Effect of hydrolysate as soil additives on soil properties

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Introduction

- Agriculture impacts soil health positively (crop rotation, cover cropping, organic farming) and negatively (overuse of chemical fertilizers, pesticide use, soil erosion).

- Soil additives play an important role in enhancing soil quality and crop productivity.

- Hydrolysates are primarily used as biostimulants, to improve plant growth and resilience, but can also be used as soil additives.

- The application of hydrolysates can improve soil health and productivity by enhancing its physical structure, chemical properties, and biological activity.

Specific objectives of research

The main focus was to test the effects of hydrolysate as a soil additive in loamy sand and sandy loam soils:

- in two states (liquid and solid)

- two application rates (4 t dw ha^{-1} (low application rate) and 8 t dw ha^{-1} (high application rate)) on the:

a) Physical properties

b) Chemical properties

c) Microbial properties

Hypothesis and predictions

- (1) The effects of the solid state will be stronger and last longer than that of the liquid state (due to lower availability)

- (2) The effects of the high application rate will be stronger and last longer than those of the low application rate (due to higher availability)

- (3) The effects of hydrolysate on soil properties will be stronger in sandy loam than loamy sand soil (due to the finer texture, which is susceptible to hydrolysate adsorption).

Experimental site

- Soils were collected from two fields under conventional farming near České Budějovice (Czech Republic; 49°01′38″N, 14°27′51″E and 49°02′13″N, 14°27′46″E, respectively) in March 2022.
- Soils were collected from 0-10 cm depth at 10 locations in a 0.25-ha area.
- The collected soils were thoroughly mixed, passed through a 2-mm sieve, and stored at 4 °C before being used in the experiment.

Materials and methods

- **Material:**
- The hydrolysate was prepared from the waste chicken feather with water content of approximately 35% wt.
- Exactly 2 kg of feather was put into the reactor of 25 L volume together with 100 g of malic acid and 15 L of water, and the batch was heated to the temperature of 115-125 °C.
- After 5 hours, the reactor was cooled down and the reaction product separated by filtration to liquid hydrolysate and solid residue (below 3% wt).
- Approximately 15-17 L of liquid hydrolysate was prepared from one batch and stored at 4 °C before use.
- A part of the hydrolysate was freeze dried and stored as solid in a dry and dark location before use.

The chemical properties of the soils and hydrolysate are listed in Table 1

Table 1. The properties of the soils and hydrolysate

Soil/hydrolysate	OMC ($g.g^{-1}$)	DOC ($mg.g^{-1}$)	DN ($mg.g^{-1}$)	DP ($\mu g.g^{-1}$)	pH	Cmic ($\mu g.g^{-1}$)	Nmic ($\mu g.g^{-1}$)	WHC (%)
Loamy sand	0.04 ±0.00 a	0.03 ±0.01	0.02 ±0.00 b	3.91± 0.34 b	6.57 ± 0.07	680.92± 43.61	43.96± 0.17 b	54.88± 3.35
Sandy loam	0.05± 0.00 b	0.03±0.0	0.01± 0.00 a	1.76± 0.05 a	6.60 ± 0.09	735± 58.25	38.28± 1.89 a	52.13± 8.19
Hydrolysate	0.88± 00	310.34± 5.18	100.10± 1.55	5.67± 0.10	4.07 ± 0.01	--	--	--

Values are means ± SEM (n=3). Chemical properties of the loamy sand and sandy loam soil and hydroisolate.
Different lowercase letters in a column indicate significant differences among means based on T-test ($P < 0.05$).

Methodology for soil analysis

- Organic matter (**OM**) content was determined based on loss on ignition at 450 °C for 5h
- Dissolved organic C (**DOC**), dissolved N (**DN**), and dissolved P (**DP**) were extracted in deionized water (dH_2O) (1:10 sample:dH_2O ratio) and analyzed in leachates using a TOC-$L_{CPH/CPN}$ analyzer (Shimadzu) (DOC and DN) and spectrophotometry according to Murphy and Riley (1962) (DP).
- **pH** was assessed in a 1:10 sample: dH_2O suspension using a glass electrode.
- Microbial biomass C and N (**C_{mic} and N_{mic}**) were extracted using the fumigation-extraction method (Vance et al., 1987)
- Water holding capacity (**WHC**) was calculated as the difference between the weight of a sample saturated with water over 1h and allowed to drain over 3h and the weight of an oven-dried sample divided by the weight of the oven-dried sample.

Methodology for soil analysis

- Physical fractionation was used to separate the soil samples into three organic and/or mineral fractions as described in Kellerová et al. (2024).

- **Free particulate** OM; i.e. the fPOM fraction

- **Light fraction** represented POM

- **Heavy fraction** represented mineral-associated OM (the MAOM fraction).

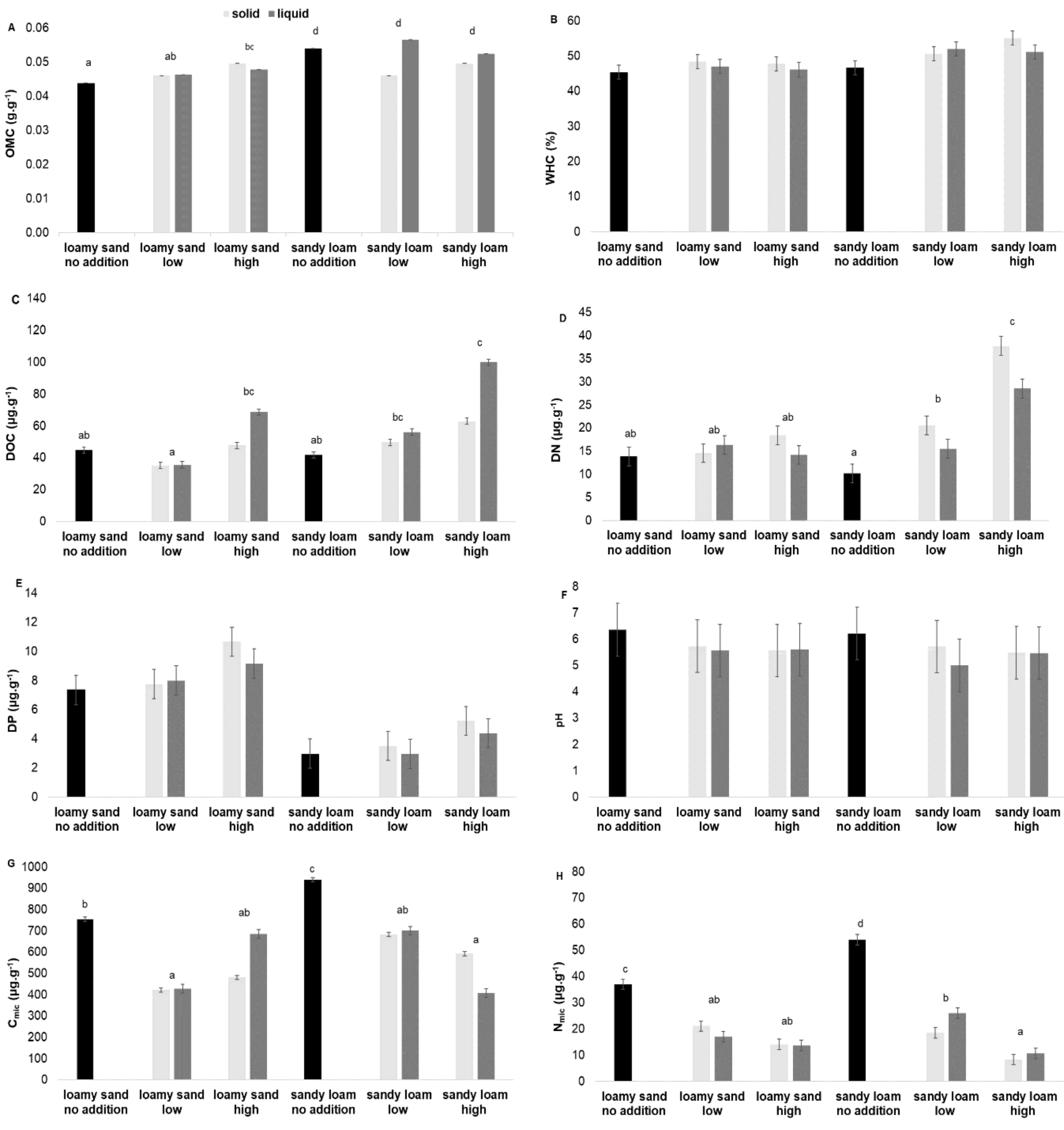
Methodology for soil analysis and statistical analysis

- All three fractions were washed thoroughly with dH_2O until the conductivity decreased below 5 µS for the POM fractions and below 50 µS for the MAOM fraction. The fractions were dried at 40 °C to a constant weight, ball milled and analysed for TOC using a TOC-LCPH/CPN model TOC analyser coupled with an SSM-5000A solid sample module (Shimadzu).

- Data were evaluated by two-factor factorial ANOVAs using PC software Statistica CZ v. 12 (StatSoft). Vertical bars denoted standard error. Results of factorial ANOVAs for effects of soil type (ST), application rate (AR), and state (S) and their interactions on soil properties were estimated with Tukey test at $p<0.05$.

Results

Fig.1 The effect of hydrolysate on soil properties

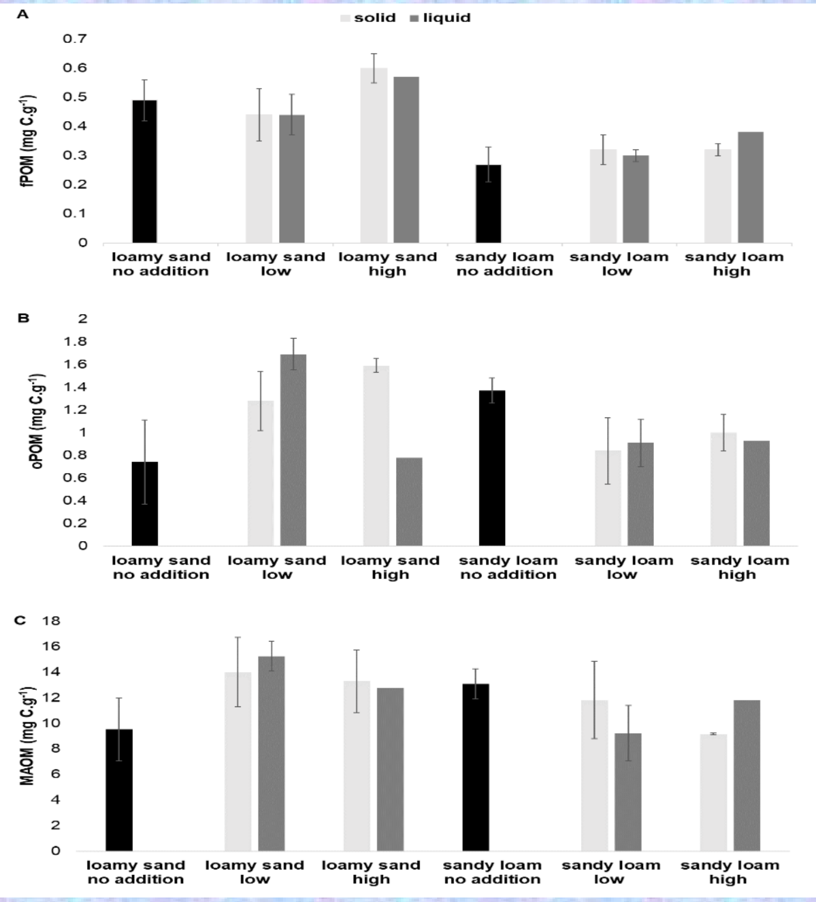


Organic matter (OM) was 15% higher in sandy loam than loamy sand. In loamy sand, hydrolysate application raised OM by 25% (low rate) and 27% (high rate) compared to no application (Fig 1A). For sandy loam, the low rate increased OM by about 2%, while the high rate reduced it by approximately 7% compared to no application. Water holding capacity (WHC) was 8% higher in sandy loam than loamy sand. WHC increased with higher application rates: in loamy sand, by 5% (low) and 3% (high); in sandy loam, by 9% (low) and 13% (high) compared to no application (Fig 1B). Dissolved organic carbon (**DOC**) content was unaffected by soil type but increased with application rate (Fig 1C).The high rate yielded 35% more DOC than the low rate and 38% more than no application. In loamy sand, DOC decreased 20% at the low rate and increased 30% at the high rate. Dissolved nitrogen (**DN**) was 34% higher in sandy loam than loamy sand (Fig 1D). The high application rate produced the most DN, with 48% and 105% increases compared to low and no application, respectively. At the high rate in sandy loam, DN was 103% higher than in loamy sand. Dissolved phosphorus (**DP**) was 128% higher in loamy sand than in sandy loam (Fig 1E). DP was lowest with no application and highest with the high application rate, which increased DP by 34% over the low rate and 44% over no application. Soil **pH** was unaffected by soil type but decreased with higher application rates. No application resulted in the highest pH, 10-14% higher than low and high rates ((Fig 1F). In loamy sand, pH was 12-13% higher with no application compared to low and high rates; in sandy loam, it was 16% and 13.5% higher.

Cmic content was 19% higher in sandy loam than loamy sand, with no application yielding the highest levels, 54-57% above high and low rates (Fig 1G). In loamy sand, Cmic dropped 43% at the low rate and 22% at the high rate. In sandy loam, Cmic decreased 26% at the low rate and 46% at the high rate. **Nmic** was 22% higher in sandy loam, with the highest levels at no application, 120% and 289% above low and high rates. In loamy sand, Nmic fell by 48% at the low rate and 62% at the high rate, while in sandy loam, it dropped by 58% and 82%, respectively (Fig 1H).

Results

Fig 2. TOC content in loamy sand and sandy loam soil type



Total organic C (TOC)

Free particulate OM content (fPOM) was affected by soil type and application rate. The content of **Free particulate** (fPOM) in loamy sand soil was 66% higher for compared to sandy loam (Fig.2A). Regarding application rate it was 24% and 25% higher with high application rate compared to low and no aplication rate. **Light fraction** (oPOM) was affected by soil type and it was 6% higher in loamy sand soil than sandy loam (Fig.2B). MAOM fraction was not affected by any parameters (Fig.2C).

Supplementary table 1.

Source of variance	df	OMC ($g.g^{-1}$)	DOC ($\mu g.g^{-1}$)	DN ($\mu g.g^{-1}$)	DP ($\mu g.g^{-1}$)	pH	Cmic ($\mu g.g^{-1}$)	Nmic ($\mu g.g^{-1}$)	WHC (%)	fPOM (mg $C.g^{-1}$)	oPOM (mg $C.g^{-1}$)	MAOM (mg $C.g^{-1}$)
ST	1.36	75.97 ***	3.47 NS	13.59 ***	338.46 ***	2.84 NS	5.766 *	5.56 **	15.71 **	18.28 **	5.26 *	4.13 NS
AR	2.36	1.76 NS	11.48 ***	26.99 ***	27.9 ***	60.9 2 ***	17.69 ***	81.74 ***	7.16 *	4.97 *	0.35 NS	0.24 NS
S	1.36	0.04 NS	1.69 NS	3.80 NS	2.91 NS	0.10 NS	0.094 NS	0.14 NS	1.04 NS	0.00 NS	0.29 NS	0.01 NS
ST × AR	2.36	5.28 *	3.67 **	18.21 ***	0.70 NS	0.10 NS	4.37 *	7.27 *	2.30 NS	1.37 NS	1.18 NS	0.22 NS
ST × S	1.36	0.67 NS	0.89 NS	1.9 NS	0.008 NS	0.23 NS	1.32 NS	1.17 NS	0.00 NS	0.17 NS	0.30 NS	0.01 NS
AR × S	2.36	0.50 NS	0.66 NS	2.03 NS	1.99 NS	0.11 NS	0.04 NS	0.04 NS	1.06 NS	0.11 NS	3.50 NS	0.28 NS
ST × AR × S	2.36	2.11 NS	2.13 NS	0.50 NS	0.67 NS	0.09 NS	2.11 NS	0.59 NS	0.62 NS	0.48 NS	2.22 NS	1.15 NS

Results of factorial ANOVAs for effects of soil type (ST), application rate (AR), and state (S) and their interactions on soil properties. F values are shown and *, **, and *** indicate significance at $P < 0.05$, < 0.01, and < 0.001, respectively; NS indicates $P > 0.05$.

Conclusion

- **Soil properties were generally affected by the soil type and application rate.**
- **The content of OM was increased in loamy sand for low and high application rate.**
- **WHC was increased in both soil type with low and high application rate compared to no application rate**
- **DOC content was affected by application rate. The highest content of DOC was found at high application in loamy sand, while in sandy loam, both low and high application rate increased significantly compared to no application rate.**
- **DN content was higher in sandy loam than loamy sand soil. The highest DN content was found at high application rate in both soil type.**
- **DP content was higher in loamy sand compared to sandy loam. The lowest DP content was found with no application rate, while with the high application rate it was the highest content of DP.**
- **Soil pH was affected by application rate. The highest pH was found with no application rate in both soil types.**
- **C_{mic} content was higher in sandy loam than loamy sand. The highest content of C_{mic} was found with no application rate**
- **N_{mic} content was higher in sandy loam soil 22% compared to loamy sand. The highest content was found in no application rate**
- **TOC content (fPOM was affected by soil type and application rate, oPOM was affected by soil type, and MAOM fraction was not affected by any parameters**.

THANK YOU FOR ATTENTION