillonite (Figure 5.1) was in agreement with the results of Mikutta *et al.* (2007) and Meier *et al.* (1999). They reported larger amounts of adsorbed DOM for goethite in comparison to 2:1 phyllosilicates (pyrophyllite and vermiculite) or 1:1 phyllosilicates (kaolinite). This is mainly driven by the positive charge of the goethite in nearly neutral conditions, while kaolinite and montmorillonite are overall negatively charged (Table 5.1). The more negative ζ potential upon DOM adsorption illustrates the predominant negative charge provided by both types of DOM at pH 6. For kaolinite and

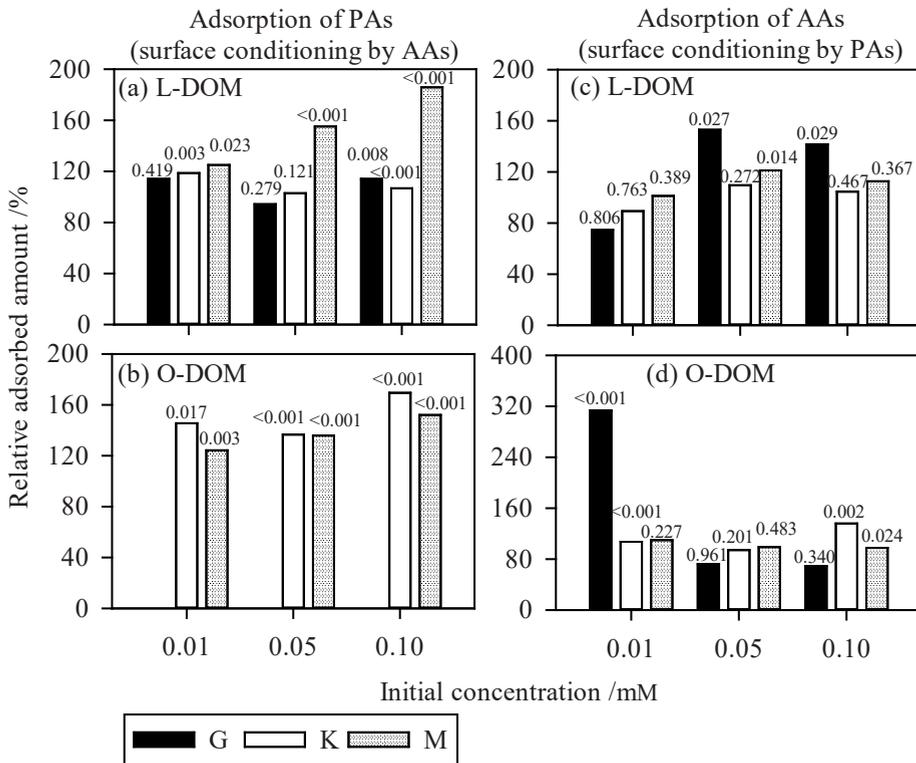


Figure 5.4 Effects of surface conditioning on the adsorption of: (a) PAs on coated minerals with L-DOM, (b) PAs on coated minerals with O-DOM, (c) AAs on coated minerals with L-DOM and (d) AAs on coated minerals with O-DOM. Dotted lines refer to the reference values (control experiments). The results are presented as mean values ($n = 3$). P value above each column is from the analysis of variance of the absolute adsorption results and it indicates the significance of the difference compared with the corresponding reference. The adsorption of PAs on O-goethite (b) is not displayed because the values of the reference are zero. G, goethite; K, kaolinite; M, montmorillonite.

montmorillonite less DOM can be bound due to the limited number of positively charged sites (protonated hydroxyls) existing on the edges of phyllosilicates in slightly acidic conditions (Tombácz *et al.*, 2004; Zhao *et al.*, 2008; Barré *et al.*, 2014). Furthermore, the positively charged functional groups (e.g. protonation of amino groups) of DOM allow binding to negatively charged sites of the minerals. However, these compounds are scarce in natural DOM solution (Kalbitz *et al.*, 2003). The relatively higher adsorption of DOM on kaolinite than on

montmorillonite (Figure 5.1) could be attributed to larger pore volume and size (Table 5.1). A positive relationship between the associated OC with Fe oxide and the average pore radius was proved by Mikutta *et al.* (2014). Another reason was possibly the massive uncharged and hydrophobic regions on kaolinite surface, which attract strongly hydrophobic moieties of DOM (Sposito, 2008). A consistent increase of equilibrium solution pH after DOM adsorption was observed for all combinations of DOM and minerals (Figure C7, Appendix), hinting to ligand exchange of DOM with hydroxyl groups of mineral surface as an important binding mechanism (Murphy *et al.*, 1990; Chorover & Amistadi, 2001; Polubesova *et al.*, 2008; Zhang *et al.*, 2012).

The SSA was a poor predictor of DOM adsorption to different minerals. Montmorillonite had the highest SSA, but the lowest degree of adsorption for both types of DOM, and kaolinite adsorbed more DOM than montmorillonite in spite of its smaller SSA (Figure 5.1 and Table 5.1). These observations possibly further highlighted the significance of pore and hydrophobic interactions for kaolinite. The literature does not give a consistent picture related to differences in adsorption of DOM on different phyllosilicates (Feng *et al.*, 2005; Saidy, *et al.*, 2013; Wang & Xing, 2005; Saidy *et al.*, 2013). The same increasing order of TPV and DOM adsorption for the different minerals (Table 5.1) highlights the potential role of TPV in predicting adsorption potential of DOM on minerals.

Higher adsorption of O-DOM relative to L-DOM was observed for goethite (Figure 5.1 and Table C6). Extracts from the forest floor litter (O-DOM) typically contain more aromatic compounds in comparison to leaf litter (Kalbitz *et al.*, 2003; Hansson *et al.*, 2010; Klotzbücher *et al.*, 2013), as also confirmed by the specific UV absorbance at 280 nm (Figure C6, Appendix). Many studies have shown the preferential adsorption of aromatic compounds to mineral surfaces (Kaiser & Guggenberger, 2000; Kalbitz *et al.*, 2005; Kothawala *et al.*, 2012). Dissolved OM from O horizon is expected to have higher negative charges than that from leaf litter due to oxidative changes during litter decomposition, as reflected by the more acidic water extract for the former (pH 4.4). This enrichment of negative charge promotes their binding on positively charged surfaces (e.g. goethite) and thus underpins the observed stronger decrease in the ζ potential after adsorption of O-DOM on goethite in comparison to L-DOM (Table 5.1). However, changes in the specific UV absorbance do not confirm a preferential adsorption of aromatic compounds for the adsorption cases of O-DOM on goethite and kaolinite (Figure C6, Appendix). Aliphatic fractions and nitrogenous components might adsorb preferentially as well according to Wang & Xing (2005) and Aufdenkampe *et al.* (2001).

5.4.2 Effects of OM coatings on the adsorption of PAs and AAs on minerals

The binding of OM reversed the surface charge of goethite from positive to negative and increased the negative charge of the phyllosilicates (Table 5.1). This is in agreement with findings from other studies (Davis, 1982; Ramos & McBride, 1996; Zhuang & Yu, 2002; Tombácz *et al.*, 2004). The development towards larger negative charges upon DOM adsorption can result in more homogeneous adsorption behaviour of the different soil minerals towards PAs and AAs (Davis, 1982; Sposito, 2008).

5.4.2.1 Phenolic acids

The increase in negative surface charge by OM coating is likely to be the most important reason for the observed reduced adsorption of the negatively charged PAs via enhancing the repulsion effects. This is supported by the fact that the strongest changes in surface charge coincided with the largest decrease in PAs adsorption (goethite) and that the O-DOM with a larger negative charge had a stronger effect than L-DOM with a smaller negative charge (Table 5.1 and Figure 5.2). The combination of both, a more negative charge of DOM (O-DOM) and a strong increase in surface negative charge for goethite deriving from coating by O-DOM, even resulted in a complete suppression of PAs adsorption (Figure 5.2b). This was unexpected as the OM loading of the mineral surface was just about 50% of the adsorption capacity (Table 5.1). On the other hand, when DOM molecular is adsorbed on mineral surfaces, the hydrophilic portions associate directly with mineral surface and the hydrophobic portions are exposed outwards (Kleber *et al.*, 2007). This would result in mineral surfaces with strong hydrophobicity thus preventing adsorption of polar organic compounds. These results suggest that the overall charge characteristics are more important for adsorption of PAs than the theoretical number of available adsorption sites. We can only speculate whether new mineral surfaces are still available after saturation of 50% of the adsorption capacity. Our results most likely indicate that PAs were still primarily attached to the adsorption sites of the mineral surface not yet occupied by DOM, but the bonds were weaker as indicated by a larger desorption in comparison to pure minerals (Gao *et al.*, 2017 and Table C4). The association of PAs with adsorbed OM might occur through polar- π interactions (Keiluweit & Kleber, 2009), but its contribution should be negligible. Therefore, metal (hydr)oxides play a dominant role in the adsorption of PAs rather than organic components (Huang *et al.*, 1977; Cecchi

et al., 2004). Regardless of the exact mechanism involved, our results clearly show that OM coatings have a distinctly different effect on the adsorption of PAs on mineral surfaces than for non-polar organic compounds of which the adsorption is proposed to be enhanced by such coatings (Murphy *et al.*, 1990; Wang *et al.*, 2008; Polubesova *et al.*, 2009).

5.4.2.2 Amino acids

The effects of OM coatings on the adsorption of AAs were found to mainly depend on the changes in surface charges of the minerals and the charge characteristics of the AAs. This explains why we found opposite effects on adsorption for the basic Lys and the acidic Glu. Increasing negative charge by the OM coating is most likely responsible for the increased adsorption of Lys on all minerals (Figure C12, Appendix) and decreased adsorption or no adsorption at all of Glu on L- and O-kaolinite and O-goethite (Figure C11, Appendix). The large negative charge of montmorillonite most likely caused the lack of any adsorption of Glu on this mineral independent of adsorbed OM. The small changes in adsorption of Glu on L-goethite might be related to low adsorption of this type of DOM (i.e., low surface coverage) having a lower negative charge than O-DOM (Table 5.1 and Figure 5.1). The slightly increased adsorption of neutral AAs (Leu and Phe) on L-goethite which were not adsorbed to pure and O-DOM-coated goethite illustrates the importance of surface charge which was closest to zero at the surface of the L-goethite (Table 5.1). Our results thus underline the importance of soil OM for the adsorption of AAs, especially for basic and neutral ones. Many studies also reported natural soil and sediments possessed much higher adsorption capacity for basic AAs (Lys) than acidic (Glu) and neutral ones, e.g. leucine, glycine and alanine (Henrichs & Sugai, 1993; Wang & Lee, 1993; Jones & Hodge, 1999; Liu & Lee, 2007). Mikutta *et al.* (2010) showed stronger association (higher abundance) of acidic AAs with metal-organic precipitates and variable-charge minerals obtained via separating bulk soil samples. Based on these results, we propose that soil OM and negatively charged minerals contribute to the immobilization of basic AAs, while the retention of acidic AAs is primarily controlled by soil minerals with positive charges, i.e. oxides and hydroxides. This rationale would underpin the varied dynamics and distribution pattern of PAs and AAs across soil profiles characterized by large differences in soil OM contents between top and sub soil horizons. The low desorbability of AAs bound to OM-coated mineral surfaces (Table C4, Appendix) possibly hints to the formation of stable inner sphere complexes particularly between Lys and adsorbed OM via interaction between

protonated amino groups of AAs and carboxyl or hydroxyl groups of adsorbed OM rather than electrostatic interaction and outer sphere complexes. This need to be verified by further study considering the relatively short period of time (2 hours) for the desorption process.

5.4.3 Competitive adsorption of PAs and AAs on OM-coated minerals

According to the results of Gao *et al.* (2017), PAs and AAs compete for adsorption sites on mineral surface with larger competitiveness of AAs compared to PAs resulting in smaller adsorption of PAs under competitive conditions (in slightly acidic condition). The authors explained the larger competitiveness of AAs than PAs (mainly Sal as the predominant adsorbed PA) by the different number of bonds between the organic acid and the mineral surface. Bidentate complexes between Sal and mineral surfaces require two adjacent adsorption sites (Yost *et al.*, 1990; Kubicki *et al.*, 1997), while the tested AAs probably need only one binding site at the mineral surface (Kitadai *et al.*, 2009; Yang *et al.*, 2016). Taking into account the less favourable conditions for adsorption of PAs on organically coated mineral surfaces (Figure 5.2a and b), the slightly decreased adsorption of PAs under competitive conditions (Figure 5.3a and b) seems to be reasonable. The enhanced adsorption of PAs on O-kaolinite in the presence of AAs contradicts the trend of decreased adsorption observed for PAs alone, and could be attributed to the surface conditioning by AAs, which promoted the adsorption of PAs (Figure 5.4b).

However, the hypothesized larger competitiveness of AAs than PAs on OM-coated minerals must be refuted based on our results. In contrast to pure minerals (Gao *et al.*, 2017), competition between PAs and AAs resulted in a reduced adsorption of AAs to mineral surfaces coated with OM, except for O-goethite and O-kaolinite (Figure 5.3). The reduction in adsorption was even larger for AAs than for PAs. The strongest decrease in the adsorption of AAs (i.e. Glu) under competition with PAs (i.e., Sal) was observed for L-goethite, where we found the strongest increase in adsorption of AAs induced by the OM coating in absence of PAs (Figure 5.2c). In turn, the largest increase in adsorption of AAs under competition with PAs coincided with high abundance of O-DOM coatings (O-goethite; Figure 5.2d). Therefore, we can conclude that coating the mineral surfaces with DOM changes the competition between different organic components for adsorption sites and the competitive strength of AAs disappears. It seems that the coating of the mineral surface is

quantitatively more important for adsorption than competition between single molecules.

5.4.4 Adsorption of PAs and AAs on OM-coated minerals with surface conditioning by AAs and PAs

Surface conditioning by AAs generally enhanced the adsorption of PAs on all OM-coated minerals, except partly (some concentrations) for L-goethite and L-kaolinite (Figure 5.4a and b). This is in accordance with the results for pure minerals (Gao *et al.*, 2017) and supports the conceptual model of Kleber *et al.* (2007) proposing that N-rich organic material bound on mineral surfaces may offer more reactive binding sites for other organic compounds (e.g. PAs). The strength of the effects seems to be related to the amount of adsorbed AAs, and is larger for phyllosilicates than for Fe oxides. Since PAs are negatively charged in slightly acidic condition, the amino groups of attached AAs could provide additional binding sites for PAs explaining an increased adsorption of PAs (mainly Sal) on O-goethite and O-kaolinite as well (Figure 5.4). Earlier we showed Lys was selectively adsorbed on OM-coated minerals, thus only basic AAs possibly could perform this bridging function.

Surface conditioning by PAs generally had little influence on the adsorption of AAs on OM-coated minerals (Figure 5.4c and d). Obviously, the largely reduced adsorption of PAs on OM-coated minerals in comparison to the pure ones prevented any effect of adsorbed PAs on the subsequent adsorption of AAs.

5.5 Conclusion

This study shows that the adsorption of natural DOM on soil minerals depended highly on both the charge characteristics of mineral surfaces and the composition of natural DOM. DOM adsorption was more strongly related to the total pore volume of soil minerals than to the SSA, to which the link was weak. Organic matter coating on mineral surfaces had remarkable influence on the surface charge properties and the adsorption of PAs and AAs. Coating of various mineral surfaces (goethite, kaolinite and montmorillonite) by natural DOM consistently increased their negative charges and consequently altered the adsorption of PAs and AAs, i.e. promoting adsorption of basic AAs and suppressing the PAs and acidic AAs under slightly acidic conditions. Binding sites on metal oxides not yet occupied by OM (positive charges) were

particularly important for adsorption of PAs and acidic AAs, while the retention of basic AAs was strongly dependent on negative charges of clay minerals and can be further increased by adsorption of DOM. The binding of basic AAs as Lys was particularly strong on OM-coated mineral surfaces. These stable bonds of adsorbed Lys on OM-coated mineral surface resulted in an enhanced adsorption of PAs thus providing indirect evidence for the multilayer model. The amount and composition of the OM coatings on mineral surfaces were quantitatively more important for adsorption of PAs and AAs than the competition between single molecules or effects of surface conditioning by the other class of compounds. Future studies should focus on direct evidence of the formation of a multilayer structure and the functions of specific compound classes using molecular spectroscopy (e.g. infrared or X-ray photoelectron spectroscopy) and modelling approaches.