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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 4

Competition and surface conditioning alter the adsorption of phenolic and amino acids on soil minerals

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Abstract

Adsorptive interactions of organic molecules with soil minerals often impair their bioavailability. However, little is known about the adsorption behaviour of phenolic and nitrogenous compounds on different minerals and their mutual interaction with respect to competition and surface conditioning, i.e. surface modification induced by preceding adsorption of the other class of compounds. Therefore, batch adsorption experiments were done to study the interaction between phenolic acids (PAs; salicylic acid, Sal; syringic acid, Syr; ferulic acid, Fer; vanillic acid, Van) and amino acids (AAs; lysine, Lys; glutamic acid, Glu, leucine, Leu; phenylalanine, Phe) during adsorption on goethite and Ca²⁺-montmorillonite at pH 6 by applying adsorbate concentrations of 0.01, 0.05 and 0.1 mM. Larger adsorption of PAs was observed on goethite than montmorillonite, whereas the phyllosilicate was a better adsorbent for AAs than the oxide. Among all tested PAs, Sal was preferentially adsorbed on both minerals. For the AAs, Glu was preferentially adsorbed on goethite and Lys on montmorillonite. The AAs were more competitive than PAs and partially suppressed the adsorption of PAs on both minerals. The adsorption of PAs or AAs on both minerals was enhanced by surface conditioning with the other group, with larger effects for goethite than montmorillonite. For goethite, surface conditioning by PAs enhanced the adsorption of AAs more (by 97–161%) than did AAs for PAs (9–48%). The results support the hypothesis that pre-adsorption of one class of organic compound can enhance the retention of another class. This suggests that adsorbed organic matter on soil mineral phases might be subject to a self-strengthening effect.

4.1 Introduction

Soil stores three to four times as much organic carbon (OC) as the atmosphere or plants (Lal, 2004), which represents an important role in global C cycling. Recently emerging concepts of soil organic matter (SOM) dynamics suggest that the long-term persistence of OC in soil depends on the complex interplay between SOM and environmental factors, e.g. accessibility to decomposer organisms, interaction with soil minerals and soil moisture (Schmidt *et al.*, 2011; Lehmann & Kleber, 2015). It has been shown that mineral–organic associations (MOAs) serve as an important reservoir of soil OC (Kögel-Knabner *et al.*, 2008) and the turnover of SOM is suppressed by its adsorption on mineral surfaces (Kalbitz *et al.*, 2005; Mikutta *et al.*, 2007).

Adsorptive interactions between soil minerals and organic compounds are governed by mineral surface structural and charge properties, chemical properties of the organic compounds, solution chemistry (pH, ionic strength and composition) and the presence of competing organic or inorganic compounds (Thomas & Kelley, 2010; Zhang *et al.*, 2012). Many studies have shown that soil minerals can preferentially bind aromatic compounds, thus increasing their turnover time in soil (Kaiser & Guggenberger, 2000; Kalbitz *et al.*, 2005). The primary sources of aromatic acids in soil are plant cell walls and lignin polymers of vascular plants, which can represent 20–30% of the C cycling in the biosphere (Croteau *et al.*, 2000). During the first step of lignin degradation (depolymerization) water-soluble aromatic degradation products are released into the soil solution and these compounds can be mineralized or adsorbed on mineral surfaces as a precondition for long-term stabilization (Kalbitz *et al.*, 2005; Hagedorn *et al.*, 2015).

This classical view of a preferential bonding of carboxylated phenolic compounds on many soil minerals (Kaiser & Guggenberger, 2000; Kalbitz *et al.*, 2005) has been challenged by the ‘onion’ or ‘multilayer’ model proposed by Kleber *et al.* (2007). This conceptual model suggests that the adsorbed organic compounds on mineral surfaces are self-organized into a multilayer structure with amphiphilic N-containing components enriched on the mineral surface, thereby forming the so called inner layer (Kleber *et al.*, 2007). However, experimental information about the adsorption of carboxylated phenolic and N-containing compounds on different mineral surfaces and their interaction with respect to competition and surface conditioning is lacking. There is no direct experimental support for the functioning of N-containing compounds for the development of a multilayer structure of organic compounds on mineral surfaces. This might be achieved with model compounds that represent

nitrogenous and lignin-derived SOM species.

Amino acids (AAs) are the most active pool of soil organic N and play an important role in soil N cycling (Schimel & Bennett, 2004). Although free AAs in soil are often quite limited, organic N retained in polymeric AA compounds can account for 30–40% of the total soil N pool (Martens & Loeffelmann, 2003). Interaction of AAs with soil mineral surfaces has been recognized as an important process that might affect the bioavailability of AAs in soil (Yeasmin *et al.*, 2014). Competitive interaction between AAs and other organic compounds (e.g. phenolic compounds) during adsorption on mineral surfaces, which are expected to occur frequently in natural environments, are still not well understood because previous studies examined interactions only between AAs and soil minerals in mono-component systems (Yeasmin *et al.*, 2014).

Phenolic acids (PAs), a natural constituent of soil solutions, are the simplest water-soluble products of lignin degradation. Numerous studies have shown that they have a strong affinity for soil or soil minerals (Celis *et al.*, 2005; Chefetz *et al.*, 2011). However, we do not know whether these monomeric aromatic acids are preferentially adsorbed with respect to N-containing compounds. Different soil mineral surfaces also have different charge properties, providing strongly different adsorption sites for PAs. Furthermore, experimental evidence is lacking for the notion that the proposed surface conditioning by AAs would increase the adsorption of aromatic moieties.

The main objective of this study was, therefore, to study the interaction between PAs and AAs during their adsorption on goethite (α -FeOOH) and montmorillonite. The latter are representatives of Fe oxides and 2:1 clay minerals respectively that are common in many soils and have different surface properties: goethite with variable charges and montmorillonite with permanent negatively charged silicate layers (Sposito, 2008). We aimed to test whether AAs were preferentially adsorbed compared with PAs and if surface conditioning of minerals by AAs affected the adsorption of PAs, and *vice versa*. We examined (i) the adsorption behaviour of different AAs representing acidic, neutral, basic and aromatic AAs (glutamic acid, leucine, lysine and phenylalanine) and PAs as model compounds for lignin degradation products (salicylic, syringic, ferulic and vanillic acid) on goethite and montmorillonite, (ii) the adsorption behaviour of PAs on soil minerals after surface conditioning by AAs, (iii) the adsorption behaviour of AAs on soil minerals following surface conditioning by PAs and (iv) the competitive adsorption of PAs and AAs by performing batch adsorption experiments.

4.2 Material and methods

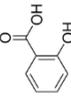
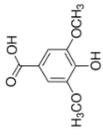
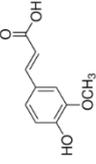
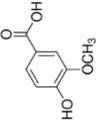
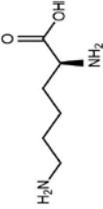
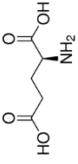
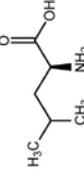
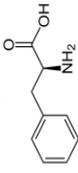
4.2.1 Minerals

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 by sedimentation from Morocco bentonite. Exchange sites were saturated with Ca²⁺ by suspending the clay twice in 1 M CaCl₂ (1 g clay in 10 ml CaCl₂). Finally the montmorillonite was washed until salt free, freeze-dried and passed through a 200- μ m sieve. The X-ray diffraction patterns (Siemens, D500, Karlsruhe, Germany, Cu-K α radiation) indicated the presence of well-crystallized goethite and a high purity of the < 2- μ m fraction of the Morocco bentonite (Figure B1, Appendix). The specific surface area (SSA) and total pore volume (TPV) of minerals were derived from duplicate N₂ adsorption isotherms recorded with a Quantachrome Autosorb1-MP analyser (Quantachrome, Boynton Beach, FL, USA). An aliquot (20–200 mg) of 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 from up to 10 adsorption points (Brunauer *et al.*, 1938). The TPV was taken at relative pressure P/P₀ ca. 0.995, where P and P₀ are the equilibrium and the saturation pressure respectively of N₂. The SSA of goethite and montmorillonite was 70 and 95 m² g⁻¹, and the TPV of goethite and montmorillonite was 0.73 and 0.19 cm³ g⁻¹, respectively. The anion exchange capacity (AEC) of goethite, calculated from the surface charge density at pH 6 in NaCl background electrolyte (Walsch & Dultz, 2010), was 13 mmol_c kg⁻¹. The cation exchange capacity (CEC) of montmorillonite determined with cobalt-hexamine trichloride was 888.2 mmol_c kg⁻¹ (Daou *et al.*, 2015).

4.2.2 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 structures and properties are listed in Table 4.1. Nva and Eva were used as internal standards (ISs) in the measurement of AAs and PAs, respectively.

Table 4.1 Structure and properties of the selected low molecular weight (MW) phenolic acids and amino acids.

Compound	Abbreviation	Structure	MW	Water Solubility /g l ⁻¹ , 25 °C	Acidity constants			pI ^a
					pK _{a1}	pK _{a2}	pK _{a3}	
Salicylic acid	Sal		138.1	1.14 ^b	2.8	13.4 ^c		
Syringic acid	Syr		198.2	5.75 ^d	4.34	9.49 ^e		
Ferulic acid	Fer		194.2	0.78 ^b	4.56	9.39 ^e		
Vanillic acid	Van		168.1	1.53 ^d	4.42	9.39 ^e		
L-Lysine	Lys		146.2	> 1000 ^f	2.18	9.12	10.53	9.82 ^f
L-Glutamic acid	Glu		147.1	8.4 ^f	2.16	9.67	4.32	3.24 ^f
L-Leucine	Leu		131.2	23 ^f	2.36	9.60		5.98 ^f
DL-Phenylalanine	Phe		165.2	29.7 ^f	1.83	9.13		5.48 ^f

^a Isoelectric point; ^b Mota *et al.* (2008); ^c Yost *et al.* (1990); ^d Noubigh *et al.* (2007); ^e Ragnar *et al.* (2000); ^f Barrett (1985).

4.2.3 Preparation of PA and AA solutions

Separate stock standard solutions of each AA (2000 mg l^{-1}) were prepared in 0.1 M HCl and separate stock standard solutions of each PA were prepared in water at a concentration of 1000 mg l^{-1} for Sal, Syr and Van and 500 mg l^{-1} for Fer, respectively. A different concentration was used for Fer due to its low solubility in water. The stock solutions were stored at $4 \text{ }^\circ\text{C}$ for not longer than one month. Working mixture solutions with equal concentrations for individual PAs and AAs were freshly prepared by mixing and diluting the stock standard solutions.

4.2.4 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) was done in triplicate with both goethite and montmorillonite at three different initial adsorbate concentrations of the individual compounds, i.e. 0.01 (small), 0.05 (intermediate) and 0.1 mM (large). The solutions were added to each mineral separately (control experiment), together (competition experiment) or after adsorption of the other mixture (conditioning experiment). For the latter, two conditioning experiments were carried out: adsorption of PAs on goethite or montmorillonite after adsorption of AAs (conditioning experiment I) and adsorption of AAs on the minerals after adsorption of PAs (conditioning experiment II). To our knowledge these experimental procedures were adopted for the first time.

Each 40 ml of adsorbate solution were added to a 50-ml Falcon tube containing either 200 mg goethite or 600 mg montmorillonite. A reliable measurement of AAs and PAs in the equilibrium solution could be achieved only with different mineral masses according to our pre-test, illustrating the different adsorption capacity of goethite and montmorillonite. The suspensions were shaken horizontally (1.58 s^{-1}) for 2 hours in the dark at $4 \text{ }^\circ\text{C}$, centrifuged (3500 g , 20 minutes) and filtered through $0.45\text{-}\mu\text{m}$ cellulose ester membranes (ME25, GE Healthcare Life Sciences, Whatman, Little Chalfont, UK). A short reaction time of 2 hours and a low temperature of $4 \text{ }^\circ\text{C}$ were chosen to minimize microbial effects. The filtered samples were stored at $4 \text{ }^\circ\text{C}$ before measurement. To inhibit microbial activity further and assure a constant electrolyte background, 10 mM NaN_3 was used as background electrolyte and the pH was adjusted to 6 before mixing with the minerals to simulate a common natural condition by the dropwise addition of 0.1 M NaOH or HCl . In the conditioning experiment, the

mineral remaining in the tube after centrifugation was re-suspended in 40 ml of the second adsorbate solution with a vortex mixer or glass rod before equilibration.

The pH of the filtrate after adsorption was measured with a multi-channel analyser (CONSORT C831, Abcoude, The Netherlands). In addition, the possible loss of mineral between the two adsorption steps after filtration was taken into account by calculating the weight difference of the membrane filter before and after filtration. The wet membrane filter was dried for ca. 16 hours at 80 °C before weighing. Loss of PAs or AAs during filtration was also calculated by comparing the concentration of the standard mixture before and after filtration. In brief, the maximum loss of minerals was ca. 1%, but there was a relatively large loss during filtration for Fer (12%) and Lys (11%). The adsorption of each adsorbate on the minerals was calculated as the difference between its concentration in the initial and equilibrium solution with compensation for the loss of mineral and adsorbate during filtration. During the conditioning experiments, desorption of previously adsorbed PAs or AAs was evaluated by measuring the concentration of PAs or AAs in the filtrates after the second adsorption step.

The adsorption of PAs on Fe³⁺-containing minerals could be accompanied by their oxidative transformation including the formation of Fe²⁺ (Polubesova *et al.*, 2010), and soil minerals can also catalyse the reaction of AAs and the formation of peptides (Zaia, 2004). Since these transformation reactions would bias the adsorption results, they were tested under anoxic conditions. For PAs that reacted with goethite, no Fe²⁺ was detected in either liquid or solid samples to indicate phenol transformation by oxidation. Furthermore, concentrations of total organic carbon (TOC) in equilibrium solutions were no larger than those derived from compound-specific concentrations measured by high performance liquid chromatography (HPLC; see below), which also indicated that PAs and AAs remained unaltered during the experiments.

4.2.5 Measurement of PAs and AAs

Liquid chromatography/mass spectroscopy grade acetonitrile (ACN), methanol (MeOH), formic acid (FA) and analytical NH₄HCO₂ were used in the measurement of PAs and AAs. For the HPLC measurement, the mobile phase, AAs and PAs solutions were prepared using sterile ultrapure water.

The PAs in the filtrates were directly analysed with a Prominence HPLC system equipped with a diode array detector (Shimadzu, Kyoto, Japan). Separation was done with a LiChrospher/Superspher RP-18 100-Å column (125 mm × 2 mm, 4 µm; Phenomenex, Torrance, CA, USA) and an RP-18 4-µm guard column. The mobile phase was solvent A (MeOH, 0.1% FA, v/v%) and solvent B (ultrapure H₂O, 0.1% FA, v/v%) with an isocratic elution of 27% solvent A for 15 minutes. Solvent A (90%) was used to wash the column between injections for 5 minutes followed by a 15 minutes equilibration period. The flow rate of the mobile phase was 0.3 ml minute⁻¹. The injection volume was 10 µl and the oven temperature was 35 °C. Sal, Van, Syr, Fer, and Eva were detected at 240, 260, 270, 320 and 280 nm, respectively. The IS (Eva, final concentration 5 mg l⁻¹) was added to the aqueous sample before the injection.

Prior to measurement, the AAs in the filtrates were diluted 10 or 20 times (90% ACN, 10% H₂O, 8 mM NH₄HCO₂, 0.12% FA, 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 electrospray mass spectrometry was used to analyse the AAs without derivatization (Gao *et al.*, 2016). Separation was with an ACQUITY UPLC BEH Amide column (100 mm × 2.1 mm, 1.7 µm, Waters, Milford, MA, USA), together with an ACQUITY UPLC BEH Amide 1.7-µm VanGuard guard column. The MS detection was carried out with a 4000 QTRAP LC–MS/MS System (AB SCIEX, Milford, MA, USA), coupled with an ESI interface. The mobile phase was solvent A (90% H₂O, 10% ACN, 8 mM NH₄HCO₂, 0.12% FA, v/v%) and solvent B (90% ACN, 10% H₂O, 8 mM NH₄HCO₂, 0.12% FA, v/v%). Gradient elution was used: 94–88% B from 0 to 6 minute, 88–50% B from 6 to 11 minute, constant 50% B from 11 to 12 minute. The separation system was reconstituted to the start condition within 1 minute and equilibrated for 10 minutes between injections. The injection volume was 20 µl; samples were eluted at 0.4 ml minute⁻¹. The column temperature was set at 35 °C.

4.2.6 Zeta potential measurement of pure and surface conditioned minerals

Zeta (ζ) potential measurements were carried out with phase-analysis light scattering (ZetaPALS, Brookhaven Instruments Corp., Holtsville, NY, USA). The ζ potential was calculated from electrophoretic mobility by the Smoluchowski equation. Following the adsorption experiment, moist mineral pastes were used to prepare mineral suspensions of 100 mg l⁻¹ in ultrapure water in 25 ml flasks without pH adjustment, except for the pure goethite suspension for which the

pH was adjusted to the same value as the suspension of surface-conditioned goethite. After gentle shaking, 1.6 ml were sampled with a pipette, transferred to a cuvette and measured at 20 °C. Each sample was measured five times and each of the five replicates (runs) consisted of 20 single measurements (cycles). The electrical conductivity of the suspensions was determined simultaneously and used to control electrolyte concentration. The pH was determined in the cuvette by a miniaturized electrode (BlueLine 16pH, Si Analytics, Mainz, Germany).

4.2.7 Statistics

Statistical analysis was performed with SigmaPlot (version 13.0). A two way analysis of variance (ANOVA) was used to evaluate the effect of mineral types, 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 (see Appendix, Tables B1, B2, B5–B12). Fisher's least significant difference (LSD) was used for multiple comparisons of the means. The significance level of the difference was set at $P < 0.05$.

We introduced the relative adsorbed amount in the competition and conditioning experiments because this approach showed the effects of competition and conditioning more clearly on the adsorption of PAs and AAs. The relative adsorbed amount is the percentage value from the adsorption results relative to that obtained from the control experiment. The same mean values from the control experiments were used for each of the three replicates in calculating the relative values of adsorbed amounts.

4.3 Results

4.3.1 Control experiment – Adsorption of PAs and AAs on minerals

Both the mineral type and adsorbate concentration had significant effects ($P < 0.001$) on the adsorption of PAs and AAs, and the effects of them were interdependent (Tables B1 and B2, Appendix). The adsorption of PAs and AAs on goethite and montmorillonite at slightly acidic conditions increased with increasing initial concentrations from 0.01 to 0.1 mM (Table 4.2). The increased adsorption of PAs was, however, less than the increase in adsorbate

Table 4.2 Mean values of total adsorption of PAs and AAs on goethite and montmorillonite, respectively, in the control experiment.

Compounds	Mineral	Initial concentration /mM	Mean ^a / $\mu\text{mol g}^{-1}$	SEM ^b	LSD ^c
PAs	G ^d	0.01	1.07 (1.92)	0.01	0.02
		0.05	1.82 (5.18)		
		0.10	2.05 (6.75)		
	M ^e	0.01	0.33 (0.39)		
		0.05	0.73 (1.06)		
		0.10	0.86 (1.37)		
AAs	G	0.01	0.03 (0.34)	0.03	0.08
		0.05	0.12 (1.03)		
		0.10	0.19 (1.61)		
	M	0.01	0.05 (0.49)		
		0.05	0.31 (2.36)		
		0.10	0.86 (4.63)		

^a The data are transformed mean values with original values in parentheses; ^b Standard error of the transformed mean determined using the residual mean square (12 d.f.); ^c Least significant difference, $P < 0.05$; ^d Goethite; ^e Montmorillonite.

concentration, indicating a limited number of adsorption sites. Although it was difficult to plot an adsorption isotherm for PAs or AAs on soil minerals because of the limited adsorption points, goethite appeared to have a larger adsorption capacity for PAs than AAs. In contrast, montmorillonite was a much better adsorbent for AAs than for PAs (Table 4.2). Salicylic acid was the predominantly adsorbed PA for both minerals (contribution of Sal to adsorbed PAs: 70–75% for goethite and 96–100% for montmorillonite). However, its adsorption on goethite was much greater than on montmorillonite (Figure B2, Appendix). Adsorption of other PAs (Syr, Fer and Van) on goethite was rather limited and no or negligible adsorption occurred on montmorillonite (Figure B2, Appendix). Among the different AAs, goethite adsorbed only Glu (acidic AA), whereas montmorillonite adsorbed only Lys (basic AA).

The net surface charge of goethite (positive) was opposite to that of montmorillonite (negative, Table 4.3). The ζ potential of montmorillonite decreased considerably by surface conditioning of PAs or AAs, whereas it tended to increase for goethite (Table 4.3). For both of the minerals, minor

Table 4.3 Effect of surface conditioning by PAs or AAs on the zeta (ζ) potential of goethite and montmorillonite.

Mineral	Initial concentration /mM	ζ potential /mV		EC ^a / μ S cm ⁻¹		pH	
		Mean	SEM ^b	Mean	SEM	Mean	SEM
G-PAs ^c	Blank ^g	33.9	1.6	14.3	0.83	5.7	0.10
	0.01	36.5		5.0		5.8	
	0.05	38.6		5.0		5.9	
	0.10	38.3		4.7		5.8	
M-PAs ^d	Blank	-22.9		4.3		6.2	
	0.01	-51.6		4.3		6.1	
	0.05	-56.1		5.0		6.2	
	0.10	-53.8		4.0		6.3	
G-AAs ^e	Blank	33.9	2.1	14.3	0.86	5.7	0.03
	0.01	35.5		7.0		5.7	
	0.05	37.5		5.3		5.6	
	0.10	45.6		4.7		5.8	
M-AAs ^f	Blank	-22.9		4.3		6.2	
	0.01	-48.6		4.0		6.1	
	0.05	-45.6		4.3		6.2	
	0.10	-49.6		4.0		6.2	

^a Electrical conductivity of mineral suspension; ^b Standard error of the mean determined using the residual mean square (16 d.f.); ^c Goethite surface-conditioned by PAs; ^d Montmorillonite surface-conditioned by PAs; ^e Goethite surface-conditioned by AAs; ^f Montmorillonite surface-conditioned by AAs; ^g Pure mineral.

differences in ζ potential were observed among different initial concentrations (Table 4.3). The solution pH increased after adsorption of PAs and AAs (Table B3, Appendix). This increase was larger for goethite than montmorillonite and differed only slightly between the treatments including the blanks.

4.3.2 Competition experiment – Adsorption of PAs and AAs on minerals

Competition between PAs and AAs at slightly acidic conditions decreased the adsorption of PAs on goethite and montmorillonite by 8 and 12%, respectively, for the intermediate initial concentration, and by 29 and 15%, respectively, for the large initial concentration (Figure 4.1a and Table B4). All these differences

were statistically significant ($P < 0.05$). There were small changes at the small initial concentration for both minerals. The general patterns of individual PA uptake on goethite and montmorillonite were affected little by AAs. Salicylic acid remained the predominantly adsorbed PA on both minerals. Competition with AAs resulted in reduced adsorption for all the PAs on goethite except for Fer, for which the adsorption increased markedly by 419 and 255% at intermediate and large initial concentrations, respectively (Figure B3a, Appendix). Despite this huge relative increase, the absolute amount of adsorbed Fer remained small (0.87 and $0.99 \mu\text{mol g}^{-1}$). For montmorillonite there was also a reduction in the adsorption of Sal, especially for the intermediate and large initial concentrations (Figure B3b, Appendix). Negligible or no adsorption of Syr, Fer and Van occurred on montmorillonite even in the absence of AAs.

Adsorption behaviour of the individual AAs was similar to that observed without the presence of PAs, i.e. goethite adsorbed only Glu, whereas montmorillonite adsorbed only Lys. The presence of PAs resulted in significantly increased adsorption of AAs on montmorillonite by 25, 21 and 25% for the small, intermediate and large initial concentrations, respectively ($P < 0.05$; Figure 4.1b and Table B4). We also observed a significant increase in the adsorption of AAs ($P < 0.05$) on goethite for the large initial concentration. However, there were no significant changes for the small and intermediate concentrations (Figure 4.1b and Table B4). The large variation in the adsorption of AAs could be attributed to the relatively small amounts adsorbed, which caused a small reduction only in the equilibrium concentrations ($< 10\%$).

4.3.3 Conditioning experiment I – Adsorption of PAs following adsorption of AAs

The adsorption of PAs on goethite after adsorption of AAs increased significantly compared with the control experiment by 41 and 48% for the intermediate and large initial concentrations, respectively ($P < 0.05$; Figure 4.1c and Table B4). An increase of 9% was also observed for the small initial concentration, but with no significant difference. Similarly, the adsorption of PAs on montmorillonite after preceding adsorption of AAs was significantly enhanced by 18 and 30% at the intermediate and large concentrations, respectively ($P < 0.05$; Figure 4.1c and Table B4). Although the adsorption of PAs on montmorillonite was reduced significantly at the small initial

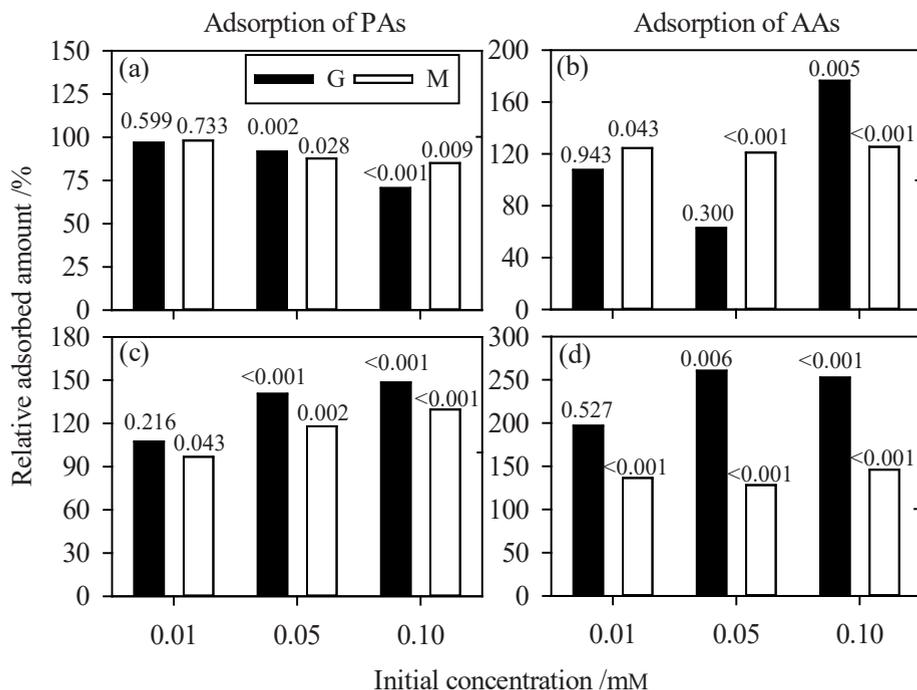


Figure 4.1 Relative effects of: (a) competition on the adsorption of PAs, (b) competition on the adsorption of AAs, (c) surface conditioning by AAs on the adsorption of PAs and (d) surface conditioning by PAs on the adsorption of AAs on goethite (G) and montmorillonite (M). Dotted lines refer to the reference values (control experiment). 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.

concentration, the change was small ($0.01 \mu\text{mol g}^{-1}$). The adsorption of PAs on goethite was much greater than on montmorillonite (Table B4, Appendix) as for the control experiment. The retention of all individual PAs was generally greater on goethite after adsorption of AAs than on pure goethite (Figure B4a, Appendix). Salicylic acid remained the predominantly adsorbed PA and its adsorption was ca. 18% greater than on pure goethite for the different initial concentrations (Figure B4a, Appendix). Although the adsorption of PAs on montmorillonite increased after adsorption of AAs, the uptake of Sal on the AA-coated montmorillonite was 8–9% less than on pure montmorillonite for all initial concentration values (Figure B4b, Appendix). This also means that the adsorption of Syr, Fer and Van was particularly promoted by surface

conditioning with AAs. Nevertheless, the adsorption of Syr, Fer and Van remained small ($< 0.27 \mu\text{mol g}^{-1}$; Figure B4b, Appendix). Depending on the initial concentration, 52–78% of the previously adsorbed AAs on goethite were desorbed or displaced by PAs during their adsorption. This proportion was much smaller for montmorillonite, with 14–19% only of the previously adsorbed AAs mobilized on interaction with PAs (Table 4.4).

4.3.4 Conditioning experiment II – Adsorption of AAs following adsorption of PAs

The adsorption of AAs on goethite and montmorillonite after conditioning with PAs increased by 97–161% and 28–46%, respectively, for different initial concentrations (Figure 4.1d and Table B4). All the differences were significant ($P < 0.05$) except for goethite at the small initial concentration. The differences in the adsorption of AAs between goethite and montmorillonite after surface conditioning with PAs tended to increase with increasing initial concentration (Figure B5, Appendix). For individual AAs, the adsorption of Glu on goethite after conditioning with PAs increased significantly by 97–114% compared with pure goethite at all initial concentrations ($P < 0.05$; Figure B6, Appendix). A small amount of Leu ($< 0.62 \mu\text{mol g}^{-1}$) was also adsorbed on surface-conditioned goethite, but no retention occurred for Lys and Phe. Although a small amount of Glu, Leu and Phe was adsorbed on montmorillonite after adsorption of PAs, it was much less than Lys with an increase in adsorption of 15–19% ($P < 0.05$; Figure B6, Appendix). Addition of AAs resulted in a similar desorption of previously adsorbed PAs from montmorillonite (29–41%) and goethite (17–39%; Table 4.4).

4.4 Discussion

4.4.1 Adsorption of specific PAs and AAs to pure soil minerals

4.4.1.1 Phenolic acids

Adsorption of Sal on both goethite and montmorillonite was much stronger than Syr, Fer and Van (Figure B2, Appendix). This could be ascribed to the adjacent position of the carboxyl and hydroxyl group of Sal, which resulted in the formation of bidentate inner-sphere complexes with the mineral (Yost *et al.*, 1990; Kubicki *et al.*, 1997). Chefetz *et al.* (2011) also found relatively little

adsorption of Syr, Van and Fer on Fe^{3+} - and Ca^{2+} -montmorillonite at pH 6. The larger adsorption of Sal on goethite than montmorillonite can be attributed to the opposite charge of PAs to the goethite at pH 6. The carboxyl groups of the PAs are deprotonated and negatively charged at pH 6, whereas the goethite is positively charged (pH_{PZC} of goethite, 7.6; Mikutta *et al.*, 2007); this was supported by the positive ζ potential (Table 4.3). By contrast, montmorillonite is negatively charged overall at pH 6 (Table 4.3), like Sal and the other PAs. In addition to the permanent negative charges, pH-dependent charges exist at the broken edges of the montmorillonite and become positively charged at pH 6 (Tombácz *et al.*, 2004). Thus, a small amount of Sal can be attracted by the montmorillonite, resulting in the formation of outer- and inner-sphere complexes (Yeasmin *et al.*, 2014). Moreover, since the montmorillonite was saturated with Ca^{2+} , cation bridges possibly also contributed to the retention of Sal (Mikutta *et al.*, 2007). A similar desorption of Sal from goethite and montmorillonite might support a similar bonding mechanism of PAs. Nevertheless, the different charge characteristics of goethite and montmorillonite are probably responsible for the greater adsorption capacity of PAs on goethite than on montmorillonite.

4.4.1.2 Amino acids

Glutamic acid (acidic AA) was preferentially adsorbed on goethite and Lys (basic) on montmorillonite. The negatively charged Glu was probably selectively bound by positively charged goethite and the positively charged Lys by negatively charged montmorillonite at pH 6 through electrostatic attraction. These results corroborated those of Yeasmin *et al.* (2014), who reported a stronger affinity of Lys to phyllosilicates than to goethite and *vice versa* for Glu. Neither a single carboxyl group nor a single amino group is sufficient for efficient binding of AAs on positively and negatively charged minerals, respectively. This means that the net charge of AAs (acidic or basic) arising from extra carboxyl or amino groups was decisive for their electrostatic bonding. The greater desorption of Glu from goethite (52–78%; Table 4.4) than Lys from montmorillonite (14–19%; Table 4.4) after addition of PAs indicates a stronger binding of AAs on negatively charged minerals by amino groups than on positively charged mineral surfaces by carboxylic groups in outer-sphere coordination.

Table 4.4 Desorption of previously adsorbed PAs and AAs after addition of AAs and PAs, respectively, in the conditioning experiments.

Compounds	Mineral	Initial concentration for adsorption /mM	Desorption / $\mu\text{mol g}^{-1}$		Desorbed proportion /%	
			Mean	SEM ^a	Mean	SEM
PAs	G ^b	0.01	0.33	0.03	17	0.9
		0.05	2.00		39	
		0.10	2.64		39	
	M ^c	0.01	0.11		29	
		0.05	0.44		41	
		0.10	0.52		38	
AAs	G	0.01	0.17	0.03	52	8.5
		0.05	0.79		71	
		0.10	1.41		78	
	M	0.01	0.07		14	
		0.05	0.42		18	
		0.10	0.88		19	

^a Standard error of the mean determined using the residual mean square (12 d.f.); ^b Goethite; ^c Montmorillonite.

4.4.2 Competitive adsorption of PAs and AAs on pure soil minerals

The adsorption of PAs (i.e. mainly Sal) on goethite and montmorillonite under slightly acidic conditions was partially suppressed in presence of AAs at intermediate and large initial concentrations. These results demonstrate a larger competitiveness of AAs than PAs. At small initial concentrations, competition was negligible probably because of the sufficient and large number of adsorption sites, whereas at intermediate and large initial concentrations the adsorption sites became limited and competition became evident. The predominant adsorption of Sal among different PAs did not change in the presence of AAs. The formation of bidentate complexes between Sal and mineral surfaces requires two adjacent adsorption sites (Yost *et al.*, 1990; Kubicki *et al.*, 1997), whereas any of the AAs used probably needs only one binding site (Kitadai *et al.*, 2009; Yang *et al.*, 2016). Therefore, it is more likely that one binding site required by AAs is available than two adjacent ones for PAs (i.e. mainly Sal), particularly at large concentrations. Furthermore, the Glu can displace some weakly bound PAs (Gu *et al.*, 1996), e.g. Syr, Fer and Van.

The adsorption of AAs on montmorillonite and goethite was enhanced by PAs, and the effect on montmorillonite was stronger than on goethite (i.e. at large initial concentrations only). Taking the competitiveness of AAs into account, we can assume a surface conditioning effect of PAs that enhances the adsorption of AAs.

4.4.3 Effect of surface conditioning on adsorption of PAs and AAs – evidence for multilayer adsorption?

At present there is little conclusive evidence for the multilayer model in the formation of natural soil MOAs. Mikutta *et al.* (2009) investigated the vertical structure of MOAs along a mineralogical soil gradient and found no evidence of enrichment of organic N compounds at mineral surfaces. Du *et al.* (2014) used depth-profiling Fourier transform infrared photo-acoustic spectroscopy and identified three different layers (ca. 100 μm thick) for the associated OM on soil particles, with enrichment of N moieties in the inner layer (ca. 60 μm deep). The spatial resolution of this study, however, does not enable any conclusion about the validity of a molecular-based multilayer adsorption model.

Our model experiments showed clearly that the adsorption of organic monomers on minerals modified their surface properties and subsequently altered their adsorption behaviour for other organic compounds. The adsorption of AAs on goethite and montmorillonite was enhanced by surface conditioning with PAs, despite partial desorption or displacement of PAs during adsorption of AAs. Therefore a fraction of the adsorbed components (the strongly bound one) was responsible for the enhanced adsorption of AAs. Preceding adsorption of PAs increased the positive charges on the goethite surface and negative ones on montmorillonite indicated by the more positive and negative ζ potentials for goethite and montmorillonite, respectively, after adsorption of PAs (Table 4.3), and therefore the number of binding sites for Glu on goethite and Lys on montmorillonite. This effect was not expected for goethite and might be caused primarily by dispersion of aggregates during the batch adsorption procedure (Mikutta *et al.*, 2014). Light scattering measurements revealed a decrease in the average particle diameter of goethite from 220 nm of the untreated mineral to 40 nm after adsorption (tested for 0.1 mM AAs). In addition, the ζ potential of goethite became more positive with the adsorption of PAs and AAs. Thus, dispersion of goethite possibly occurred during the batch experiments. Walsch & Dultz (2010) also reported that dispersion of goethite occurred at $\text{pH} \leq 6.5$ in batch adsorption experiments. Furthermore, the negative charges added by

adsorption of organic acids (Table 4.2) were much smaller than the surface charge density of goethite (e.g. $48.8 \mu\text{mol}_c \text{g}^{-1}$ at a specific surface area of $70 \text{m}^2 \text{g}^{-1}$ and assuming a charge density of $0.7 \mu\text{mol}_c \text{m}^{-2}$; Walsch & Dultz (2010)). Both factors might be responsible for the adsorption of organic acids on goethite that resulted in slightly increased positive charges (Table 4.3). The dispersion-induced increase in number of adsorption sites might explain why surface conditioning by PAs did not change the character of the adsorbed AAs, i.e. it increased mainly the adsorption of AAs with the opposite charge.

In addition to the effects of dispersion, aromatic rings of PAs might be important for increased adsorption of AAs. Once PAs (predominantly Sal) were adsorbed on mineral surfaces primarily by their carboxyl (and hydroxyl) groups (Guan *et al.*, 2006), the aromatic ring of PAs probably offered some additional binding sites for AAs. Although no additional charged sites could be created by Sal, molecular interactions of aromatic rings with ionizable AAs through polar- π interaction could occur (Keiluweit & Kleber, 2009). In turn, surface conditioning by Sal increased the adsorption of negatively and positively charged AAs on goethite (Glu) and montmorillonite (Lys), respectively. The negligible adsorption of Phe (aromatic AA) suggests weak π - π interaction. Moreover, the smaller effect of surface conditioning by PAs for montmorillonite on the immobilization of AAs relative to goethite corresponded to the much smaller adsorption of PAs (Table 4.2). Therefore, the additional retention of AAs appears to depend on the abundance of aromatic rings, but this hypothesis remains to be tested in a forthcoming study. The larger effect of surface conditioning for goethite than montmorillonite can be explained by the dispersion of aggregates, which probably occurred for goethite only.

Although the stimulating effect of surface conditioning by PAs on the adsorption of AAs was quite unexpected, we anticipated an enhanced adsorption of PAs on montmorillonite after adsorption of AAs. The enhanced retention of PAs might provide an example of a more general mechanism through which the adsorption of one class of SOM components enhances the uptake of another. Such enhanced adsorption might be interpreted as an indirect experimental indication of the multilayer model (Kleber *et al.*, 2007), where preferential adsorption of N-containing compounds modify the binding environments on mineral surfaces in a way that renders them more attractive for other compound classes. These effects might be observed theoretically for both the negatively and positively charged mineral. Interestingly, the stimulating effect of surface conditioning by AAs on the binding of PAs was less than the effects of conditioning by PAs on the adsorption of AAs (Figure 4.1 and Table B4). The

effect of surface conditioning by AAs on montmorillonite was less than that for goethite, despite stronger adsorption of AAs on montmorillonite and less desorption after the addition of PAs. This suggested that the larger accessible surface induced by the dispersion of goethite aggregates was a potential mechanism. The minor effect of surface conditioning by AAs for montmorillonite might be explained by the partial intercalation of Lys into the interlayer spaces (Parbhakar *et al.*, 2007; Kitadai *et al.*, 2009). The intercalation probably prevented desorption and crosslinking to other compounds. Because PAs were negatively charged at pH 6, the amino groups of attached AAs could provide additional binding sites to retain more PAs. Aromatic AAs (Phe) did not result in an additional effect on the adsorption of PAs compared with Glu (goethite) or Lys (montmorillonite), which further supported the role of amino groups as additional binding sites for PAs.

4.5 Conclusions

Our results demonstrate clearly that at slightly acidic conditions the adsorption of PAs and AAs depend strongly on the type of mineral, adsorbate properties and concentrations. Amino acids, although the more labile carbon source, are more competitive than PAs in binding on mineral surfaces such as goethite and montmorillonite. Furthermore, surface conditioning with AAs enhanced the adsorption of PAs. These results illustrate the important role of free acidic and basic AAs for surface conditioning in relation to adsorption and probably stabilization of SOM by soil minerals, irrespective of the surface charges of the mineral. Our results also showed the potential contribution of PAs (i.e. lignin degradation products) in the formation of mineral–organic associations. Aromatic structures in PAs can enhance the adsorption of AAs and probably of other organic components, independently of net charge characteristics. The PAs resulted in a larger increase in the adsorption of AAs than AAs did for PAs. From our results we can conclude that adsorption of one class of SOM components might alter the adsorption, and potential preservation, of other classes. Nevertheless, we still lack understanding of the true molecular-scale arrangements of individual compounds which requires a combination of molecular spectroscopy (e.g. synchrotron-based mass spectrometry or X-ray photoelectron spectroscopy) and quantum chemical modelling approaches. We recommend further batch experiments to assess possible altered adsorption site availabilities induced by mineral dispersion.