



## Rapid mineralization of mineral-bound carboxyl-carbon of salicylic acid and phenylalanine



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### ABSTRACT

Ligand-bound carboxylic acids are considered a stabilized fraction of mineral-adsorbed carbon in soil. Carboxyl-<sup>14</sup>C labeled phenylalanine or salicylic acid were adsorbed onto goethite, kaolinite, or illite, and subsequently incubated in both loamy and sandy arable topsoil for three weeks. Contrary to our expectations, more mineral-adsorbed carboxyl-C was mineralized than remaining C in salicylic acid and phenylalanine irrespective of mineral type or soil due to competitive desorption followed by preferential mineralization. Factors that control the desorbability of organic molecules are more important for their stabilization in the soil than sorption strength.

In a recent experiment (Konrad et al., 2025), we found that microbial mineralization and assimilation of carbon (C) from mineral-adsorbed salicylic acid and L-phenylalanine increased with desorbability from goethite, kaolinite and illite. Desorbability is the amount desorbed indexed to the amount adsorbed for a given extractant (0.01 M NaN<sub>3</sub> in Konrad et al., 2025). This concurs with past research that protection of organic matter against mineralization increases with sorption strength—e.g., van-der-Waals forces < polyvalent exchangeable cation bridging < ligand exchange (Mikutta et al., 2007)—only when desorbability is inversely related to sorption strength. Furthermore, the question remains whether desorbability regulates the microbial processing of the entire molecule or only the adsorbed C atoms. If the latter, covariantly bonded C atoms in carboxyl groups ought to be retained in soil to a greater extent than other moieties such as substituted phenyl or aliphatic structures.

We tested this proposition by incubating carboxyl-<sup>14</sup>C-labeled salicylic acid and L-phenylalanine adsorbed to goethite, kaolinite or illite parallel to the uniform-<sup>14</sup>C label incubation experiment in Konrad et al. (2025). Sorption was performed in 0.01 M NaCl at pH 5.5 and the sorbed compounds were incubated in the same arable topsoils from Dikopshof and Thyrow. Dikopshof is a loamy, aggregated Luvisol (pH 6.0), while Thyrow is a sandy Haplic Retisol (pH 5.2). Both soils were fertilized with farmyard manure (see Lorenz et al., 2024 for further details). At pH 5.5,

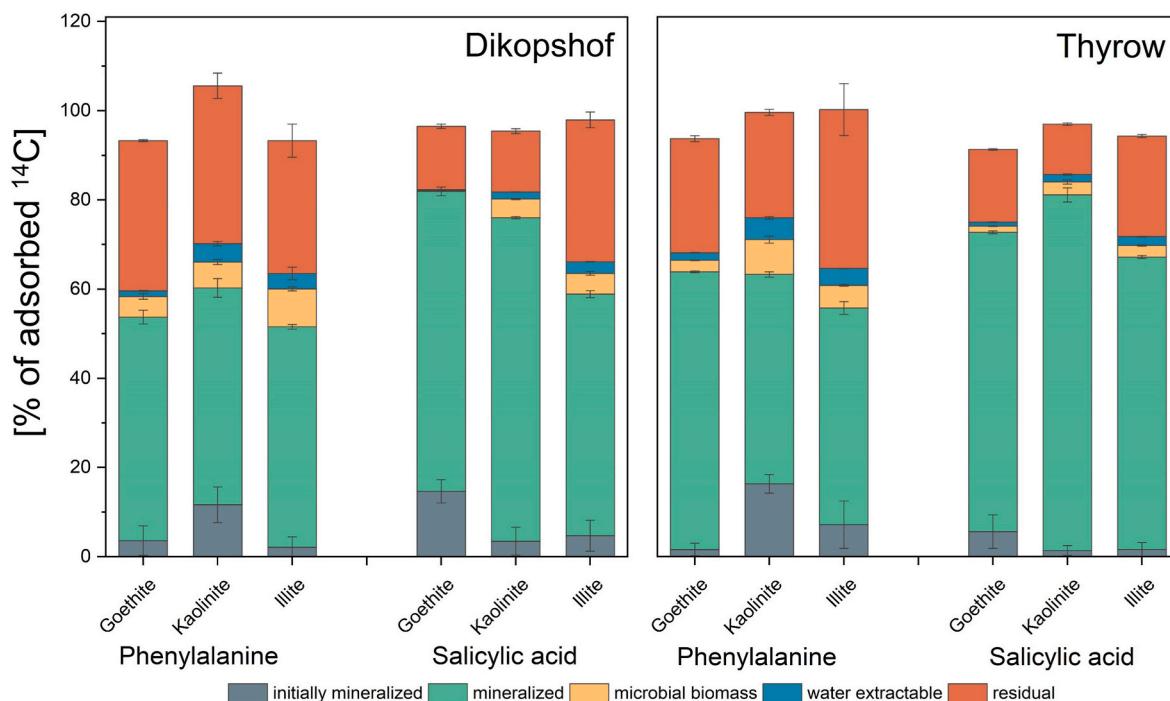
both monomers can perform ligand exchange reactions via their carboxyl group.

Minerals were identical to Konrad et al. (2025). Carboxyl-<sup>14</sup>C labeled monomers came from American Radiolabeled Chemicals (St. Louis, Missouri, USA). In agreement with the earlier experiment, sorption of phenylalanine- and salicylic acid-carboxyl-<sup>14</sup>C decreased from goethite > kaolinite > illite. Sorbed amounts of salicylic acid carboxyl-<sup>14</sup>C equaled 87.7 nmol m<sup>-2</sup> for goethite, 43.4 nmol m<sup>-2</sup> for kaolinite and 12.1 nmol m<sup>-2</sup> for illite. Those of phenylalanine carboxyl-<sup>14</sup>C were 20.6 nmol m<sup>-2</sup> for goethite, 17.4 m<sup>-2</sup> for kaolinite, and 5.6 nmol m<sup>-2</sup> for illite.

Soils were pre-incubated for 10 days at 25 °C and 60 % water-holding capacity in the dark. Fifty six gram of soil were subsequently mixed with either 240 mg of <sup>14</sup>C-loaded goethite or 300 mg of <sup>14</sup>C-loaded kaolinite or illite, and separated into three aliquots into 250 mL Schott flasks containing a scintillation vial with 1 mL 1 M NaOH to capture <sup>14</sup>CO<sub>2</sub>. Twenty minutes after the addition of minerals and before being closed airtight, triplicate aliquots of mineral-amended soil were taken, treated with ethanol to prevent further microbial processing, and combusted to quantify initial mineralization of <sup>14</sup>C. For three weeks, mineralization was calculated from the total accumulation of <sup>14</sup>CO<sub>2</sub> in NaOH via liquid scintillation counting (TriCarb 3300, PerkinElmer Inc., Shelton, Connecticut USA) with the same double pseudo-first-order kinetic equation in Konrad et al. (2025, Eq. (2)). At the end of the three weeks,

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**Fig. 1.** Fate of carboxyl- $^{14}\text{C}$  after three weeks of incubation in loamy Dikopshof (left) and sandy Thyrow soil (right), partitioned into microbial biomass, water-extractable  $^{14}\text{C}$ , residual  $^{14}\text{C}$  in the soil matrix, and mineralized  $^{14}\text{C}$ . All fractions were measured independently and total recovery may therefore slightly exceed 100 % due to analysis errors.

microbially-assimilated and water-extractable carboxyl- $^{14}\text{C}$  were extracted in triplicate in 2 M KCl with and without chloroform-fumigation, respectively (Murage and Voroney, 2007; Vance et al., 1987). Residual carboxyl- $^{14}\text{C}$  in the soil matrix was determined by combustion of soil in triplicate using a Hidex OX 600 oxidizer (Hidex Oy, Turku, Finland) and subtracting the  $^{14}\text{C}$  activity in fumigated and water extracts. Mineralization over time and mass balances were plotted using Origin Pro 2024b (OriginLab Corporation, Northampton, Massachusetts, USA). Microbial carbon use efficiency (CUE) equaled the amount of  $^{14}\text{C}$  in microbial biomass relative to microbially processed  $^{14}\text{C}$ . The differences in the fates of the carboxyl- $^{14}\text{C}$ - and uniform- $^{14}\text{C}$ -labeled monomers (Konrad et al., 2025) were analyzed for significance using Welch's *t*-test (Welch, 1947) in R (R Core Team, 2022).

The majority of carboxyl- $^{14}\text{C}$  in both substrates was mineralized in both soils (Fig. 1). For all substrate-mineral-soil combinations, mineralization rates for carboxyl- $^{14}\text{C}$  were higher than uniform- $^{14}\text{C}$  labels in Konrad et al. (2025) (+7 to +33 percentage points) and retention on minerals was mostly lower (-24 to +2; Table 1). The rate and extent of microbial processing of carboxyl- $^{14}\text{C}$  was similar in both soils (Fig. 2a-d), with initial mineralization often being too rapid for accurate fits even with double pseudo-first-order kinetics. When comparing substrates, carboxyl- $^{14}\text{C}$  from salicylic acid was generally retained less and always mineralized more than from phenylalanine (Table 1).

Three main observations can be made in comparison to Konrad et al. (2025): i) More mineral-bound carboxyl- $^{14}\text{C}$  was microbially processed compared to  $^{14}\text{C}$  in other functional groups (Table 1), ii) differences in the magnitude of carboxyl- $^{14}\text{C}$  cycling between the two soils were smaller than the effects of monomer (phenylalanine vs. salicylic acid) and mineral type (Figs. 1 and 2); and iii) microbial CUE of carboxyl- $^{14}\text{C}$  correlated with desorbability by substrate (salicylic acid:  $y = 0.19x$ ,  $p <$

$< 0.05$ ,  $R^2 = 0.88$ ; phenylalanine:  $y = 0.85x$ ,  $p < 0.001$ ,  $R^2 = 0.95$ ) (Supplementary material S1), but not by soil type (Dikopshof:  $R^2 = 0.6$ ; Thyrow:  $R^2 = 0.53$ , both  $p > 0.05$ ).

The majority of carboxyl- $^{14}\text{C}$  being mineralized within the first days of incubation indicating that mineral adsorbed carboxyl-C, including ligand-exchanges, is quickly mineralized either on or near mineral surfaces (Figs. 1 and 2). The substantial loss of carboxyl- $^{14}\text{C}$  compared to  $^{14}\text{C}$  in other functional groups suggests that mineralization occurs after desorption. Desorption happens when changes in solution composition cause other solutes to diffuse towards mineral surfaces, some of those solutes to replace inner-sphere complexes within hours via competitive desorption (i.e. displacement of an adsorbed ligand by a different ligand, like dissolved organic matter or *ortho*-phosphate; Kaiser and Zech, 1997), and displaced monomers to diffuse away from mineral surfaces. Microbial processing occurs simultaneously with these competitive adsorption-desorption processes and thus are rate-limited by diffusion from minerals to decomposers (Moyano et al., 2018; Pignatello and Xing, 1996).

The comparably low retention of carboxyl- $^{14}\text{C}$  via mineral-binding also highlights the role of other associations such as electrostatic interactions, H-bonding and hydrophobic exclusion in determining the balance between adsorption, desorption, and microbial processing. For instance, chelation is the preferred adsorption process for salicylic acid to goethite via its de-protonated carboxyl and *ortho*-hydroxy groups at the mineral loading used in this experiment (87.7 vs. conservative max. 50–95 nmol m<sup>-2</sup> reported in Yost et al., 1990). However, salicylic acid is easily displaced by dissolved organic matter (Gu et al., 1994, 1996). In contrast, both the de-protonated carboxyl group and protonated amino group in zwitterionic phenylalanine can adsorb via electrostatic interactions (Yeasmin et al., 2014) as well as self-assemble with

**Table 1** Fate of carboxyl- $^{14}\text{C}$  ( $1-^{14}\text{C}$ ) in salicylic acid and phenylalanine adsorbed to goethite, kaolinite, or illite after three weeks of incubation in loamy Dikopshof (Dik) or sandy Thyrow (Thy) topsoil, and its difference to the fate of uniformly labeled phenylalanine and salicylic acid ( $\Delta^{14}\text{C}(\text{U})$ ) in Konrad et al. (2025).  $1-^{14}\text{C}$  values are percentage of C mineralized, assimilated into biomass, extractable by water (WEOC), and retained as residues on minerals.  $\Delta^{14}\text{C}(\text{U})$  values represent the absolute difference in percent (i.e., percentage points) between  $1-^{14}\text{C}$  and  $\Delta^{14}\text{C}(\text{U})$  are indicated (\*  $p < 0.1$ , \*\*  $p < 0.05$ , \*\*\*  $p < 0.01$ ).

			mineralized [%]				biomass [%]				WEOC [%]				residual [%]				recovery [%]		microbially processed [%]		CUE [%]	
			$1-^{14}\text{C}$	$\Delta^{14}\text{C}(\text{U})$	$1-^{14}\text{C}$	$\Delta^{14}\text{C}(\text{U})$	$1-^{14}\text{C}$	$\Delta^{14}\text{C}(\text{U})$																
Dik	Goethite	81.9 ± 2.1	+33.0	***	0.2 ± 0	-6.0	*	0.2 ± 0	-2.0	**	14.2 ± 0.5	-24.5	***	96.5 ± 1.8	82.1 ± 2.1	+29.1	***	0.3 ± 0	-11.1	*				
	Kaolinite	76 ± 2.9	+23.1	*	4.2 ± 0.2	-5.7	**	1.6 ± 0.1	-2.8	**	13.7 ± 0.5	-14.4	**	95.5 ± 2.3	80.2 ± 2.8	+17.4	*	5.2 ± 0.3	-10.7	*				
	Illite	58.9 ± 3.1	+9.7	*	4.7 ± 0.4	-3.4	*	2.6 ± 0.1	-0.4	*	31.8 ± 1.8	-6.3	*	98 ± 1.1	63.5 ± 2.7	+6.2		7.4 ± 0.9	-6.8	**				
Thy	Goethite	72.7 ± 3.7	+24.1	**	1.4 ± 0.1	-3.7	**	0.9 ± 0	-2.9	***	16.3 ± 0.2	-19.4	***	91.3 ± 3.5	74.1 ± 3.7	+20.4	*	1.9 ± 0.2	-7.6	**				
	Kaolinite	81.1 ± 0.8	+19.9	*	2.9 ± 0.5	-5.5	**	1.6 ± 0.1	-4.1	***	11.3 ± 0.3	-15.2	**	97 ± 1.1	84 ± 1.2	+14.3		3.5 ± 0.5	-8.8	**				
	Illite	67.2 ± 1.7	+15.9	*	2.6 ± 0.2	-3.9	***	2 ± 0.1	-2.2	**	22.5 ± 0.3	-12.1	*	94.3 ± 2	69.7 ± 1.7	+11.9		3.7 ± 0.2	-7.6	**				
Dik	Phenylalanine	53.7 ± 1.8	+7.2	*	4.6 ± 0.6	+0.4		1.3 ± 0.1	+0.0	*	33.7 ± 0.2	-7.2		93.3 ± 2.5	58.4 ± 2.3	+9.1	*	7.9 ± 0.8	-0.3					
	Kaolinite	60.3 ± 6.1	+13.8	*	5.8 ± 0.5	-3.3		4.1 ± 0.5	+1.6	*	35.4 ± 2.8	+2.0		105.6 ± 2.3	66.1 ± 5.6	+10.5		8.9 ± 1.5	-7.4	*				
	Illite	51.5 ± 2.7	+13.5	*	8.5 ± 0.4	+4.8	***	3.5 ± 1.4	+2.1		29.8 ± 3.7	-19.8	**	93.3 ± 8.1	60 ± 3.2	+18.4	**	14.1 ± 0.2	5.2	*				
Thy	Goethite	63.8 ± 1.5	+26.1	***	2.6 ± 0.1	-0.7	***	1.7 ± 0	+1.1	***	25.6 ± 0.7	-23.9	***	93.7 ± 0.8	66.4 ± 1.4	+25.4	***	3.9 ± 0.2	-4.2	**				
	Kaolinite	63.3 ± 2.2	+17.9	*	7.8 ± 0.8	-0.4		4.9 ± 0.3	-0.7		23.7 ± 0.7	-12.5	**	99.6 ± 1.8	71.1 ± 1.5	+17.5	*	11.0 ± 1.3	-4.5					
	Illite	55.7 ± 6.7	+15.8	*	5.1 ± 0.2	-6.5	*	3.8 ± 0	-0.4		35.6 ± 5.8	-15.5	*	100.2 ± 1.7	60.8 ± 6.5	+18.3		8.5 ± 1.3	-18.8	*				

hydrophobic phenyl edges (Mossou et al., 2014). When displaced by dissolved organic matter, even more phenylalanine can adsorb to mineral surfaces due to more negative surface charges in soil compared to added minerals (Gao et al., 2018).

When accessible, carboxyl-C is often the first C mineralized due to decarboxylation (Dippold et al., 2014; Dippold and Kuzyakov, 2013). Decarboxylation typically has low activation energy since heterolytic cleavage next to carboxyl-C releases thermodynamically stable  $\text{CO}_2$  and it is further lowered by microbial decarboxylases. The remaining monomer-C undergoes other biochemical pathways, sometimes in combination with carboxyl-C as evidenced by the greater assimilation and higher CUE of phenylalanine carboxyl-C—a proteinogenic amino acid—into microbial biomass than for salicylic acid carboxyl-C (Table 1). Kinetics also differentiate these C moieties since breaking less polar or non-polar aliphatic or aromatic C–C bonds requires higher activation energies and thus is expected to proceed more slowly. Furthermore, after desorption as whole molecules and preferential mineralization of carboxyl-C, partitioning and van-der-Waals interactions between silicon surfaces and aromatic rings can occur, resulting in re-adsorption and reduced bioavailability of remaining C-atoms (Keiluweit and Kleber, 2009). However, since the incubation was only three weeks, the long-term retention of both mineral-bound and microbially assimilated C can only be speculated.

In both instances, the propensity to dissociate—that is, desorbability—is determined by factors beyond sorption strength. The addition of substrate-coated minerals to soil leads to changes in boundary conditions, new interactions and thus altered desorbability of adsorbates. Mineral characteristics likely matter more than soil cation exchange capacity, texture, pH and nutrient availability as indicated by similar patterns between the two soils. Yet it should be noted that both soils are agricultural topsoils with a narrow pH range and regular farmyard manure additions with shared litter composition (Simon et al., 2025), and thus the potential role of soil and entire ecosystems in determining desorbability and thus C mineralization may be larger than observed (Stutz, 2023).

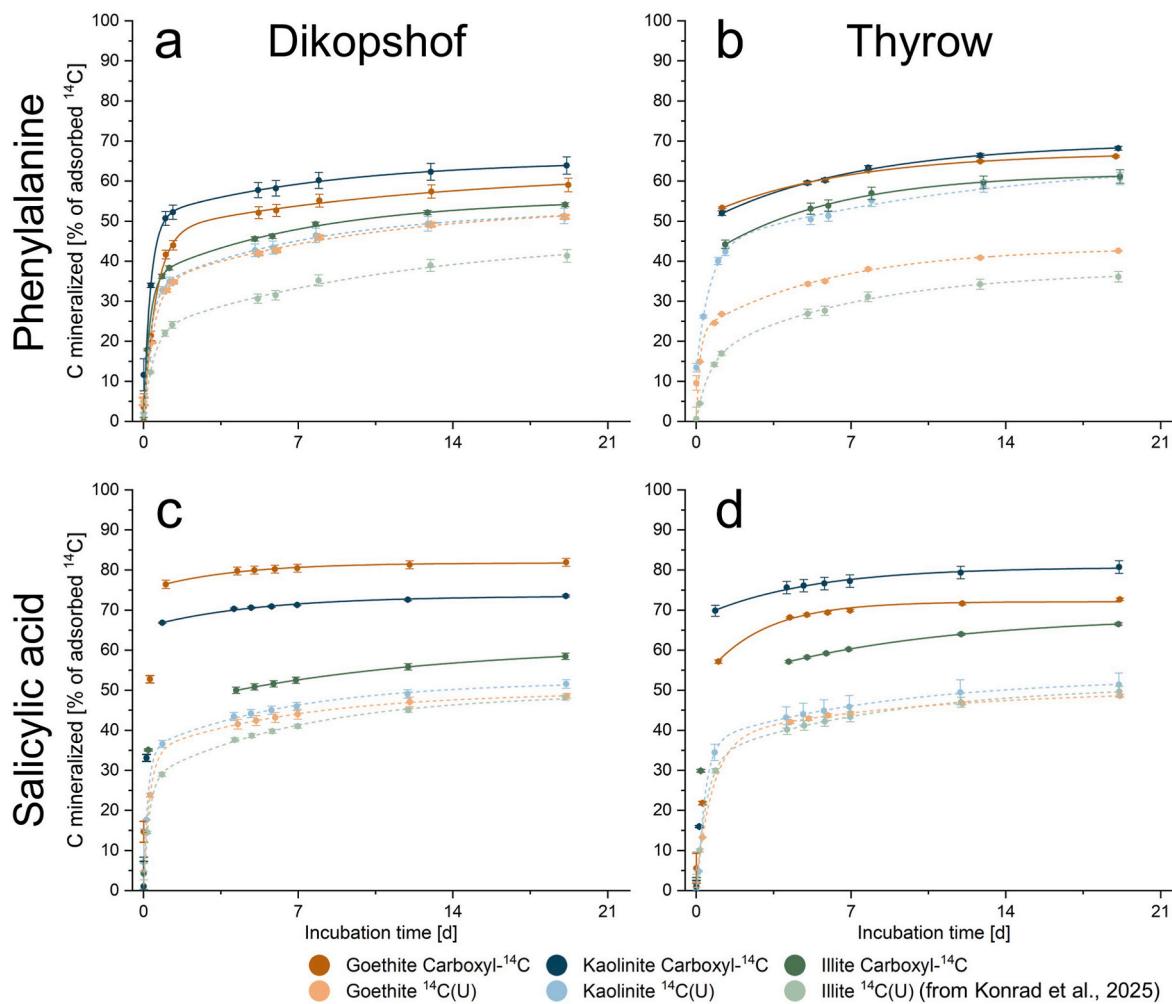
Our results demonstrate that even ligand binding to mineral surfaces does not necessarily protect the C involved in coordination with the mineral surface from microbial processing. Competitive desorption followed by partial mineralization best explains how inner-sphere associations of carboxyl groups can be overcome and mineralized while other functional groups persist. Mineralogy seems to matter more than other soil properties, but any factor that alters the tendency for mineral-bound compounds to desorb—e.g., increasing pH, or increased concentrations of oxyanions in solution—could lead to mineralization at the expense of sequestration.

#### CRediT authorship contribution statement

**Alexander Konrad:** Writing – original draft, Visualization, Software, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Diana Hofmann:** Writing – review & editing, Visualization, Validation, Supervision, Methodology, Investigation. **Jan Siemens:** Writing – review & editing, Validation, Supervision, Resources, Investigation, Funding acquisition, Conceptualization. **Friederike Lang:** Writing – review & editing, Supervision, Project administration, Funding acquisition. **Ines Mulder:** Writing – review & editing, Validation, Supervision, Project administration, Methodology, Funding acquisition, Conceptualization. **Kenton P. Stutz:** Writing – review & editing, Visualization, Validation, Supervision, Resources, Project administration, Methodology, Investigation, Funding acquisition, Conceptualization.

#### Declaration of competing interest

The authors declare that they have no known competing financial interests of personal relationships that could have appeared to influence the work reported in this paper.



**Fig. 2.** Cumulative mineralization of <sup>14</sup>C labeled carboxyl groups of phenylalanine (panel a, b) or salicylic acid (panel c, d) adsorbed to either goethite, kaolinite or illite incubated in loamy Dikopshof (panel a, c) or sandy Thyrow (panel b, d) topsoil in comparison to uniformly labeled phenylalanine and salicylic acid from Konrad et al. (2025).

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## Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.soilbio.2025.110016>.

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