The contribution of earthworms to soil aggregate formation



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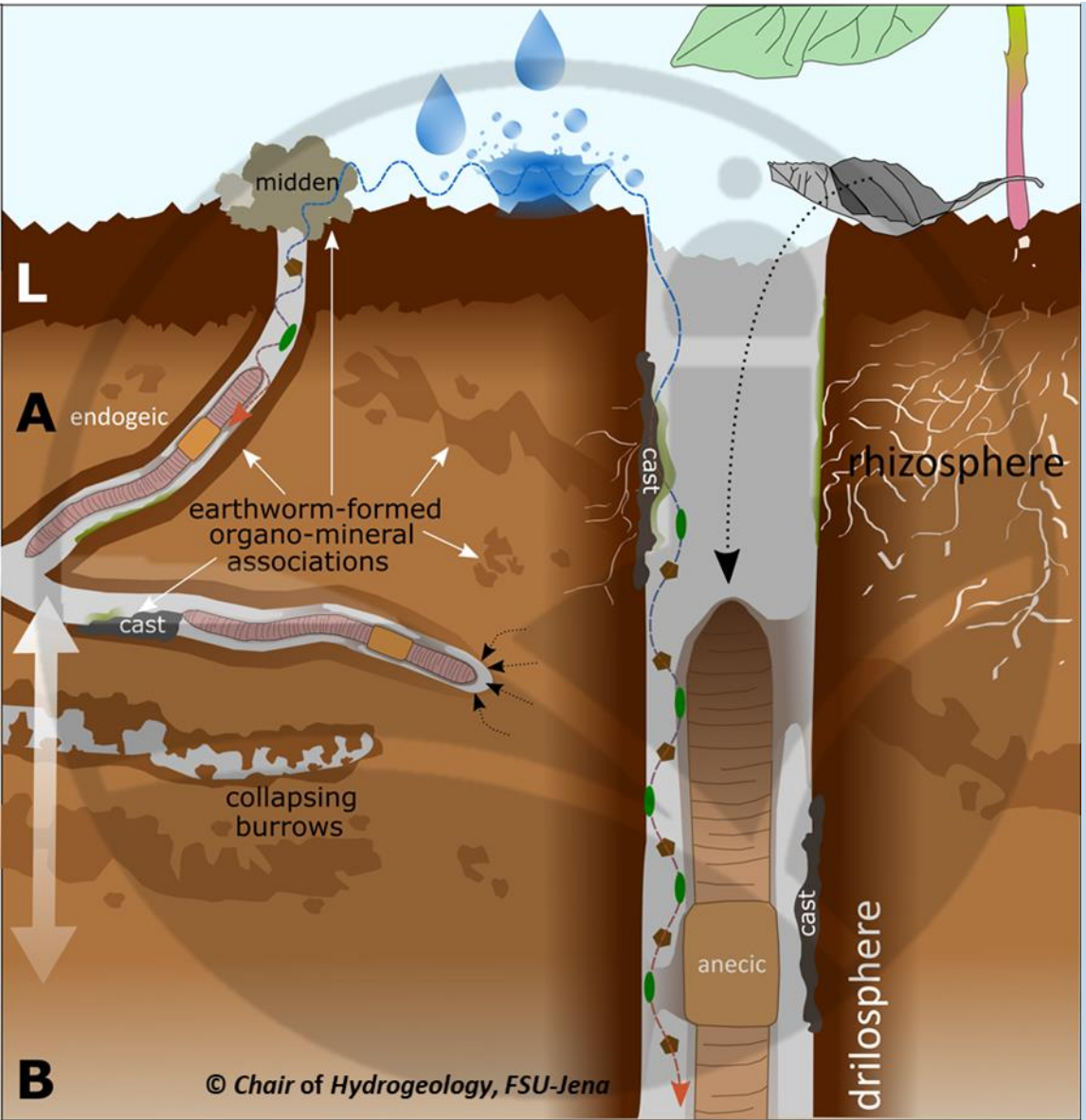
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Introduction

EJSS paper: Guhra et al. (2019)
SBB paper: Guhra and Stolze et al. (2020)



Plant-derived mucilage and microbial produced extracellular polymeric substances (EPS) are known to be crucial for the formation and stabilization of organo-mineral associations and soil aggregates. Especially the interactions between microbial EPS and typical constituents of the soil mineral phase like iron oxides and clay minerals were intensively investigated. Although the role of earthworms in improving soil quality has been stated, surprisingly little is known on the interactions of earthworm mucus with the soil mineral phase and experimental studies are vastly lacking. We contribute to closing this knowledge gap by studying the interactions between soil minerals and cutaneous earthworm mucus with completely mixed batch reactor experiments and compare to microbial EPS as well studied biogenic organic-matter found in soil.

Results and Discussion

EJSS paper: Guhra et al. (2019)
SBB paper: Guhra and Stolze et al. (2020)

Mucus compositional features

- Cutaneous mucus (MU) of anecic (AN) (***L. terrestris***) and endogeic (EN) (***A. caliginosa***) earthworms is mainly compost of **proteinious molecules** and in lesser amount of carbohydrates → specific composition to serve as lubricant
- Mucus showed **similarities to microbial EPS** of the early stationary phase in comparison to the late phase → equivalent pathways in the soil, similar functionalities for soil microorganisms and interactions with the soil mineral phase could be suggested
- **Narrow C:N ratio** → beneficial for microbial activity
- **High potassium values** were detected for both mucus types → inorganic plant nutrient

Sample	percent [%]					
	chemical shift [ppm]					
	200-160	160-110	110-90	90-65	65-45	45-0
	amides, carboxyl C	aromatic, olefinic C	anomeric C	O-alkyl C	αC of amino acids	aliphatic C
anecic mucus	16.6	18.4	5.6	11.7	18.4	29.3
endogeic mucus	17.6	12.1	5.6	17.0	19.4	28.2
EPS (early growth)	21.7	7.5	3.4	10.9	23.8	32.8
EPS (late growth)	7.4	4.4	12.0	43.7	21.2	11.3

Tab. Guhra and Stolze et al. (2020): Integral regions of solid-state 13C-nuclear magnetic resonance spectroscopy (NMR) of the freeze-dried mucus of anecic *L. terrestris* and endogeic *A. caliginosa* after extraction. For comparison, additional spectra of microbial EPS were added from previous studies: Bacillus subtilis 168 (DSM 402) (EPS), late (Narvekar et al., 2017; Guhra et al., 2019) and early (Liu et al., 2013) stationary phase.

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Results and Discussion

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Mucus compositional features

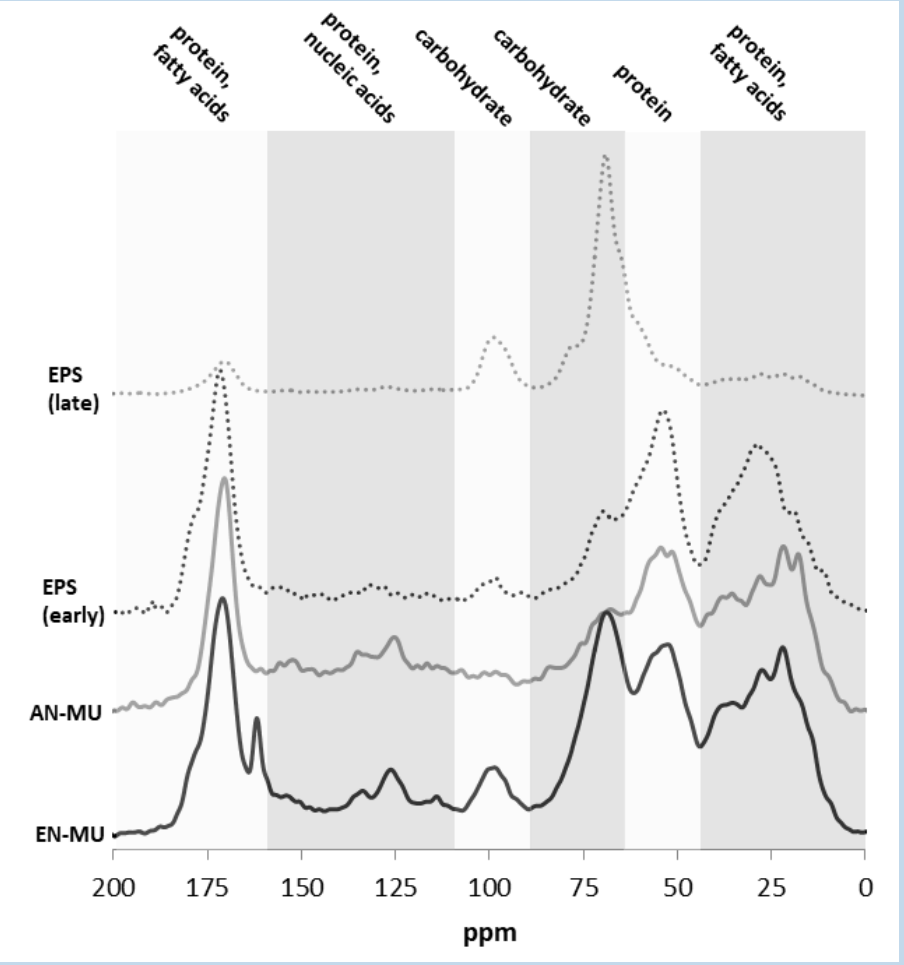


Fig. Guhra and Stolze et al. (2020): Solid-state 13C-nuclear magnetic resonance spectra of anecic *L. terrestris* mucus (AN-MU), endogeic *A. caliginosa* mucus (EN-MU), extracellular polymeric substances (EPS) of Bacillus subtilis obtained from the early stationary phase (early) (Liu et al., 2013) and from the late stationary phase (late) (Guhra et al., 2019). Integral regions are shown by alternating grey values and classified according to Metzger et al. (2009).

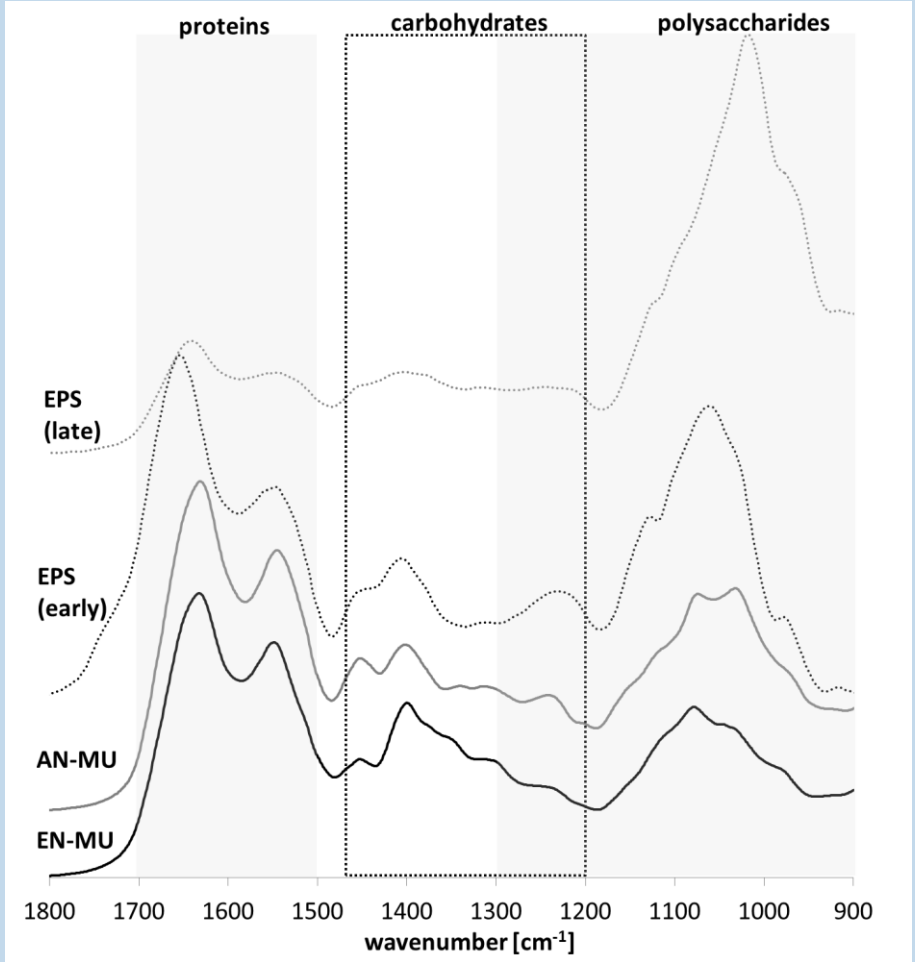


Fig. Guhra and Stolze et al. (2020): Attenuated total reflection – Fourier-transform infrared spectroscopy (ATR-FTIR) spectra of anecic *L. terrestris* mucus (AN-MU), endogeic *A. caliginosa* mucus (EN-MU), extracellular polymeric substances (EPS) of Bacillus subtilis obtained from the early stationary phase (early) (Liu et al., 2013) and from the late stationary phase (late) (Guhra et al., 2019). Main composites were classified in proteins (1700 to 1500 cm -1), carbohydrates (1460 to 1200 cm-1) and polysaccharides as well as nucleic acids (1300 to 900 cm-1) according to Jiao et al. (2010) and Socrates (2010).

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Results and Discussion

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Mucus adsorption to secondary soil minerals

Illite (Il):

- Samples showed an **increase of the P:C** and **S:C ratios** → phosphorous-containing MU constituents as well as sulphur-containing (e.g. cysteine and methionine) are unfavoured for adsorption
- Adsorption during **electrostatically unfavourable conditions** implies additional adsorption processes e.g. hydrophobic interactions and hydrogen bonding

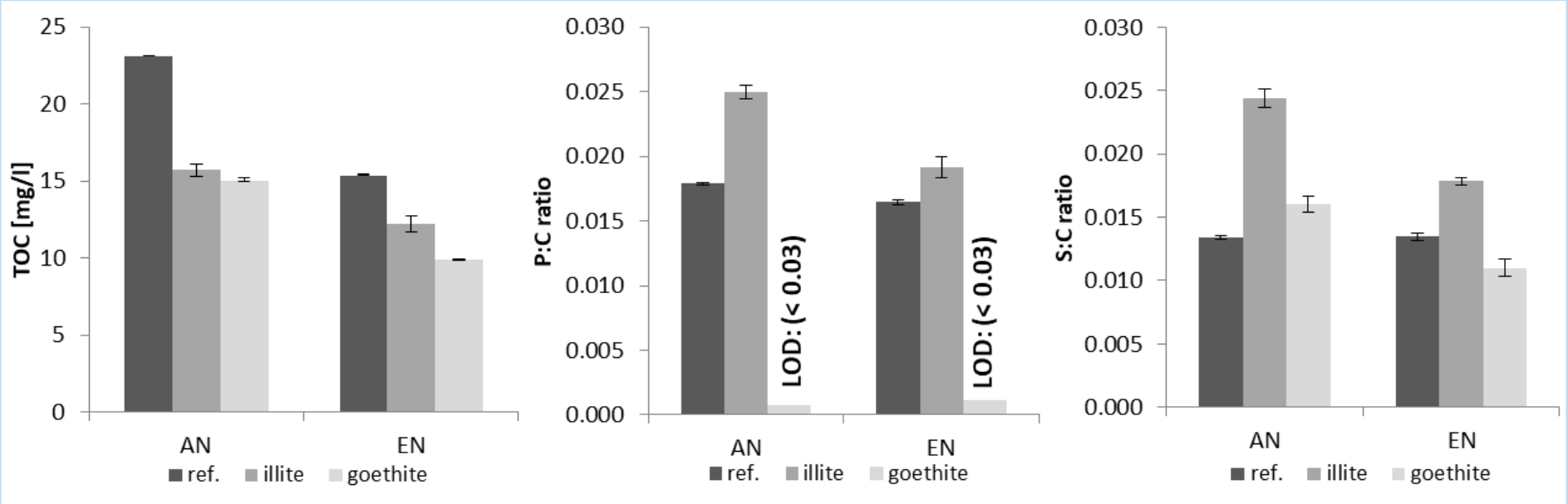


Fig. Guhra and Stolze et al. (2020): Total organic carbon (TOC) and inductively coupled plasma optical emission spectrometry (ICP-OES)-measured P and S from the mineral phase separated supernatants after batch adsorption experiments of treatments containing anecic *L. terrestris* (AN-MU) and endogeic *A. caliginosa* (EN-MU) mucus. The reference (ref.) is assigned to the mineral-free solution which was processed in the same way then the other treatments. The P:C ratio and S:C ratio was calculated for mineral-free and -containing treatments of AN and EN-MU. For the calculation of the P:C ratio of the goethite-containing treatment the limit of detection (LOD) of the device was used for the estimation for P concentration.

Results and Discussion

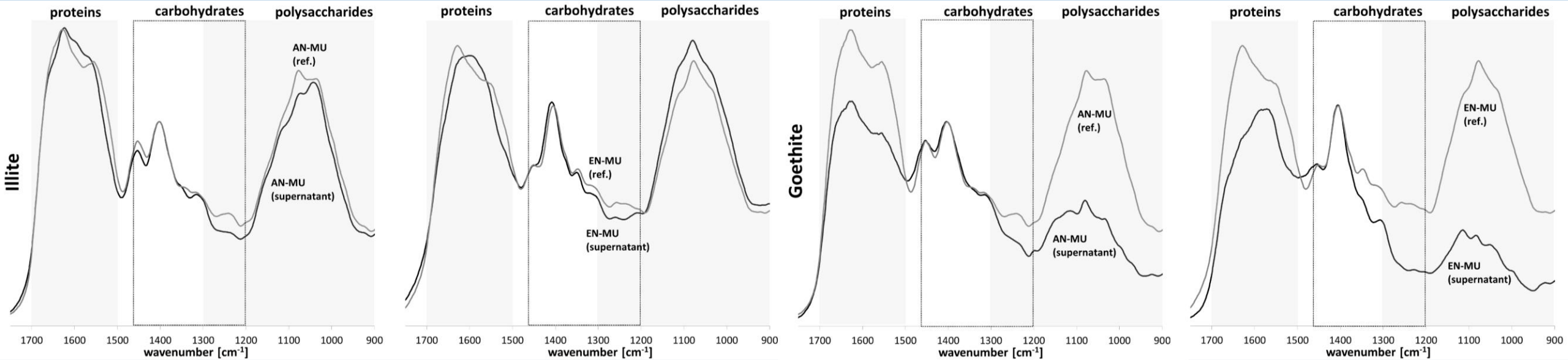
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Mucus adsorption to secondary soil minerals

Goethite (Gt):

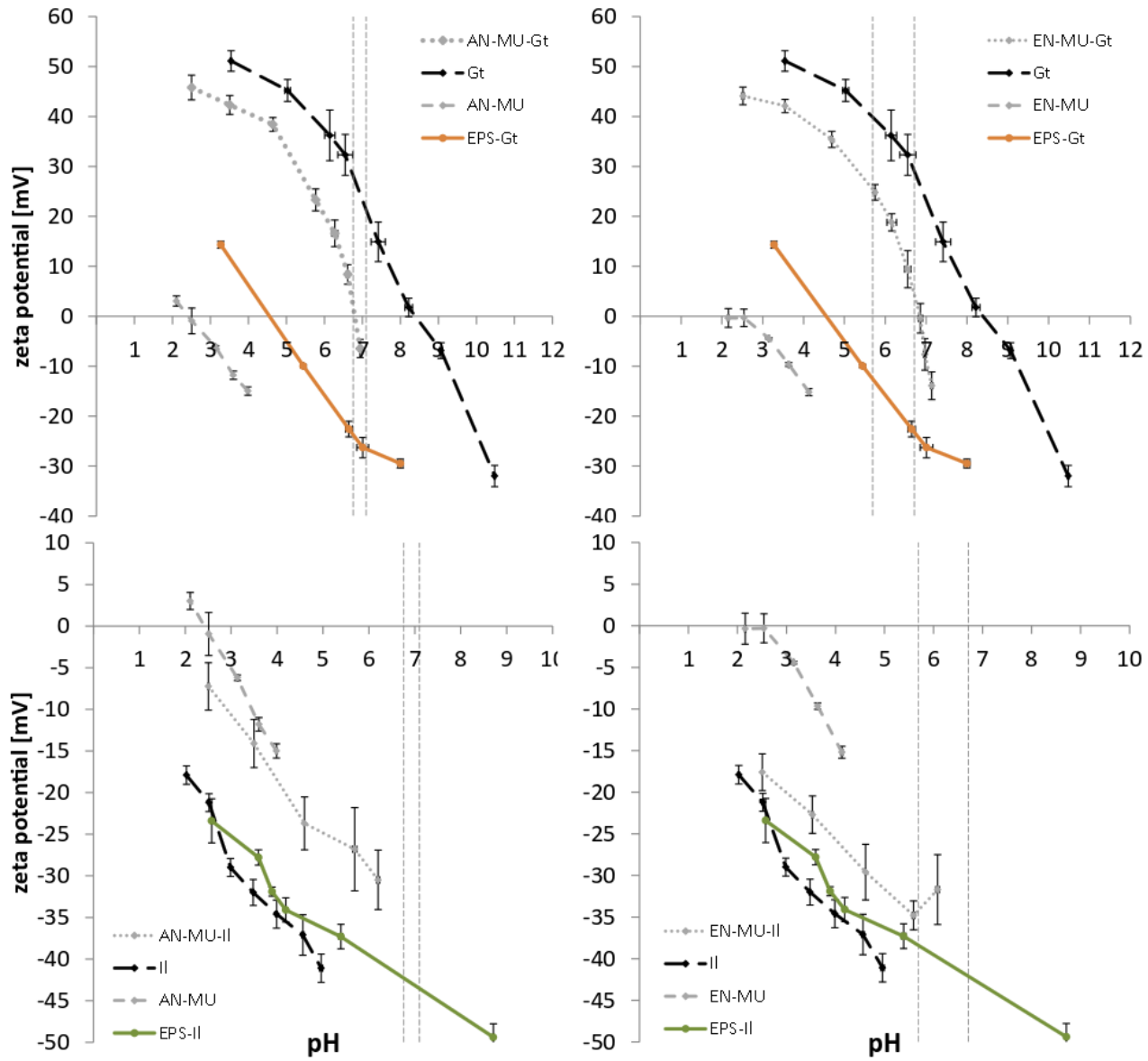
- Dominant adsorption of **proteins**, **polysaccharides** and **nucleic acids** in relation to carbohydrates → contribution of carboxylic groups for the adsorption to Gt is not evident
- Low P:C-ratio → **preferred adsorption of phosphorus**-containing mucus constituents
- **Increased pH** due to adsorption → formation of Fe-O-P bonds under the release of surface hydroxyl groups

Fig. Guhra and Stolze et al. (2020): Attenuated total reflection – Fourier-transform infrared spectroscopy (ATR-FTIR) spectra of the anecic *L. terrestris* (AN-MU: left) and endogeic *A. caliginosa* (EN-MU: right) mucus treated (black) with goethite (Gt: top) and illite (Il: bottom) as well as reference (ref.) without a mineral treatment (grey). For comparison of spectra, intensities were normalized to the carboxylic group (1402 cm-1). Main composites were classified in proteins (1700 to 1500 cm -1), carbohydrates (1460 to 1200 cm-1) and polysaccharides as well as nucleic acids (1300 to 900 cm-1) according to Jiao et al. (2010) and Socrates (2010).



Results and Discussion

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The formation of organo-mineral associations

- mucus-mineral associations with goethite are formed under electrostatic attractive and with illite under repulsive conditions → **specific adsorption**
- Associations were stable against subsequent washing → potentially **preserved against elution** and enhanced dwell time inside soil system
- Surface charge is altered in comparison to the bare mineral phases → mucus coating of mineral surfaces
- late stationary phase EPS screen the surface charge of goethite more and of illite less efficient then mucus

Fig. Guhra and Stolze et al. (2020): Zeta potential measurements as function of pH for bare minerals (black dashed line), mucus (grey dashed line) and mucus-mineral association (grey pointed line) for anecic *L. terrestris* (AN-MU) mucus (left) and endogeic *A. caliginosa* (EN-MU) mucus (right) as well as for goethite (Gt) (top) and illite (Il) (bottom). Areas between vertical grey dashed lines indicate pH during the experiments. For comparison we add the EPS-goethit (EPS-Gt: brown) and EPS-illite (EPS-Il: green) associations found in Guhre et al. (2019).

Conclusions

EJSS paper: Guhra et al. (2019)
SBB paper: Guhra and Stolze et al. (2020)

- earthworm cutaneous mucus of *L. terrestris and A. caliginosa* **similar in their chemical composition**, adsorption behavior and ability to form organo-mineral associations as microbial EPS of the early stationary phase → the functions of earthworm mucus in soil could be consistent and predictable
- adsorption of earthworm cutaneous mucus **depended on the type of soil mineral**, which could determine how much and which fraction of mucus becomes associated → formation of mucus–mineral associations
- **preferential adsorption** of the organic **carbon** and organic **phosphorus** compounds in the mucus on soil minerals could enrich the newly-formed organo-mineral associations with biogenic nutrient elements → contributes to nutrient redistribution throughout the soil profile
- unassociated mucus potentially **remains in the soil liquid phase** → available for microbial metabolism or transport in the soil pore system
- **Bioturbation**: active soil processing of anecic (vertical preferential flowpaths formation) and endogeic (horizontally burrowing and OM-enriched aggregate formation) → mucus deposition throughout the soil profile

Sources

EJSS paper: Guhra et al. (2019)
SBB paper: Guhra and Stolze et al. (2020)

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