µCT monitoring of growth of *S. commune* in artificial pore space

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Objective

RENA project: Development of ex-situ bioremediation strategies for radionuclide and heavy metal contaminated soils.

Fig. 1: Mycoremediation of soil. [1]

- Related reactive transport models require a comprehensive physico-chemical database
- The growth of fungi within pore space on µm-scale has not been monitored *in-situ* so far
- State of the Art µCT/XRM should enable monitoring and quantification of fungal growth processes as well as caused pore space modifications

XRM/µCT

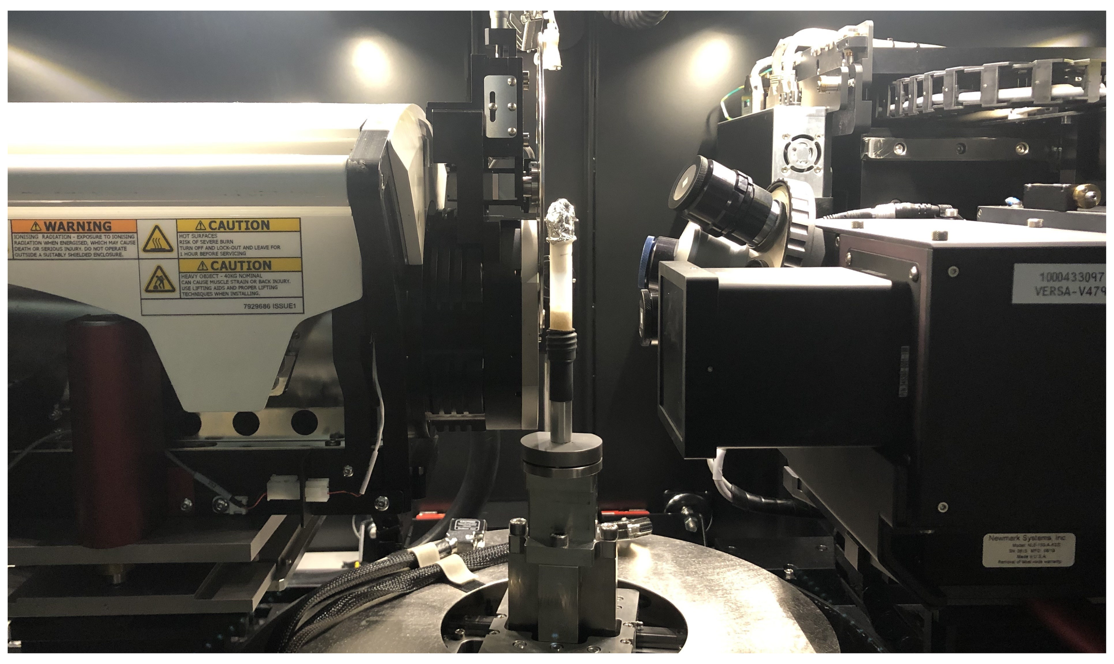

Fig. 2: Internal set up of Zeiss Xradia 620 Versa: X-ray source (left), sample holder (middle) and detector (right).

- XRM: high-resolution non-destructive 3D imaging of internal structures in the sub-micrometer range
- Xradia 620 Versa: a two-stage magnification architecture using high flux beam source technology as well as a high-array detector enable high resolution of fine details. [2]
- Data treatment: Dragonfly 4.1

Proof of concept — Artificial soil consisting of silica beads compared to grain size distribution of real soil material:

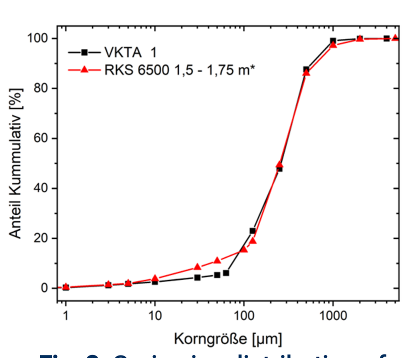

Fig. 3: Grain size distribution of soil material relevant for RENA.

Table 1: Silica beads composition to compile representative artificial soil.

Size [mm]	Fraction [%]
0.25 – 0.5	75
0.75 – 1.0	10
1.25 – 1.65	10
1.7 – 2.2	5

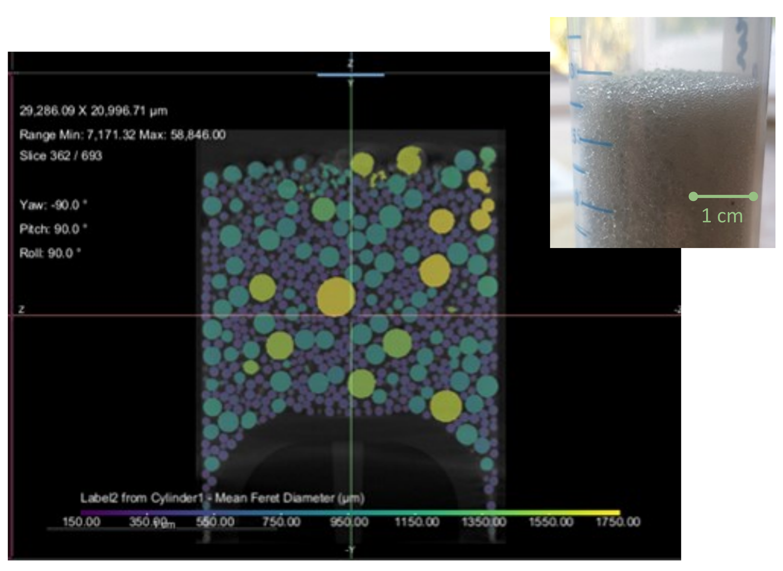

Fig. 4: XRM visulisation of artificial silica matrix with water satured pore space. Grains labeld in different colours according to size. Images recorded with 0.4x objective, resolution: 19.5 µm, image size 3 mm. Small picture up right: silica beads composition.

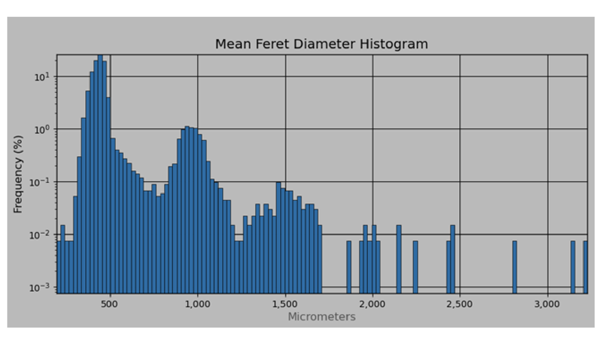

Fig. 5: Calculated Grain size distribution from XRM images.

➔ Validation of experimental set up and verification of grain size distribution from XRM data

Growth of *S. commune* and image segmentation

Sample preparation

Fig. 6: *S. commune* 12-43 x 4-39 cultivated on Agar plate (left) by Maximilian Herold. Sample inoculated with 1cm x 1cm section from agar plate (right).

Table 2: Composition of nutrient medium.

Chemical	m [g]
Glucose	2
Aspartate (Aspartic acid)	0.2
K_2HPO_4	0.2
KH_2PO_4	0.05
$MgSO_4$	0.05
Thiaminium dichloride	$12 \cdot 10^{-6}$

XRM/µCT— Measurement parameter

Table 3: Measurement settings applied for all shown experiments.

Measurement	Objektive	Voltage [kV]	Filter	Exposure time [s]	Pixel size [µm]	Projections
Overview scan	0.4x	80	LE 4	1	19.5	1601
Fungal growth monitoring	4x	80	LE 6	2	1.8	1601

XRM/µCT Monitoring of fungual growth

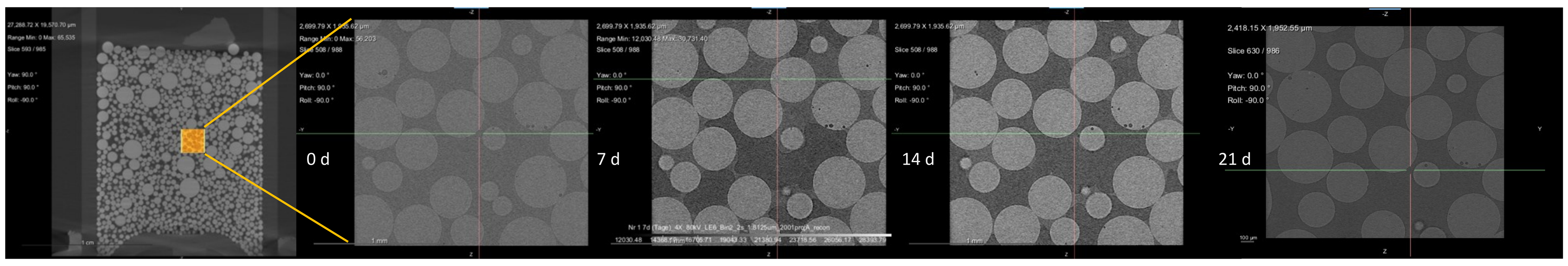

Fig. 7: Monitoring of growth over 3 weeks. Different gray values correspond to density differences. Sample before inoculation of *S. commune* (0 d). After 7 and 14 days expanding fungal biomass is visible. After 21 days pore space is completly occupied.

Histographic vs. Machiene learning segmentation

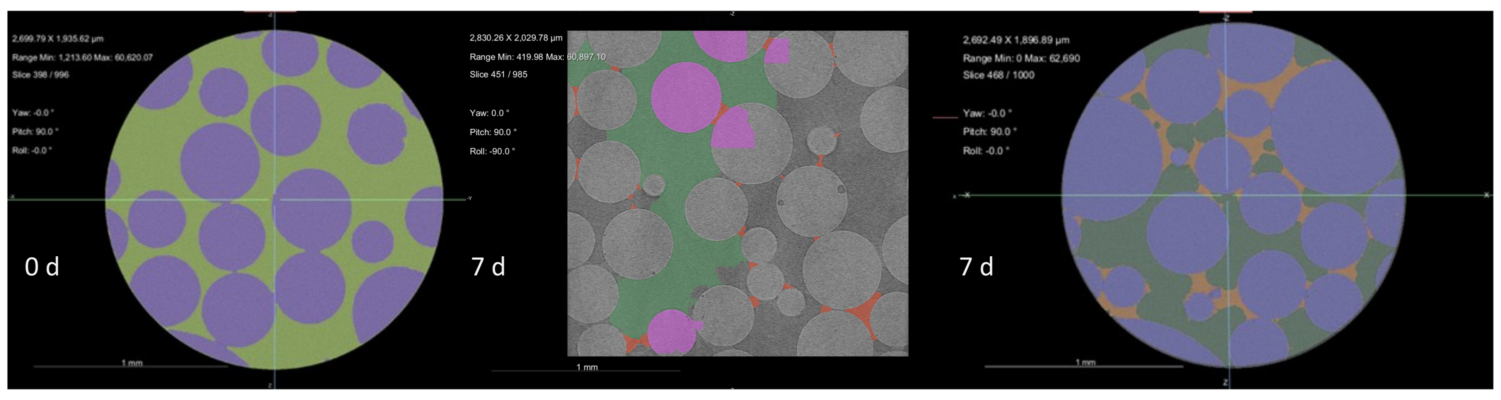

Fig. 8: Left: Histographic segmentation of grey values used for two parameter systems (0 d/21d). Middle: Labeled pixels on a slide to feed segmentation trainer for meachine learning. Right: Manuelly corrected meachine learning Segmentation used for three parameter systems (7 d/ 14d).

Machiene learning segmentation:

- Labeling pixels by interactive "painting" on the dataset to train a classifier, wich can assign the remaining pixels by the intensity, Algoritmus: ExtraTrees
- Error correction by manual post-processing with morphological operators: Smoothing, Dilate, Erode and Close

Biomass quantification

Table 4: Percentages of grains, nutrient medium and fungus over 21 days fungal growth.

Proportion	0 d	7 d	14 d	21 d
Grains [%]	62.9	64.3	65.3	64.6
Nutrient medium [%]	37.1*	20.0	3.6	-
Fungus [%]	-	15.7	31.1	35.4

*Comparable pore volume to sandy soils (37,0 – 39,5 %).[3]

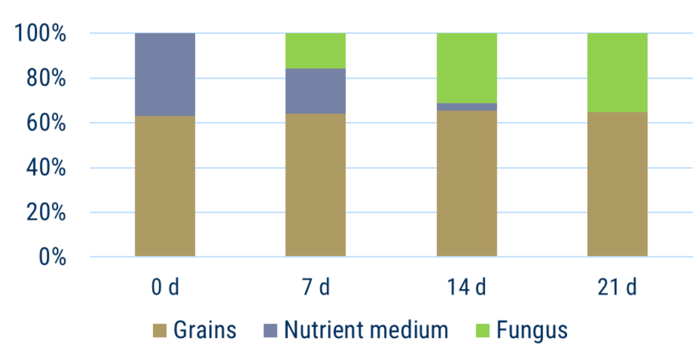

Fig. 9: Fractions of grains, nutrient medium and fungus over 21 days fungal growth.

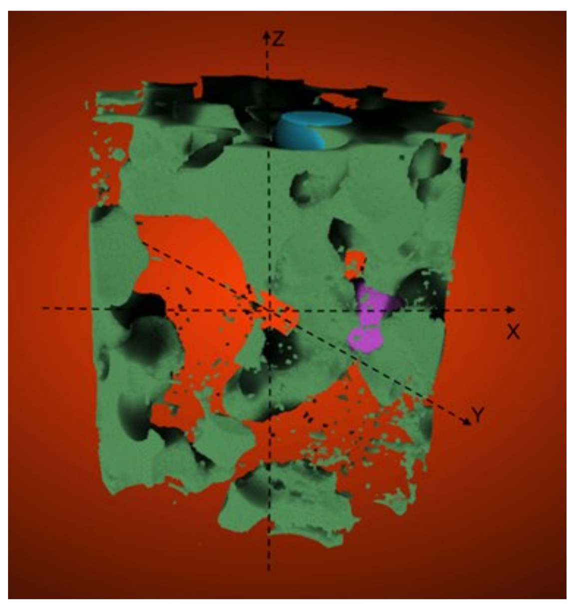

Fig. 10: 3D visualisation of the positions of grain A (blue) and grain B (purple) within the fungal mass (green) after 7 d of fungal growth. Resolution: 1.8 µm.

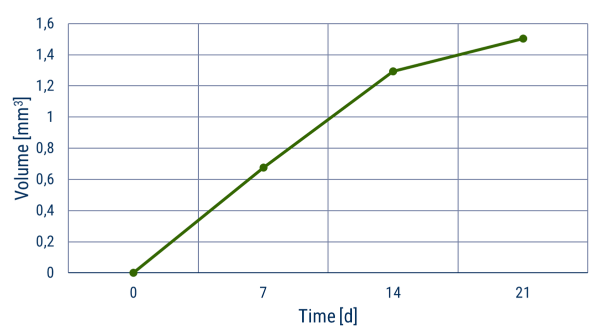

Fig. 11: Volume of biomass over 21 days fungal growth.

Grain shifts

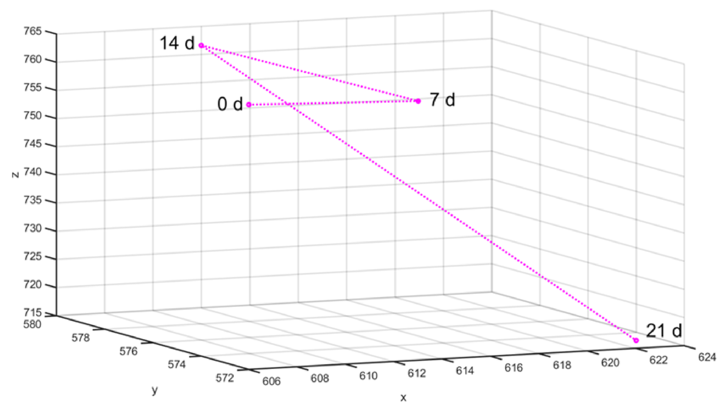

Fig. 12: Voxel positions of grain B in relation to grain A (coordinate origin) over fungal growth.

Table 5: Relative movement of grain A and B in regard to each Other.

Grain B		X	Y	Z
Distance to Grain A before fungal growth		353	923	225
	7 d	15	4	5
Relative shift	14 d	20	-2	5
	21 d	-156	-455	-107

CONCLUSION

- Despite the small density difference the growth of *S. commune* was monitored and the biomass was quantified via XRM/µCT.
- After 21 days the pore space was completely occupied by fungual biomass.
- Increasing grain shifts due to growing biomass according to decreasing open pore space.
- The transferability to real soil materials is assumed to be reasonable.

OUTLOOK

- Calculation of pore radii distribution
- Adaption on real soil material
- Smaler sample geometries to enable higher mycel resolution
- Application on mapping of transport experiments with Nanoparticels

REFERENCES
[1] https://educalingo.com/de/dic-en/mycelium
[2]https://www.zeiss.de
[3] AG Boden, 1994: Bodenkundliche Katieranleitung, 4. Auflage Schweizerbart, Stuttgart.

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