

Microscale spatial distribution and soil organic matter persistence in top and subsoil

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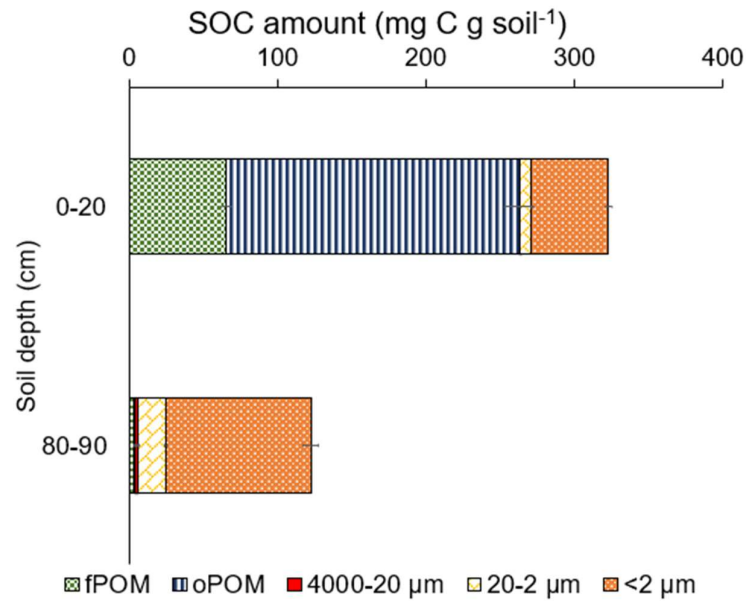
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Supplementary Figures



Supplementary Fig. 1: **Soil organic carbon (SOC) amount in different fractions of the top and subsoil samples used in the experiment.** Different composition emphasizes the presence of light fractions in the topsoil (fPOM = free particulate organic matter; oPOM = occluded particulate organic matter) and mineral heavy fractions in the subsoil (20-2 and < 2 μm mineral fractions). Error bars indicate the standard error between three technical replicates. Further soil properties are listed in Table 1.

¹³C and ¹⁵N enriched amendments

Hot-spot OM
(1 - 2 mm)

¹³C = 1.77 atom %

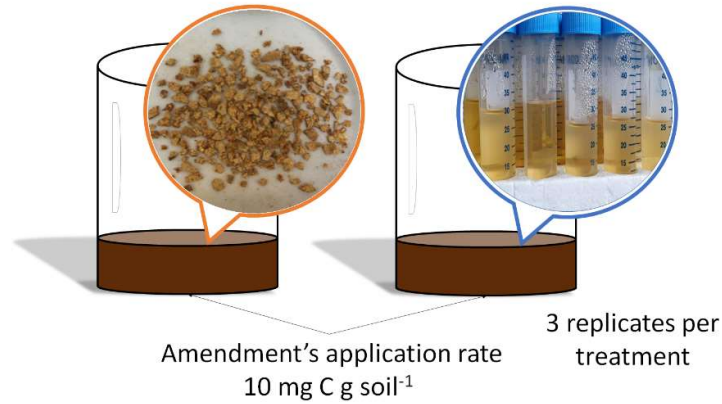
¹⁵N = 7.59 atom %

Distributed OM
(< 0.7 μm)

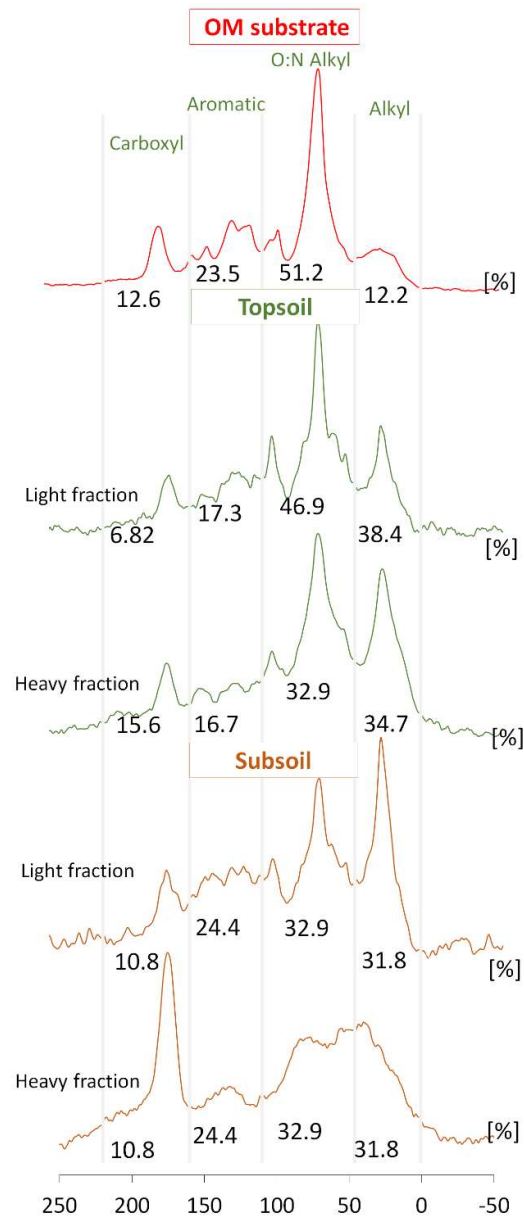
¹³C = 1.77 atom %

¹⁵N = 7.62 atom %

C: 300 mg g⁻¹, N: 12 mg g⁻¹, C:N ratio: 25, pH (CaCl₂): 4.6

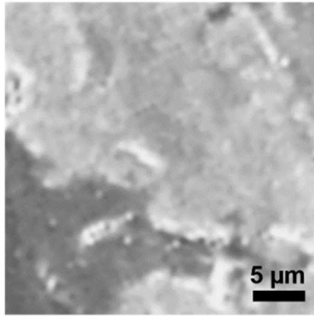


Supplementary Fig. 2: **Summary figure of the incubation experiment.** The two units represent the C inputs used in the incubation as follows: 1 – Hot-spot organic matter (OM); and 2 – Distributed OM. The OM was extracted from the willow leaves by shaking them in water for 72h at 32°C. After this period, the material was filtered with a 0.7 μm filter and freeze-dried. The point source OM treatment was pelleted in 1-2 mm size pellets and the distributed OM treatment was re-suspended in water.

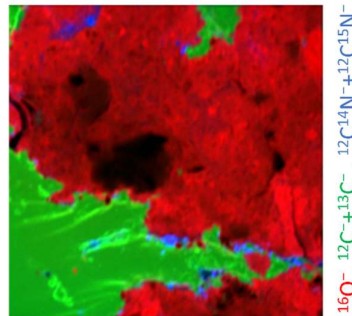


Supplementary Fig. 3: ^{13}C NMR spectra and integrals. Here we present the light and heavy fraction of control samples from the top (0 – 0.2 m) and subsoil (0.8 – 0.9 m) before the incubation experiment and the microbially derived organic matter (DOM) used in the experiment as amendments before the incubation.

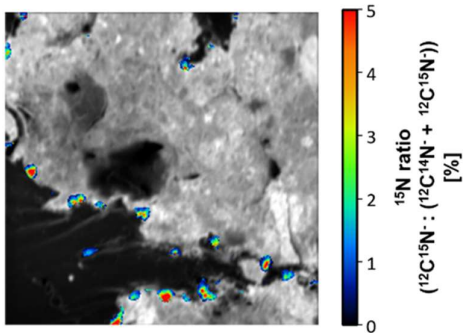
(a) Scanning electron microscopy



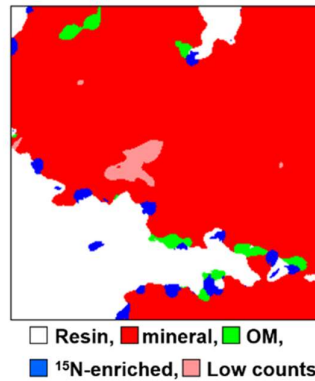
(b) OM-related NanoSIMS measurement



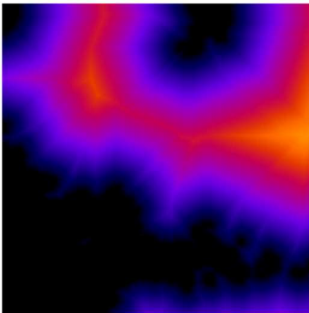
(c) ¹⁵N enrichment (¹⁶O⁻)



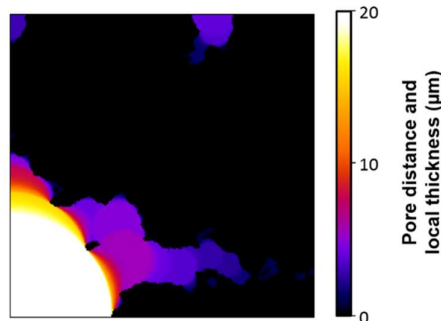
(d) OM-related image segmentation



(e) Pore distance



(f) Local pore thickness



Supplementary Fig. 4: **Workflow of the NanoSIMS image analysis.** After (a) preliminary scanning electron microscopy analyses, measurements of ion distributions were conducted using nanoscale secondary ion mass spectrometry (NanoSIMS) to reveal (b) the distribution of $^{16}\text{O}^-$ (red), $^{12}\text{C}^-+^{13}\text{C}^-$ (green), $^{12}\text{C}^{14}\text{N}^-+^{12}\text{C}^{15}\text{N}^-$ (blue) and (c) overlay of the ^{15}N enrichment on the $^{16}\text{O}^-$ distribution (grey). (d) The ion distributions were used to compute the image segments of mineral surfaces, OM, and resin based on a machine-learning algorithm. (e,f) Distance into the soil matrix based on Euclidean distance maps according to the resin-filled pore distribution.

Supplementary Video Legend

Animated version of the 3D data from Fig. 3. The composite 3D image shows C, Si, and Al (red, green, and blue color, respectively) distribution across a sectioned topsoil microaggregate measured by energy-dispersive X-ray spectroscopy (EDX). The Figure was created using Image J (version 1.8.0_172 <https://imagej.nih.gov/ij/download.html>)