Supplementary material

Comparison of commonly used extraction methods for ergosterol in soil samples

Appendix A: Methods

1. Alkaline extraction

According to Bååth (2001), 1 g soil sample was mixed with 5 ml of 10% methanolic potassium hydroxide (KOH) solution in a 50 ml centrifuge tube and sonicated for 15 min. Lipids in the sample were subsequently saponified to release esterified ergosterol into free form by placing the tube in a 70°C water bath for 60 min. Between each step, the sample was thoroughly mixed using a vortex for 5 s. After cooling down in the dark to prevent photolysis, 1 ml MilliQ water and 2 ml of cyclohexane were added into the tube for sample elution and vortexed for 1 min. For phase separation, the mixture was centrifuged at 3000 rpm for 10 min at 20°C. The organic phase was collected in an amber glass vial. For podzol, the mixture was vortexed again and centrifuged once more for 2 min before the collection. The sample was eluted twice to obtain maximum ergosterol yield. The combined organic phase was evaporated on a heating block at 40°C under nitrogen gas (N_2) steam until completely dry. The vial was quickly removed from the heating block due to heat sensitivity of ergosterol. Ergosterol was resolved by well mixing with 0.5 ml methanol (MeOH) subsequently heated at 40°C for 15 min. The podzol sample with brownish colorization was diluted by 1:5 dilution factor. The resolved sample was filtered through 0.45 µm polytetrafluoroethylene (PTFE) syringe filters into 2 ml amber high-pressure liquid chromatography (HPLC) vial and stored at 4°C prior to HPLC analysis.

2. Glass bead methods

By modified method from Gong *et al.* (2001), 2 g soil sample and 4 g glass beads (comprising 2 g 212-300 µm and 2 g 710-1180 µm) were mixed with 10 ml MeOH in a centrifuge tube for the normal scale extraction. After vortexed for 10 s, the sample tube was beaten with bead-based homogenizer for 10 s 4 times. Gas was released by loosening the tube cap after each beating to avoid overpressure. The sample tube was centrifuged at 3000 rpm and 20°C for 10 min. For turbid samples (podzol soil), 1 ml supernatant was transferred into an Eppendorf tube for further centrifugation at higher speed of 10000 rpm and 20°C for 10 min. The clear supernatant from podzol was diluted with 1:10 dilution factor due to its brownish colorization. The supernatant with minimum volume 500 µl was transferred into an amber HPLC vial by using 0.45 µm PTFE syringe filter. All samples were stored at 4°C prior to HPLC analysis.

For the miniaturized extraction, the amount of sample was decreased to 0.16 g and the glass beads to 0.8 g (comprising 0.4 g 212-300 µm and 0.4 g 710-1180 µm) in a 2 ml Eppendorf screw tube. After adding 800 µl MeOH, the centrifuge tube was vortexed for 10 s and sequentially beaten for 60 s. The soil mixture was once centrifuged at 10000 rpm and 20°C for 10 min. The turbid supernatant was transferred into an Eppendorf tube and centrifuged at the same speed for further 10 min. Dilution and filtration of the supernatant as well as the storage were the same as for the normal scale.

3. Ultrasonication methods

The prepared sample (1:5 soil:MeOH (weight to volume ratio, w/v)) for the ultrasonic bath was vortex for 10 s and then placed in a rack in the middle point of the ultrasonic bath to expose to equal energy. The sample was sonicated for 15 min subsequently vortexed for 10 s and centrifuged at 4000 rpm and 20°C for 10 min. One milliliter of

supernatant was syringe filtered through a 0.45 µm PTFE membrane into an amber HPLC vial. However, 1 ml supernatant from turbid sample was transferred to an Eppendorf tube and centrifuged at 10000 rpm for 10 additional min prior to filtration. Podzol sample was diluted to 1:10 dilution prior to the filtration due to the brownish colorization of the supernatant. All samples were at stored 4 °C to prevent degradation of ergosterol before HPLC analysis.

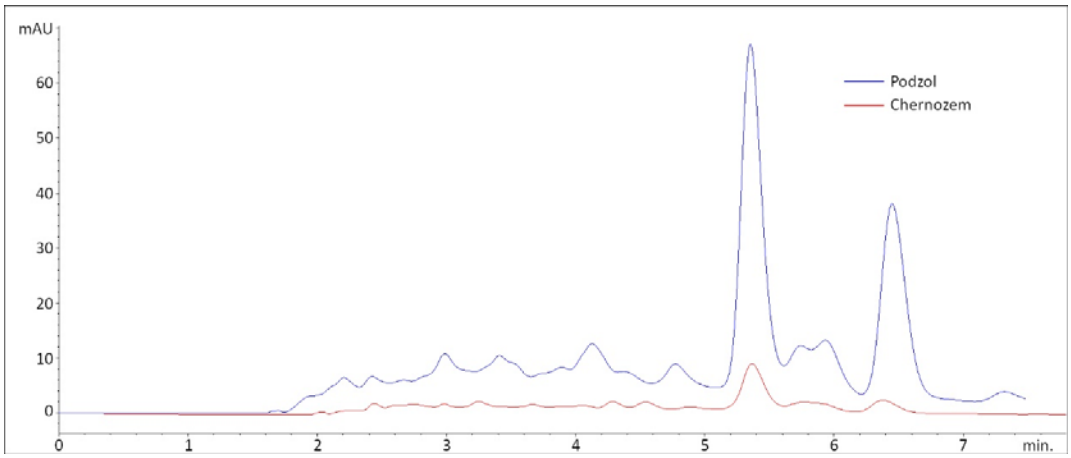
For the high energy ultrasonic probe variation, the commercial high amplitude probe (SONOPULS, BANDELIN, Berlin, Germany) with a cylindrical tip was set at 45% energy, the maximum input energy possible, with that we could obtain 38.3 W power output. An in-house ultrasonic probe described in Schomakers *et al.* (2015) was operated at 5 µm amplitude with frequency 19 kHz to produce 24.4 W for the low energy variation. The output energy was calibrated by measuring the temperature increase in 200 ml of deionized water in 15 s intervals over 2 min. The sample was strapped in a circular strainer and lowered into a 1.5 l bowl water bath (Appendix Fig. 1). The probe was set to 1 cm depth into the water bath with equidistant to all samples. The runtime was 15 min. After ultrasonication, the sample was centrifuged at 4000 rpm and 20°C for 10 min. Samples were diluted and filtrated as mentioned above for the approach using the ultrasonic bath.

Appendix **Fig. 1.** Ultrasonic probe and samples set up.

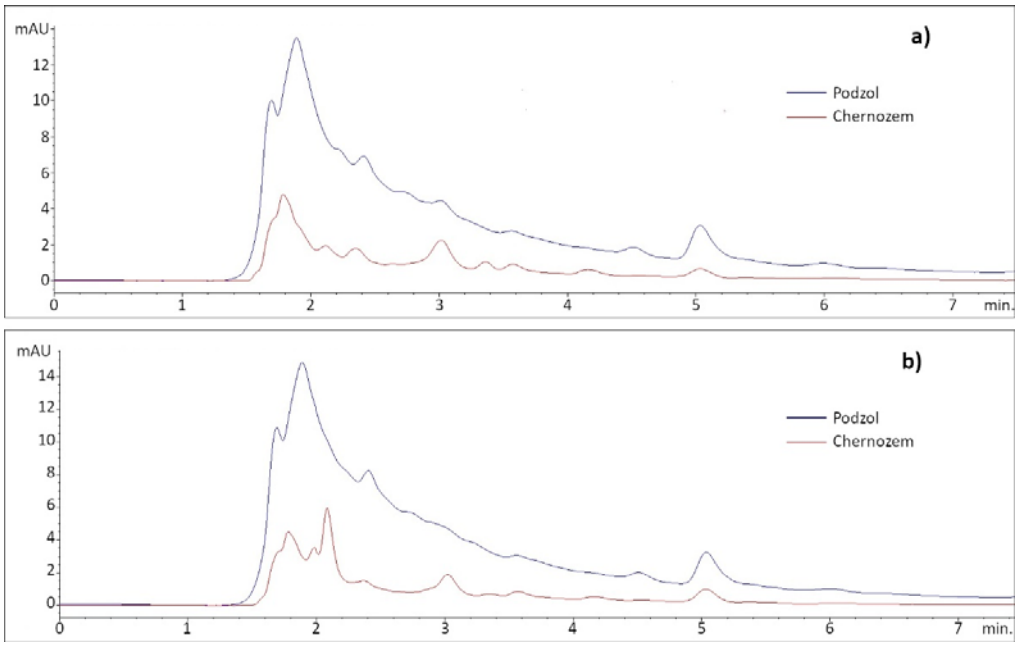
4. Simple shaking

Following the same soil:solvent ratio (1:5 w/v), 2 g soil sample was mixed with 10 ml MeOH. The sample was shaken overnight (min. 12 h) with an overhead shaker as modified from the soil dispersion methods (Mentler *et al.*, 2004). The mixture was centrifuged at 4000 rpm and 20°C for 10 min. The brownish supernatant from podzol was diluted by 1:10 before filtration. One milliliter of supernatant was transferred into an amber HPLC vial by using 0.45 µm PTFE syringe filter. All samples were stored at 4°C before HPLC analysis.

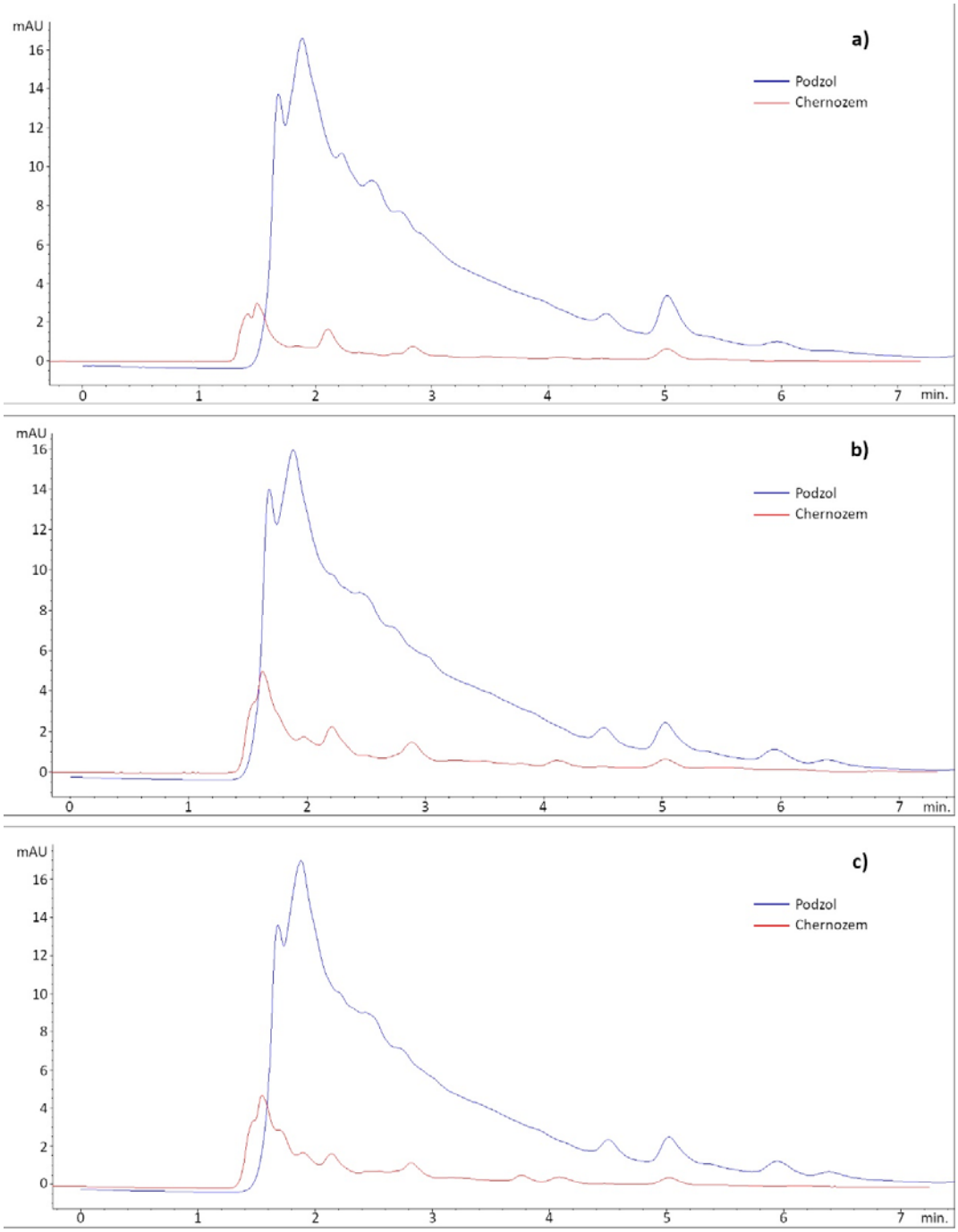
Appendix B: High-pressure liquid chromatography (HPLC) chromatograms



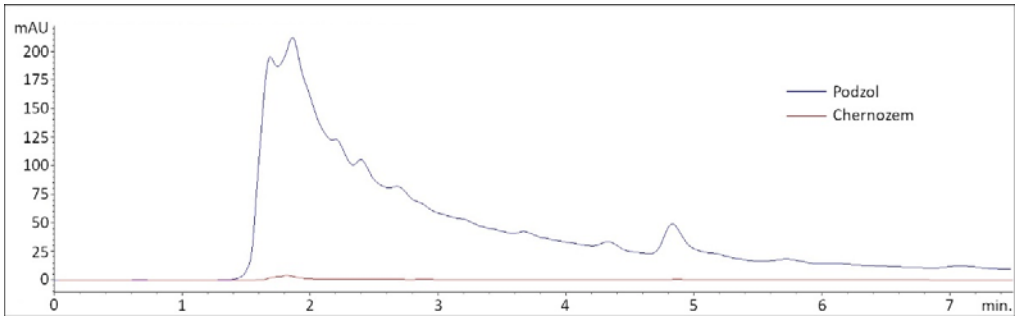
Appendix **Fig. 2**. HPLC chromatograms for ergosterol determination extracted by the alkaline method in chernozem and podzol. The x-axis indicates the retention time in minutes, the y-axis the ultraviolet (UV) light absorption.



Appendix **Fig. 3.** HPLC chromatograms for ergosterol determination extracted by the normal scale and the miniaturized glass bead methods (a) and b), respectively) in chernozem and podzol. The x-axis indicates the retention time in minutes, the y-axis the UV light absorption.



Appendix **Fig. 4.** HPLC chromatograms for ergosterol determination extracted by the ultrasonication assisted method: a) ultrasonic bath, b) high energy ultrasonic probe and c) low energy ultrasonic probe in chernozem and podzol. The x-axis indicates the retention time in minutes, the y-axis the UV light absorption.



Appendix **Fig. 5.** HPLC chromatograms for ergosterol determination extracted by the simple shaking method in chernozem and podzol. The x-axis indicates the retention time in minutes, the y-axis the UV light.