Supplementary material for

Podzolisation affects the spatial allocation and chemical composition of

soil organic matter fractions

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Figure S1: Soil profiles of successive podzolisation in north-west Germany and their classification according to IUSS Working Group WRB (2015).

Fractionation of soil organic matter

For fractionation, 100 g of soil (< 2 mm) was added to 250 ml of water and dispersed using a calibrated ultrasonic probe (Ultrasonic Processor, UP200S, Hielscher Ultrasound Technology, Teltow, Germany) with an output-energy of 22 J ml⁻¹. The dispersed suspension was wet-sieved (63 µm sieve) until the rinsing water was clear.



Figure S2: Modified bulk soil fractionation scheme (s + c, silt and clay; rSOC, resistant soil organic C; S + A, sand and sand-sized aggregates; POM > 63 μ m, particulate organic matter > 63 μ m; POM < 63 μ m, particulate organic matter < 63 μ m; DOC, dissolved organic carbon; adapted from Zimmermann *et al.* 2007).

The fraction > 63 µm was dried at 40 °C, weighed and subsequently suspended in a sodium polytungstate (Na₆(H₂W₁₂O₄₀), TC-Tungsten Compounds, Grub am Forst, Germany) solution adjusted to a density of 1.8 g cm⁻³. The suspension was centrifuged at 230 *g* for 15 min and left standing for a few days until a separation of the heavy fraction of sand and sand-sized aggregates (S + A) and the floating light particulate organic matter (POM) occurred. The light POM > 63 µm fraction was then aspirated, and both fractions were washed with deionised water to remove all Na₆(H₂W₁₂O₄₀), dried at 40 °C and weighed.

The suspension < 63 μ m was pressure-filtered through a 0.45 μ m cellulose nitrate filter (Sartorius, Göttingen, Germany). The filtrate constituted the fraction of dissolved organic C (DOC). Following the original protocol (Zimmermann *et al.* 2007), the fraction between 0.45 and 63 μ m represented the mineral silt and clay (s + c) fraction. Due to the blackish colour of the s + c, caused by plant debris (Fig.

S3), we carried out a second density fractionation of the s + c fraction at a density of 1.8 g cm⁻³, like for the fraction > 63 μ m. The original fraction between 0.45 and 63 μ m was, thus, divided into a heavy fraction < 63 μ m consisting of silt- and clay-sized particles (s + c) and a light organic fraction (POM < 63 μ m).



Figure S3: Scanning electron microscope image of the silt and clay fraction from the EA horizon of P5.

A chemically resistant carbon fraction (rSOC) was obtained from the s + c fraction by treatment with sodium hypochlorite (NaOCI). Hence, 50 ml of a NaOCI solution (6%), adjusted to pH 8 with concentrated HCI, was added to 1 g of the s + c fraction and kept at room temperature for 18 h. The residue was centrifuged at 230 g for 15 min, decanted, washed with deionised water, and centrifuged again. This procedure was repeated three times in total. The residual fraction was dried at 40 °C. The initial and residual sample masses were recorded to assess the losses by the treatment.



Figure S4: Examples of diffuse reflectance infrared spectra of particulate organic matter (POM) fractions from the AE horizon of P2. Intensities at the marked wavenumbers were used to calculate ratios as indicators of SOM composition.

Reference

Zimmermann M, Leifeld J, Schmidt MWI, Smith P, Fuhrer J (2007) Measured soil organic matter fractions can be related to pools in the RothC model. *European Journal of Soil Science* **58**, 658– 667. doi:10.1111/j.1365-2389.2006.00855.x