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## Role of heavy polar organic compounds for water repellency of sandy soils

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**Abstract** Separation and chemical characterisation of specific compounds responsible for soil water repellency has not previously been achieved. Here we describe the extraction, separation and analysis by gas chromatography-mass spectrometry of organic compounds found in wettable and water repellent sandy soils from the Netherlands and United Kingdom. Fatty acids ( $C_{16}$ – $C_{24}$ ), amides ( $C_{14}$ – $C_{24}$ ), alkanes ( $C_{25}$ – $C_{33}$ ), aldehydes/ketones ( $C_{23}$ – $C_{31}$ ) and complex ring-containing structures were detected in all samples. We found a greater abundance of high molecular mass polar compounds in the water repellent samples.

**Keywords** Hydrophobicity · Water repellency · Soils · Organics · Gas chromatography

### Introduction

Soil water repellency is caused by hydrophobic organic compounds either deposited on soil mineral and aggregate surfaces or present as interstitial matter. These compounds may originate from decomposing soil organic matter, plant root exudates, certain fungal or microbial species, and surface waxes from plant leaves. The nature

of organic compounds suggested in previous studies to cause water repellency include plant and cuticular waxes, alkanes, fatty acids and their salts and esters, phytanes, phytols and sterols (Doerr et al. 2000). Despite significant advances made in previous investigations, sufficient separation and exact chemical characterisation of these compounds has yet to be achieved (Roy et al. 1999; Doerr et al. 2000). Although generic chemical classes have been proposed, their precise chemical structures remain unknown. Furthermore, the occurrence of the compounds involved in water repellency has not been investigated in relation to wettable control samples. Hence the molecular basis of water repellency is still only poorly understood. Our ongoing research is focused on this gap in current knowledge. In order to address this problem in detail we are undertaking a systematic study of extraction and characterisation of organic materials from water repellent and wettable sandy soils from five countries, Australia, Greece, Portugal, the Netherlands and the United Kingdom, comprising a range of locations with differing climates and vegetation cover.

We have previously examined a range of extraction procedures used in published studies and demonstrated that compounds capable of causing water repellency can be extracted from a wide range of sandy soils using a mixture of isopropanol and aqueous ammonia, and that these extracts induce water repellency when applied to acid-washed sand (AWS) (Doerr et al. in press). This present paper presents the results obtained from a comparative study of soils sampled at Ouddorp, the Netherlands and the Gower Peninsula, Wales, United Kingdom. The samples from the Netherlands were taken from the same site at four different depths (0–10, 10–20, 20–30 and 30–40 cm) whereas the samples from the UK were taken from the soil surface (0–5 cm) at adjacent locations. Mixtures of compounds were extracted from the samples using isopropanol and aqueous ammonia. The water repellency of the samples was determined before and after extraction using the water drop penetration time (WDPT) method (Doerr 1998). The ability of the extracts to induce water repellency in acid-washed sand was examined, and their composition analy-

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**Table 1** Soil sample codes and origin, amounts extracted, water repellency levels (WDPT in seconds) before and after extraction, and effect of reapplication of extracts redissolved in chloroform (CHCl<sub>3</sub>) to wettable acid-washed sand (AWS). Samples with the letter *C* denote wettable control soils. WDPT classes (after Bisdom et al. 1993): *nr* non-repellent, *sl* slightly, *st* strongly, *se* severely, *ex* extremely water repellent

Code	Sampled depth (cm)	Amount extracted (g kg <sup>-1</sup> ) <sup>a</sup>	WDPT before	WDPT after	WDPT reapplied
NL1 <sup>b</sup>	0–10	9.8±1.5	st	nr	se
NL2 <sup>b</sup>	10–20	2.6±0.3	se	nr	ex
NL3 <sup>b</sup>	20–30	1.1±0.1	ex	nr	ex
NLC <sup>b</sup>	30–40	0.6±0.2	nr	nr	se
UK1 <sup>c</sup>	0–5	1.2±0.5	se	sl	st
UK2 <sup>d</sup>	0–5	2.4±0.3	st	nr	ex
UKC <sup>e</sup>	0–5	0.20±0.03	nr	nr	sl
AWS <sup>f</sup>	/	/	nr	nr	/
Soxhlet & empty thimble	/	0.01±0.01	/	/	/

<sup>a</sup> Error estimates typically based on five independent measurements

<sup>b</sup> Permanent pasture site sample

<sup>c</sup> Dune herbs and grasses site sample

<sup>d</sup> Grassland (sports turf) site sample

<sup>e</sup> Dune, un-vegetated site sample

<sup>f</sup> Supplied by Riedel-de Haën

sed by gas chromatography-mass spectrometry (GC-MS) of the tetrahydrofuran (THF) sol-uble portions.

## Experimental

### Soil samples

Soils were sampled at Ouddorp, Netherlands, UTM coordinates 51°48'N, 3°54'E; and Gower, Wales, UK, UTM coordinates 51°35'N, 4°06'W, sites which experience an oceanic humid-temperature climate with rainfall occurring throughout the year. Samples were taken from within the root zone of the soils, oven-dried at 20 °C and passed through a 2 mm sieve. All samples were of medium sand texture, predominantly 220–390 μm, with a clay content below 0.1%w. Samples were coned and quartered to reduce sub-sample variability (Doerr et al. in press). Sample characteristics are summarised in Table 1.

### Water repellency assessments

Water repellency was determined using the water drop penetration time (WDPT) method after equilibrating subsamples to a controlled atmosphere of 20 °C and 45–55%w relative humidity for 24 h. Five droplets of distilled water (~80 μL) were placed on the soil surface and the time recorded for droplet penetration. Five descriptive ratings of water repellency were distinguished (Table 1), based on the median time needed for droplet penetration: non-repellent (infiltration within 5 s), slightly (5–60 s), strongly (60–600 s), severely (600 s–1 h), and extremely water repellent (>1 h).

### Extraction and separation of organic compounds

Soil samples (240 g) were Soxhlet-extracted for 24 h using a 2.4 L isopropanol-aqueous ammonia (35%, 0.88

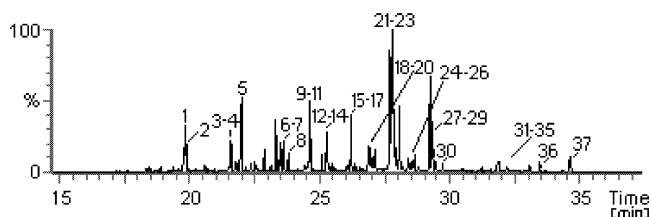
SG) (7/3 v/v) solvent mixture (Doerr et al. in press). Samples were pre-wet with the solvent mixture for 15 min prior to refluxing, as ammonia is lost during the extraction procedure. The extracted material dissolved in the solvent was then filtered, concentrated on a rotary evaporator at 45 °C and taken to dryness on a hot water bath. The dried extract was dissolved in 200 mL water-chloroform (CHCl<sub>3</sub>) (1/1 v/v). The phases were separated, washed with 2×100 mL aliquots of the other solvent and taken to dryness on a hot water bath (Horne and McIntosh 2000). The aqueous phase would contain amphipathic and the more polar material and the CHCl<sub>3</sub> phase the more hydrophobic lipid compounds. In order to establish if any alkanes were lost during the concentration of the extract, standard C<sub>15</sub>, C<sub>18</sub>, C<sub>21</sub>, C<sub>23</sub>, C<sub>26</sub>, C<sub>28</sub> and C<sub>30</sub> alkanes dissolved in tetrahydrofuran (THF) were individually applied to separate portions of AWS. After evaporation of the solvent, the loaded sand samples were subjected to the usual experimental procedure. Only alkanes with ≥23 carbon atoms were recovered.

### Extract re-applications

A portion of the extract was re-dissolved in CHCl<sub>3</sub>, filtered, and reapplied at the same mass ratio as it was extracted to 5 g of wettable (WDPT<5 s) AWS (Ma'shum et al. 1988).

### GC-MS analysis

The CHCl<sub>3</sub> soluble fraction was dissolved in THF and filtered through tightly packed glass wool to remove insoluble particles. Electron impact (EI) mass spectra were then obtained using a Fisons GC8000 gas chromatograph with a ZB5 5% phenyl polysiloxane capillary column (30 m, 0.32 mm i.d., 0.25 μm phase thickness) interfaced with a Fisons Masslab MD800. Splitless, 1 μL injections with helium carrier gas and a temperature program of



**Fig. 1** Analysis of organic extract of soil sample NL3 by gas chromatography-mass spectrometry (GC-MS). **1** C<sub>16</sub> acid, **2** C<sub>14</sub> amide, **3** C<sub>18</sub> unsaturated (*cis*) acid, **4** C<sub>18</sub> acid, **5** C<sub>16</sub> amide, **6** 4,8,12,16-tetramethylheptadecan-4-olide (C<sub>21</sub>H<sub>40</sub>O<sub>2</sub>), **7a** C<sub>20</sub> acid, **7b** C<sub>18</sub> unsaturated amide, **8** C<sub>18</sub> amide, **9a** C<sub>21</sub> acid, **9b** benzamide type compound (unknown formula), **10** C<sub>25</sub> alkane, **11** C<sub>23</sub> aldehyde/ketone, **12** benzene-1,2-dicarboxylate (unknown formula), **13** C<sub>22</sub> acid, **14** C<sub>26</sub> alkane, **15** C<sub>23</sub> acid, **16** C<sub>27</sub> alkane, **17** C<sub>25</sub> aldehyde/ketone, **18** C<sub>24</sub> acid, **19** C<sub>28</sub> alkane, **20** C<sub>22</sub> amide, **21** C<sub>29</sub> alkane, **22** C<sub>27</sub> aldehyde/ketone, **23** unidentified, **24** C<sub>30</sub> alkane, **25a** C<sub>24</sub> amide, **25b** cholesterol/stigmasterol (unknown formula), **26** unidentified, **27** C<sub>31</sub> alkane, **28** cholesterol/stigmasterol (C<sub>29</sub>H<sub>48</sub>O), **29** C<sub>29</sub> aldehyde/ketone, **30** unidentified, **31** C<sub>33</sub> alkane, **32** C<sub>31</sub> aldehyde/ketone, **33a** d-friedoolean-14-en-3-one (C<sub>30</sub>H<sub>48</sub>O), **33b** cholesterol/stigmasterol (C<sub>29</sub>H<sub>50</sub>O), **34** pentacyclic triterpenoid alcohol (unknown formula), **35** stigmasta-3,5-dien-7-one (C<sub>29</sub>H<sub>46</sub>O), **36** d-friedoolean-14-en-3-ol, acetate (3. beta.) (C<sub>32</sub>H<sub>52</sub>O<sub>2</sub>), **37** unidentified

40 °C isothermal for 2 min, then ramped at 10 °C min<sup>-1</sup> to 300 °C and held for 30 min, were used. The injection port was set at 250 °C. Compounds were identified based on retention times, and mass spectral interpretation, using the NIST mass spectral search program and library, together with comparison with authentic compounds where possible.

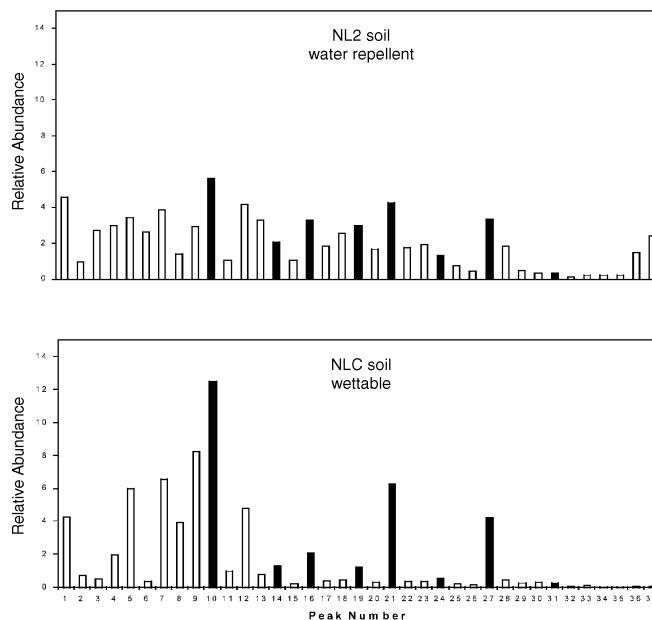
## Results and discussion

### Organic extract and water repellency

Here we comment on the amounts of organic material extracted from soil samples and the relationship with levels of water drop penetration time (WDPT). Significantly less material was extracted from the wettable samples than the water repellent samples (Table 1). A similar difference in extracted amounts between water repellent and wettable soils was observed by Hudson et al. (1994). This is in agreement with the origin of the control samples in this case. What is significant is that for the repellent samples there was no relationship between the amount of organic matter extracted, and the degree of soil water repellency. Below we discuss the separation and identification of the compounds extracted.

### Organic compound identification

A typical chromatogram of soil organic compounds is shown in Fig. 1. The main peaks are numbered 1–37. Representative relative abundance plots for a water repellent (NL2) and wettable (NLC) soil are given in Fig. 2.



**Fig. 2** Distributions of soil organic compounds in NL2 and NLC soil samples. Shaded bars denote alkanes. Relative abundance is calculated on the basis of peak height with respect to the sum of the peak heights of all the compounds shown. Note the greater abundance of high molecular mass polar compounds in the water repellent sample compared with the wettable sample

Five main types of compounds have been identified: fatty acids, amides, alkanes, aldehydes/ketones and complex containing structures.

### Fatty acids

Fatty acids (C<sub>16</sub>, C<sub>18</sub>, C<sub>20</sub>, C<sub>21</sub>, C<sub>22</sub>, C<sub>23</sub> and C<sub>24</sub>), corresponding to peaks 1, 4, 7a, 9a, 13, 15 and 18 respectively, were detected (Fig. 1). Even chain acids predominated. A similar distribution of mostly even numbered fatty acids, but with 16–32 carbon atoms, was noted by Ma'Shum et al. (1988) in organic fractions from Australian soils. Horne and McIntosh (2000) have also shown the presence of long chain fatty acids in water repellent soils.

### Amides

Amides with 14, 16, 18, 22 and 24 carbon atoms, corresponding to peaks 2, 5, 8, 20 and 25a respectively, were also detected (Fig. 1). As there was an amide of the same chain length as many of the acids, e.g. C<sub>16</sub>, C<sub>18</sub>, C<sub>22</sub> and C<sub>24</sub>, it appeared possible that the amides were being formed as a result of heating for long periods in the presence of ammonia. In order to test this hypothesis, C<sub>18</sub> and C<sub>24</sub> acids dissolved in THF were applied to acid-washed sand. After evaporation of the solvent, the loaded sand sample was subjected to the usual experimental

procedure. No amides were detected by GC-MS, implying that the amides in the extracts were originally present in the soils.

#### *Aldehydes or ketones*

Aldehydes or ketones having 23, 25, 27, 29 and 31 carbon atoms corresponding to peaks 11, 17, 22, 29 and 32 respectively were also detected (Fig. 1). However, the precise structures of these compounds could not be determined on the basis of the mass spectra obtained.

#### *Alkanes*

Alkanes, drawn as shaded bars in Fig. 2, were detected with a distribution from C<sub>25</sub> to C<sub>31</sub> and C<sub>33</sub>, corresponding to peaks 10, 14, 16, 19, 21, 24, 27 and 31 respectively. Their chain length distribution is slightly higher than that of the acids and amides. There is a predominance of alkanes having an odd number of carbon atoms, suggesting plant rather than microbial or fungal origin (Eglinton et al. 1962). However each chain length between 25 and 31 carbon atoms was detected suggesting that some alkanes may arise from microbial/fungal metabolism. Horne and McIntosh (2000) also observed the presence of alkanes.

*Polycyclic compounds.* The more complex ring-containing structures determined were predominantly cholesterol/stigmasterol derivatives: peaks 25b of unknown formula, co-eluting with C<sub>24</sub> amide; 28, C<sub>29</sub>H<sub>48</sub>O; 33a, d-friedoolean-14-en-3-one C<sub>30</sub>H<sub>48</sub>O, and 33b, C<sub>29</sub>H<sub>50</sub>O; 35, stigmasta-3,5-dien-7-one C<sub>29</sub>H<sub>46</sub>O; and 36, d-friedoolean-14-en-3-ol, acetate (3. beta.)-C<sub>32</sub>H<sub>52</sub>O<sub>2</sub>. For peaks 28 and 33b, although the molecular formula and the principal functional groups present have been established, the structures of these compounds could not be determined on the basis of their mass spectra alone.

*Miscellaneous compounds.* Other compounds detected include: an unsaturated C<sub>18</sub> acid (C<sub>18</sub>H<sub>34</sub>O<sub>2</sub>, peak 3) and its amide (C<sub>18</sub>H<sub>35</sub>NO, peak 7b, co-eluted with C<sub>20</sub> acid), 4,8,12,16-tetramethylheptadecan-4-olide (peak 6, C<sub>21</sub>H<sub>40</sub>O<sub>2</sub>), a benzamide type compound of unknown formula (peak 9b, co-eluted with C<sub>21</sub> acid), a benzene-1,2-dicarboxylate (peak 12, perhaps a contaminant from the plastic bags used for storing the soil samples) and a pentacyclic triterpenoid alcohol of unknown formula (peak 34). The molecular ions for the species in peaks 9b, 12, 25b and 34 were not detected. There were also indications of the presence of alkenes, alcohols, esters and diols, but further work is needed before they can be identified unequivocally. The identifications of peaks 4, 13, 14, 18, 19 and 24 have been confirmed by comparison of their spectra with those of authentic samples. Peaks 23, 26, 30 and 37 are currently unidentified.

#### Distributions

All extracts contained alkanes in similar relative abundances as indicated by the shaded bars in Fig. 2. The observed difference in behaviour between the wettable and water repellent samples cannot therefore be ascribed to the presence or absence of these hydrophobic compounds. The other components of the extracts are generally more polar materials with oxygen-based functional groups. Under the gas chromatographic conditions used here, compound retention times are determined by a combination of relative molecular mass and polarity with small non-polar compounds eluting first and larger polar compounds eluting last. The extracts from the wettable samples contain only small amounts of the larger polar compounds. In contrast all five extracts from the water repellent samples contain significant amounts of polar compounds (Fig. 2). This appears to be the major difference between the water repellent and wettable samples. The mechanism whereby the presence of higher molecular mass polar compounds might induce water repellency in soils is not known. We can speculate that the inherently lower water solubility and lower diffusion coefficients of these compounds are key factors. When water comes into contact with a wettable soil, the lower molecular mass polar compounds will diffuse relatively quickly into the water droplets, and solubilise hydrophobic compounds, such as alkanes, in a detergent-like fashion. In contrast, when water comes into contact with a water repellent soil, the higher molecular mass polar compounds will diffuse more slowly and remain on the soil surface for longer, keeping the hydrophobic and lower molecular mass species bound to the soil for a greater length of time. Hence, the barrier to water penetration is maintained for longer. It may also be significant that the wettable samples have a smaller overall organic content than the water repellent samples, so that the amount of hydrophobic material which needs to dissolve to allow wetting is much less.

To conclude, the following are key findings: (1) the same types of compounds were found in all extracts; however, the water repellent samples contained high molecular mass polar compounds which were virtually absent from the wettable samples, and (2) although the water repellent samples contained significantly more organic material than the wettable samples, for the repellent samples there was no relationship between the amount of extractable material and the degree of repellency. We therefore speculate that the presence of high molecular mass polar compounds in significant amounts is necessary for water repellency in soils of this type.

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