SUPPORTING INFORMATION

Organic matter identifies the nano-mechanical properties of native soil aggregates

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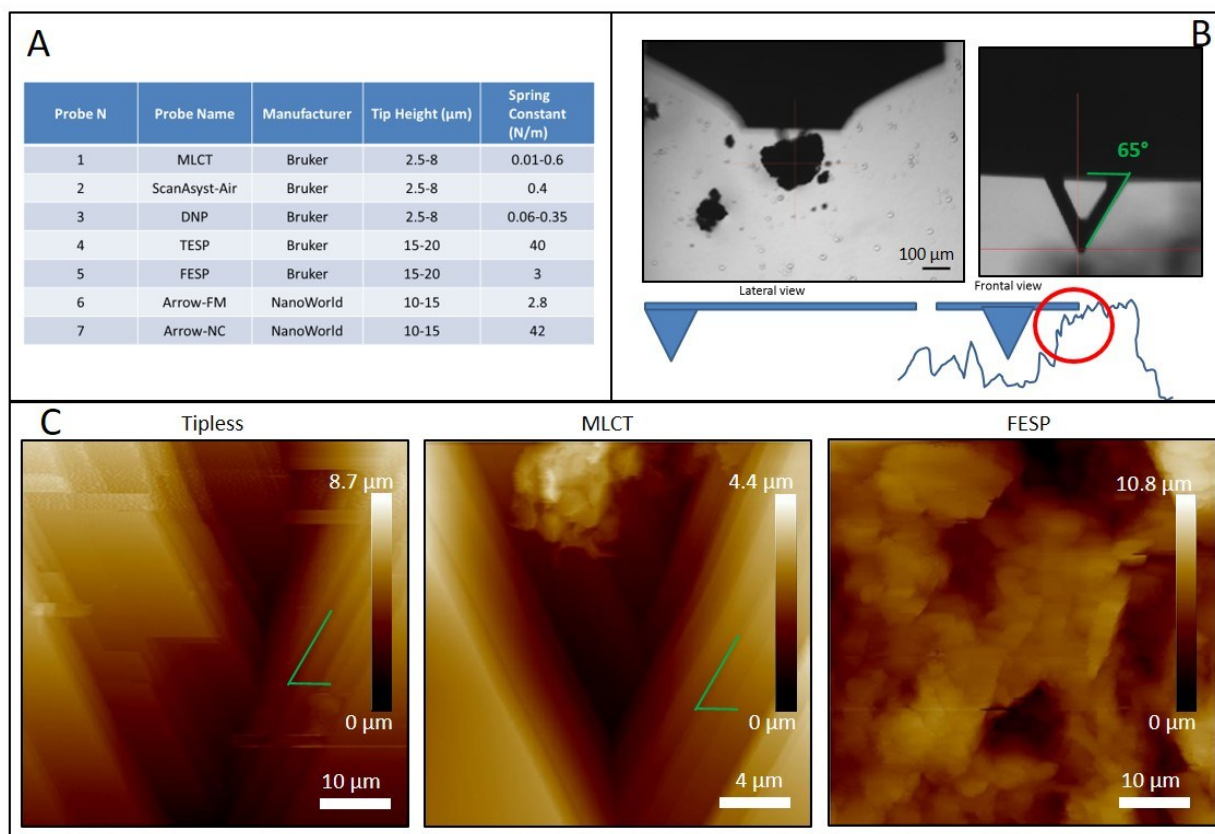
Supporting Figures

Figure S1. Performance of different cantilevers for soil aggregates. A) A list of the different probes tested, with manufacturers' values. The range of values reported for tip height is due to the inherent variability in manufacturing process, while the range of values for spring constant is due to the presence of several cantilever in the same chip having different spring constants. B) Clockwise from top-left corner, this panel represents: optical image of a cantilever positioned in the centre of a soil aggregate, which was previously immobilized on a thin layer of epoxy resin; detail of an MLCT cantilever, with the angle between the base and the cantilever; a schematic of the possible steric hindrance created by soil irregularities during cantilever scans. C) Scanning performance of different cantilevers (from left to right): a cantilever without tip produces images of terraces with the same angle reported in B; MLCT cantilevers often produce images similar to the ones of tipless cantilevers, demonstrating that the tip is unable to reach and scan the soil surface; cantilevers with longer tips are able to scan the soil surface in a more effective way and to produce images deprived of artefacts.
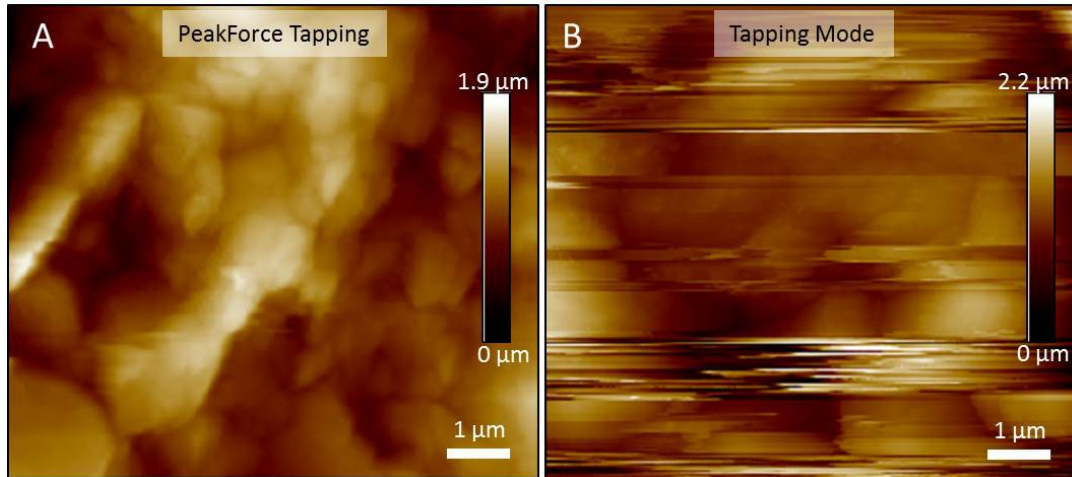
Figure S2. Performance of PeakForce Tapping (PFT) and Tapping Mode (TM) scanning. A) Peakforce Tapping is able to properly scan the soil surface, while Tapping Mode often produces streaks where the cantilever tip is unable to folllow the irregular soil surface.
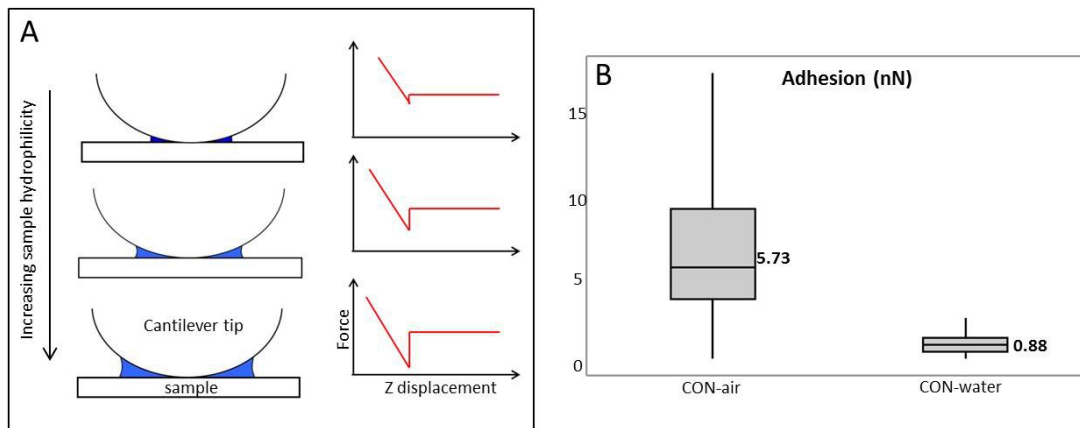

Figure S3. Surface hydrophilicity and adhesion pull-off force. A) In air an aqueous meniscus (blue in the schematic) forms between the cantilever tip and the scanned surfaces. Higher hydrophilicity increases the water meniscus and the associated pull-off force of the cantilever tip. B) Adhesion values measured on 30 soil CON areas in air (as already reported in Fig. 3D) and on 8 soil CON areas in water. The decrease in adhesion force in water is attributable to the absence of a meniscus force between the soil surface and the cantilever tip.

Supporting Tables

Coordinates	51°34'48"N 4°08'25"W
Particle Size Distribution	44.0 (±0.2)% sand 50.1 (±0.2)% silt 5.8 (±0.02)% clay
Texture	Silty loam
Organic matter (%)	5.60 ± 0.02
Total N (%)	0.210 ± 0.003
Total C (%)	3.20 ± 0.01
pH	6.27 ± 0.05
Bulk Density (g cm^{-3})	1.18 ± 0.11

Table S1. Properties of Cefn Bryn soil used in this study.

Materials and Methods

Soil Collection, Characterization and Storage.
Soil was collected from under semi-natural grasslands at Cefn Bryn, Gower, Wales (51°34'48"N 4°08'25"W). A 5 x 5 m grid was laid out, five of the 25 squares were randomly selected (using a random number generator) for sampling, and within each of the 5 squares, a 50 cm square of turf was cut away on 3 sides and the soil from 5 to 10 cm depth was collected. Soil samples from all 5 squares were mixed in large plastic bags, sieved to 2 mm and thoroughly homogenised, and then stored at 4°C until testing. Soil characterization was conducted by Forest Research-Alice Holt Research Station (Surrey, UK). Particle size distribution was measured on pooled bulk soil using a Malvern Mastersizer 2000 (Worcestershire, UK) following organic matter removal by hydrogen peroxide flushing. Nitrogen and carbon was measured according to ISO methods 13878 and 10694, respectively, using a Thermo Scientific FlashEA® 1112 Nitrogen and Carbon Analyzer (Massachusetts, USA). Organic matter was measured using loss on ignition (LOI) technique. Soil pH was measured in water using a Spectrum Technologies Inc. IQ 150 pH meter (Illinois, USA). All soil physicochemical properties, reported in Table S1, are expressed as mean ± Standard Error of the Mean (SEM).

Acid-Peroxide Cleaning. Soil aggregates were cleaned from organic matter using the protocol reported in Mortlock and Froelich, 1989[32]. Briefly, 5 mL of 10% H_2O_2 is added to soil (between 25 to 200 mg) in a Falcon tube, and after 30 min, 5 mL of 1N HCl solution is added. The tube is then sonicated and left at room T for 30 min. 20 mL of deionized water is added and the tube is centrifuged at 4300 *g* for 5min. After supernatant removal, the soil sample is left to dry overnight at 60°C.

Goniometry and WDPT. Air dry, sieved (<2 mm) and homogenised soil samples were used for all goniometry and WDPT measurements, and all tests were carried out in ambient laboratory conditions (20-22°C and 35-50% RH).

For microlitre goniometry, three samples of each sample were prepared by filling small plastic weigh boats and levelling the surface. Three 80 µL drops of distilled water were deposited onto each sample, and high resolution videos were obtained using a KRÜSS DSA 25 (Hamburg, Germany). Contact angles for each drop were measured with the KRÜSS software using a linear baseline and a sessile drop (Laplace-Young) curve fit immediately following deposition. A single contact angle for each soil was determined by averaging the replicate contact angles and taking the standard deviation.

Picolitre goniometry samples were prepared by sprinkling the smallest grains of each soil across 4 small (approx. 1 mm^2) squares of double-sided adhesive tape fixed to a microscope slide. The procedure was repeated three times to try to achieve a monolayer, and excess soil was removed by gently tapping the slide on its side after each application. HPLC grade water was dispensed onto the soil surface as 4 nL drops, and high resolution videos were obtained using a KRÜSS DSA 100M (Settings: 60V; 10,000 µs period; 100 µs pulse). Contact angles for each drop were calculated from screenshots of the drop immediately upon deposition using a linear baseline and a sessile drop (Laplace-Young) curve fit. A single contact angle for each soil was determined by averaging the replicate contact angles and taking the standard deviation.

The water drop penetration time (WDPT) of each soil sample type was determined by 30 x 80 µL drops of distilled water. A petri dish was filled with each soil and levelled before applying drops to the soil surface and noting the time from application to infiltration of each drop. A single WDPT was calculated for each soil by averaging all WDPT values. Data has been tested for normality using the Anderson-Darling test and a threshold p value of 0.05. All goniometry and WDPT values are expressed as mean (parametric data)/median (non-parametric data) ± Standard Error of the Mean (SEM).

Sample preparation for Atomic Force Microscopy (AFM). Soil samples were left to equilibrate at ambient conditions (T=21°C, RH≈ 50%) for 24 hours. Soil was gently sprinkled on an area of glass slide previously covered with a thin layer of a two-component epoxy, EPO-TEK 302-3M from Epoxy Technology Inc. (Billerica, U.S.A.), previously left to dry in air for about 6 hours in order to harden the glue enough to not engulf the soil aggregate particles. An optical microscope was used to check that the single aggregates only immobilized and were not engulfed in the epoxy glue. Samples were left to further dry in air for additional 24 hours before AFM analysis. An optical microscope was used to localize the aggregates for AFM scan and to position the cantilever tip above the soil aggregate.

AFM and Quantitative NanoMechanical Analysis (QNM). A Bruker BioScope Catalyst (Bruker Instruments, Santa Barbara, California, USA) was used to scan soil aggregates. MPP-21200-10 cantilevers (FESP, probe n.3 in Figure S1A, Bruker Instruments, Santa Barbara, California, USA) were used, with a nominal spring constant of 3 N/m, a nominal resonant frequency of 75 kHz and a silicon tip with a nominal height of 15-20 µm. The cantilever was calibrated on clean sapphire surface and spring constant and deflection sensitivity determined. All imaging was conducted using Peak Force Tapping Mode (PF-TM) in Quantitative Nanomechanical Mode (QNM) at a scan speed of 1-0.5 Hz. Each image had a pixel resolution between 128x128 and 512x512, and one area per soil aggregate was scanned, with a total of about 30 areas per soil sample. A constant force of 5 nN was applied during scans. PFT-QNM calculated adhesion maps from the force required to detach the cantilever tip during the retraction cycle ("pull-off" force). Stiffness maps were reported as Derjaguin–Muller–Toporov (DMT) and LogDMT maps, where the

modulus was obtained from the approach part of the force curves using the DMT model[21]. Nanoscope Analysis software, v1.50, was used to calculate roughness R_q using the equation:

$$R_q = \sqrt{\frac{\sum Z_i^2}{N}}$$

(1)

where, N is the number of points in the considered area and Z_i is the vertical displacement of each point i from the average data plane. Off-line analysis of topography images consisted of first order flattening and plane fitting. ImageJ® was used to calculate the areas occupied by organic matter. Raw data from QNM AFM outputs was extracted in ASCII format, plotted and analysed using Mathematica 10.0 and Minitab 17. Data has been tested for normality using the Anderson-Darling test and a threshold p value of 0.05. All AFM values are expressed as median ± Standard Error of the Mean (SEM).