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### ARTICLE INFO

# ABSTRACT

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*Keywords:* Sequential extraction Lipids Vegetation Roots Sandy soils Hydrophobic organic compounds in soils, mainly derived from plants, cause soil water repellency (SWR). The relation between such hydrophobic compounds, which we call SWR-markers, and SWR has been rarely known. We aim to understand these relations and trace the possible origin of SWR-markers. Sandy soils were collected from the field under various vegetation at different depths. Of the bulk soil characteristics, total organic carbon (TOC) strongly correlated to SWR. A new sequential extraction and hydrolysis approach was applied to our sandy soils to obtain three individual organic fractions that were analysed by gas chromatography–mass spectrometry: the D fraction was extracted from soils by DCM/MeOH, which was used to eliminate free lipids; the residual soils were extracted by IPA/NH<sub>3</sub> and were separated into a DCM/MeOH soluble fraction (AS) and an insoluble fraction (AI) which was depolymerised by trans-methylation. SWR increased after DCM/MeOH extraction and disappeared almost after IPA/NH<sub>3</sub> extraction. According to the chemical composition of each fraction, the D fractions and the AS fractions mainly originated from plant leaf waxes. Suberin-derived compounds were most abundant in the AI fractions. Based on the composition of the extracted fractions and the behaviour of SWR upon these extractions, we speculate that high molecular weight suberins were stronger SWR-markers than the low molecular weight (free) lipids. As suberin is an aliphatic biopolyester typically existing in plant roots, the influence of roots on SWR may be more important than previously thought. The results suggest that our approach is useful to extract the essential SWR-markers and enable the identification of their main sources: plant leaf waxes and roots. In particular roots may have a strong influence on SWR to affect water infiltration and, therefore, plant growth.

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### 1. Introduction

Soil water repellency (SWR) or hydrophobicity is a common and important phenomenon occurring in soils. SWR impacts soil water flow and forms dry and wet soil moisture patterns (Dekker and Ritsema, 1994). If soils dry out for a longer period due to SWR it may diminish plant growth and promote soil erosion leading to environmentally undesired situations (DeBano, 2000; Doerr et al., 2000, 2007). SWR has been observed in many countries and particularly in different soil types such as loamy soils (de Blas et al., 2010; Hansel et al., 2008), peat soils (Michel et al., 2001), clay soils (Doerr et al., 2000), volcanic ash soils (Poulenard et al., 2004) and most frequently reported in sandy soils (Doerr et al., 2005; Franco et al., 1995, 2000; Morley et al., 2005). Factors that influence SWR are seasonal change in precipitation

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(Dekker and Ritsema, 1994; Dekker et al., 2009), soil temperature (Atanassova and Doerr, 2011; Doerr et al., 2005), soil moisture (Ritsema and Dekker, 1998; Rodríguez-Alleres and Benito, 2011) and soil organic matter (SOM) (Buczko et al., 2005; Lozano et al., 2013). Wallis et al. (1990) demonstrated a linear relation between total organic carbon (TOC) and SWR, while most studies found that TOC did not well correlate to SWR (de Blas et al., 2010; Doerr et al., 2005) implying that not only the quantity but also the quality of SOM determines the severity of SWR.

SWR is caused by hydrophobic organic compounds derived from above and below ground vegetation (Bisdom et al., 1993; de Blas et al., 2010; Horne and McIntosh, 2000) and microorganisms (Bond and Harris, 1964) that cover soil particles. In this paper, we define those compounds as SWR-markers. Previous studies isolated different groups of organic compounds including fatty acids and wax esters (Atanassova and Doerr, 2010; Franco et al., 2000; Hansel et al., 2008; Horne and McIntosh, 2000; Ma'shum et al., 1988), alcohols (Atanassova and Doerr, 2010, 2011; Hansel et al., 2008), alkanes (de Blas et al., 2013; Llewellyn et al., 2004), aromatic acids (Hansel et al., 2008), amides, aldehydes and ketones (Morley et al., 2005), phytanes, phytanols and





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sterols (Franco et al., 1995, 2000), and complex polar compounds (Atanassova and Doerr, 2010). However, to date, no relation between reported hydrophobic compounds and SWR has been found.

To disentangle the various fractions of hydrophobic organic compounds, different solvents and extraction methods have been developed. Iso-propanol/ammonia solution (IPA/NH<sub>3</sub>) has been suggested as a common and effective solvent to extract a wide range of organic compounds from soils after which the SWR decreased largely or even disappeared completely (Atanassova and Doerr, 2010; Doerr et al., 2005; Franco et al., 2000; Hansel et al., 2008; Horne and McIntosh, 2000). Franco et al. (2000) used a sequential extraction procedure to obtain two different fractions from water repellent sandy soils, i.e. a 'non-polar wax' fraction and a 'polar wax' fraction, which they related to eucalyptus trees found in the same region as soils. Hansel et al. (2008) applied a similar approach to the hydrophobic soils under pine. Free lipids are organic compounds that are soluble in organic solvents without any chemical reaction (Bull et al., 2000). Ester-bound lipids are biopolymers that are non-extractable by an organic solvent (Bull et al., 2000; Nierop et al., 2006). After removing free lipids by an organic solvent, ester-bound lipids were extracted using either IPA/ NH<sub>3</sub> of which its chloroform soluble fraction was analysed, or obtained after refluxing in KOH/methanol. The ester-bound lipids included both cutins and suberins, which are biopolymers existing in plant leaves and roots, respectively (Kolattukudy, 1981, 2001).

Although much work has been conducted in identifying the different groups of SWR-markers, less is known of the origin of these markers, whereas the relations to SWR are unclear (Horne and McIntosh, 2000). It is widely accepted that SOM originates from plants and microbes. As hydrophobic components of SOM can be traced to the original input (Bull et al., 2000; Nierop, 1998; Nierop et al., 2005; Van Bergen et al., 1997), the origin of SWR-markers could be established. For example, C<sub>29</sub> alkane is typically derived from plant leaf waxes (Bull et al., 2000; Nierop et al., 2006), C<sub>24</sub> alcohol indicates the input of oak leaf (Bull et al., 2000) and C<sub>26</sub> alcohol is typical of grass (Van Bergen et al., 1997; Walton, 1990). Furthermore, C<sub>16</sub> and C<sub>18</sub> ωhydroxy fatty acids are the characteristic components of cutin (Kolattukudy, 2001; Walton, 1990), while the presence of long-chain  $(>C_{20})$   $\omega$ -hydroxy fatty acids and  $\alpha$ , $\omega$ -dicarboxylic acids implies that they originate from suberin (Kolattukudy, 1981, 2001). As yet, however, SWR-markers were hardly related to either plant leaves or roots. As SWR occurs both in top and subsoil (Dekker and Ritsema, 1994), it is expected that both plant leaves and roots will contribute to SWR and potentially influence the conditions of water uptake for plants.

Therefore, we attempt to better understand the relations between SWR-markers, their origin and their relation to SWR by investigating SWR-markers in soils. We decided to use sandy soils as it is easier to extract SOM from sandy soils than from other soil types with much higher clay and silt contents. In the latter soils, mineral particles form strong organo-mineral complexes (Kleber et al., 2007; Schulten and Leinweber, 2000) and may also contain aggregates that incorporate SOM. Consequently, relations between SWR and SWR-markers become even more complex than when such interfering factors are negligible such as in sandy soils. To this end we will use a sequential extraction to divide hydrophobic compounds into three individual fractions and by characterising these fractions to improve our understanding of SWR at the molecular level.

# 2. Materials and methods

#### 2.1. Sampling site

The sampling area is located in the coastal sand dunes of the Zuid-Kennemerland National Park in the Netherlands. For this site, no fires have been recorded, which could be a non-biotic factor affecting soil hydrophobicity and composition of soil organic material (DeBano, 2000; Reeder and Juergensen, 1979). There were two perpendicular

Table 1 Soil profile descriptions.

Profile	Sample label	Sampling depth (cm)	Horizon	Vegetation
1	WRC-1 <sup>a</sup>	0–7	А	Grass sp.
	WRC-2	7–14	Ahb <sup>b</sup>	Grass sp.
	WRC-3	14-20	В	Grass sp.
2	WRC-6	0-1	Α	Algae
3	WRC-8	0-5	Ah	Hypnum lacunosum
	WRC-9	5-10	В	Hypnum lacunosum
4	WRC-10	0-10	Ah	Hypnum lacunosum
5	WRC-13	0-16	Ah	Pinus nigra
6	WRC-14	0–9	Ah	Crataegus sp.
	WRC-15	9-15	В	Crataegus sp.
7	WRC-25	0-7	Ah	Hippophae rhamnoides
	WRC-26	7–12	В	Hippophae rhamnoides
8	WRC-30	0-2	Ah1	Quercus robur
	WRC-31	2-4.5	Ah2	Quercus robur
	WRC-32	4.5-20	В	Quercus robur

<sup>a</sup> WRC-1 consisted of a top soil, which was formed by wind-blown sand deposition at a grass covered soil.

<sup>b</sup> WRC-2 consisted of a dark brownish Ah horizon with grass roots, which was buried by wind-blown sand deposition.



Fig. 1. Sampling map with soil labels and the distances between sampled plots.

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