



Induction of water repellency by leaves of contrasting Australian native species: effects of composition and heating

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Abstract

Aims This study identifies the contribution of leaf matter from individual plant species on water repellency with a focus on the composition of organic compounds and the role of heat in releasing these compounds to soil.

Methods Leaf powder from four plant species (*Banksia menziesii*, *Eucalyptus marginata*, *Allocasuarina fraseriana*, *Xanthorrhoea preissii*) was mixed with acid-washed sand (AWS) under a range of temperatures and WR measured. Plant chemical composition was characterized by extraction of leaf powder and GC/MS analysis.

Results Increasing concentrations of plant powder mixed with AWS increased WR for three species; whereas for *X. preissii* there was no WR at any concentration. Heating increased WR for all species over the range of 30 to 120 °C. *B. menziesii* had the greatest WR, which was associated with the largest diversity of fatty acids and *n*-alcohols and highest concentration of alkanes, whereas *X. preissii* with the

smallest WR had only C₁₆ alcohol and C₁₆ fatty acids at relatively low concentration.

Conclusions Physically mixing leaf powder with AWS allows the contribution of different plant species on WR to be directly examined. WR appears to be related to differences in the concentrations and diversity of *n*-alcohols, *n*-fatty acids, and high *n*-alkanes in the leaves. The observed temperature effects on WR provide insights into the kinetics of release and dispersal of organic compounds from interstitial material.

Keywords Leaf powder · Water repellency · Heating treatments · Mass spectroscopy

Abbreviations

WR	Water repellency
AWS	Acid-washed sand
OM	Organic matter
MED	Molarity of Ethanol Droplet
GC/MS	Gas Chromatography–Mass Spectroscopy

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Introduction

Water repellency (WR) is a phenomenon that has been observed in many soils around the world (Dekker et al. 2005; Doerr et al. 2006; Roper et al. 2015; Smettem et al. 2021). Due to uneven wetting of soil, WR often inhibits seed germination leading to impaired crop production (Doerr et al. 2000; Harper et al. 2000),

and increased incidence of overland flow and surface erosion, particularly on hills and steep slopes (Müller et al. 2018; Wallis and Horne 1992).

It has been widely reported that the expression of soil WR is associated with different types of vegetation. Soil WR has been observed under broadleaf deciduous trees, conifers, shrubs, cereal and legume crops, and pastures (Doerr et al. 2000; Wallis and Horne 1992) with the quality and quantity of soil organic matter (OM) considered to contribute to WR. For example, soils under heathland and grassland were reported to be more water repellent than soils under cork oak and olive tree communities (Jordan et al. 2008; Zavala et al. 2009b). Furthermore, soil OM from *Eucalyptus* spp. (McKissock et al. 1998; Walden et al. 2015) and *Banksia* spp. (Harper et al. 2000) induced greater WR compared to similar amounts of OM from agricultural species. Other factors affecting WR include the soil clay content, with clay counterbalancing some effects of soil OM (Harper and Gilkes 1994; Harper et al. 2000), and differences in hydrophobic compounds derived from OM deposited on soil minerals (Doerr et al. 2000; Franco et al. 1995).

So far, experimental evidence has not provided a consistent explanation as to the cause of soil WR. Some investigations have reported that organic compounds inducing WR are branched and unbranched long-chain C_{16} – C_{36} fatty acids, their esters, alkanes, carboxylic acids, phytanols, phytanes and sterols (Atanassova and Doerr 2011; Daniel et al. 2019; Franco et al. 2000; Horne and McIntosh 2000; Morley et al. 2005), and long-chain polymethylene waxes (Ma'shum et al. 1988). These compounds are often derived from OM present in soil such as leaves, twigs and roots which contain considerable amount of waxes, fat, and oil (Doerr et al. 2000; Koch and Ensikat 2008) and also from soil biota (Roper 2004; Roper et al. 2015). However, these hydrophobic compounds are also present in wettable soils (Doerr et al. 2005b; Morley et al. 2005), hence, hydrophobic compounds do not always induce WR in soils (Doerr et al. 2005b). While these studies have focused on the relationship of OM to soil WR, they do not distinguish the direct contribution of leaf matter from different plant species to soil WR.

In regions with Mediterranean climate, the extremes of temperature and the long periods of exposure to elevated temperature over the summer

could be important factors influencing the degree of WR. Soil surface temperatures may reach 70 °C in those regions over the summer period (Ward and Siddique 2014), thus soil WR may increase with soil temperature. Soil WR has been studied under eucalyptus and coniferous species (Zavala et al. 2014; Zavala et al. 2009b), deciduous forest (Jordan et al. 2008), and holm oaks and shrubs (Zavala et al. 2014) in those regions. Variation in soil WR has been examined with heating treatments under pine (Jiménez-Pinilla et al. 2016; Mataix-Solera and Doerr 2004; Zavala et al. 2009a), eucalypts (Atanassova and Doerr 2011; Bailey et al. 2015; Doerr et al. 2004), shrubs (González-Pelayo et al. 2015; Jiménez-Pinilla et al. 2016), herbs (Zavala et al. 2009a), and agricultural species (Aelamanesh et al. 2014; Doerr et al. 2004), however, little is known about the soil WR response of particular native species to heating temperatures and heating duration.

To gain further insight into the contribution of OM to soil WR, and specifically to isolate the influence of individual plant species, we used a range of native plants in southwestern Australia, a region with a Mediterranean climate and where soil WR is a major feature of the soils (Harper et al. 2000; Smettem et al. 2021). We hypothesized that (1) the severity of WR depends on the types and amounts of OM, and (2) manipulation of heating (temperature and duration) results in different increases in WR among the different species. To test these hypotheses, we mixed leaf powder from four species taken from the field with acid-washed sand (AWS) to create an artificial sandy soil, measured the WR and explored the hydrophobicity based on the plant's chemical composition. AWS is inert, and using this material removed any contributions from other soil components on WR. We also investigated through heating the role of temperature on the kinetics of WR induction.

Materials and methods

Preparation of leaf powder

Healthy mature leaves of four wild indigenous plants, *Eucalyptus marginata* (EM), *Banksia menziesii* (BM), *Allocasuarina fraseriana* (AF) and *Xanthorrhoea preissii* (XP), were collected in August 2019 from bushland at Murdoch University, Perth,

Australia. Leaves were dried at 40 °C in a fan-forced oven until they reached a constant mass, ground in a hammer mill to pass through a 600 µm sieve and stored in open plastic bags in a desiccator under vacuum at room temperature. Quartz acid-washed sand (AWS) with a diameter range of 300–350 µm was sourced from a commercial supplier.

WR measurement

The severity of WR was determined in five experiments using the Molarity of Ethanol Droplet (MED) test (King 1981). Aqueous ethanol solutions were prepared in intervals of 0.2 *M* from 0 up to 7.0 *M*. Droplets (15 µL) of the solutions were applied to the samples from a 5 mm height using a micropipette. To exclude the effect of surface roughness, the sample surface was smoothed using a spatula before MED testing. The infiltration time was recorded. The lowest concentration of ethanol which allowed the droplets to infiltrate the sample surface in 5 seconds was identified as the MED value of that sample.

Effects of plant species on WR

Experiment 1 To investigate the effects of plant species on WR, plant powder was added by weight to AWS to make six treatments of 0, 2, 4, 6, 8 and 10% (w/w). Aliquots (120 g) of each mixture were transferred into 250 mL beakers, mixed slowly with a glass rod then the beaker was wrapped with plastic food wrap and shaken thoroughly by hand for 5 min. After mixing, the content was poured into four glass Petri dishes (60 mm ID × 15 mm high) to make four replicates and WR measurements were performed immediately.

Effects of heating on WR

Experiment 2 To investigate whether WR changes with heating, 120 g aliquots of each mixture in Experiment 1 and a control (AWS without plant powder) were heated in an oven at 105 °C for 24 h. After heating, the samples were cooled to room temperature (20 °C) in a desiccator with silica under vacuum for 4 h, and the WR was then determined using the MED test. There were four replicates per treatment.

Experiment 3 To investigate the effects of a range of heating temperature on WR, 4% (w/w) samples of each species were prepared as above, poured into glass Petri dishes and heated at 7 temperatures (30, 50, 75, 100, 120, 150, 200 °C) for 24 h. Samples were cooled in desiccators with silica at room temperature (20 °C) for 4 h and then tested for WR. There were four replicates per treatment. Control samples were unheated 4% (w/w) samples placed in a desiccator with silica under vacuum for 24 h at room temperature.

Experiment 4 To investigate the effect of heating time on WR, aliquots of 4% (w/w) of dried plant powder in AWS were placed in open glass Petri dishes and heated at 105 °C for 1, 2, 4, 8, 16 and 24 h. The post heating protocol was as described above. The control samples were 4% plant powder without being heated.

To further understand how WR changed for short heating times among different species, another heating experiment was set up. Samples were prepared at 4% as described above with heating treatments at 5, 10, 20, 40 and 60 min at 105 °C. The 4% control samples without heating were also tested for WR after mixing.

Chemical analysis of leaf powder

Aliquots of each dried plant powder (3 g) were placed in a Soxhlet apparatus with 200 mL of dichloromethane/MeOH (9:1, v/v) for 24 h to obtain free lipids (Mao et al. 2015). The extracts were concentrated in a rotary evaporator to complete dryness. The residuals from dichloromethane/MeOH extraction were air-dried in a fume-hood, and re-extracted with isopropanol/NH₃ solution (7:3 v/v, ammonia 30%) for 24 h in a Soxhlet apparatus. The collected extract was cooled then concentrated using a rotary evaporator at 45 °C under reduced pressure. The concentrated extracts were dried using Labconco centrivap at 50 °C under reduced pressure. Each plant extraction was repeated three times. The dried dichloromethane/MeOH (9:1) extract was dissolved in 5 µL of 2:1 dichloromethane/MeOH spiked with dodecane (2.2×10^{-4} mol L⁻¹) as an internal standard. The dried isopropanol/NH₃ extracts were dissolved in dichloromethane/MeOH (1:9, v/v) spiked

with dodecane (2.2×10^{-4} mol L⁻¹). The extracts were then filtered through 0.45 µm filter membrane to remove insoluble particles.

Gas Chromatography–Mass Spectroscopy (GC/MS) analysis was performed using a Shimadzu GC–2010 and GC/MS–QP2010S, equipped with a SGE GC BPX5 column (30 m × 0.25 mm). The injection temperature was 310 °C. The oven temperature was programmed from 60 °C for 1 min, then ramped to 100 °C at 10 °C min⁻¹, held at this temperature for 1 min before ramping to 150 °C at 4 °C for 5 min. The oven temperature was then ramped to 280 °C at a rate of 6 °C min⁻¹ and was held at this temperature for 23 min. Compounds were identified based on analysis of retention time and, MS fragmentation patterns in comparison with the NIST library database. The concentration of identified compounds was calculated as µg g⁻¹ of dried leaf powder.

Calculations and statistical analysis

Analysis of Variance (ANOVA) was used to test the effects of different species and concentrations of leaf powder on the degree of WR in Experiments 1, 2 and 3. Where there was a significant difference, overall MED mean values were compared using Fisher's least significant difference test as a post-hoc test ($P \leq 0.05$). The normal distribution and homogeneity of variance of MED data were checked using the Shapiro–Wilk and Levene tests, respectively. Where the dependent MED parameter failed to a normal distribution, the data were square root transformed. All graphs show the non-transformed

data. Statistical analysis was conducted using GenStat 18 for Windows.

Results

Changes in WR following the mixing of leaf powder with acid-washed sand

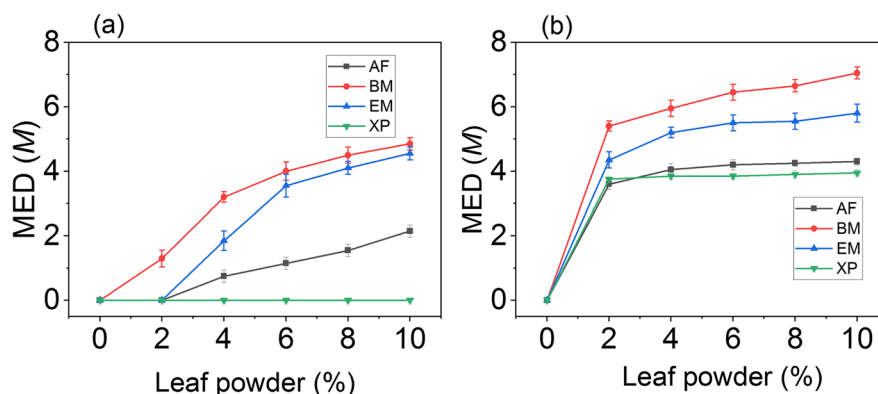
Experiment 1 This experiment examined the change in WR induced by physical mixing of AWS with plant powder for the four selected species (Fig. 1a). At 2% of organic material added, only the BM mixture exhibited WR. However, from 4%, WR increased with increasing concentration of plant powder for all species except XP, which remained wettable for all mixing concentrations. The most severe WR was observed in the BM 10% sample (MED=4.9 M), followed by EM 10% (MED=4.6 M), AF (MED=2.1 M). At each increment of OM added, the most severe WR was for BM, followed by EM, AF and XP.

Changes in WR associated with heating

Experiment 2 WR of the mixtures of AWS and plant powder increased significantly with heating at 105 °C and varied considerably among plant species (Fig. 1b). The most severe WR occurred in BM, followed by EM, AF and XP (Fig. 1b). XP exhibited a very uniform increase in MED to nearly 4 M, independent of plant powder concentrations (2–10%), from its previous zero values (Fig. 1b).

Heating of samples with 2% of OM added resulted in MED increases of 3.6–4.3 M compared to the

Fig. 1 MED values (means ± standard deviation, n=4) as a function of plant powder concentration from *Allocasuarina fraseriana* (AF), *Banksia menziesii* (BM), *Eucalyptus marginata* (EM) and *Xanthorrea preissii* (XP), (a) without heating and (b) after heating the mixture at 105 °C for 24 h



corresponding samples prepared with physical mixing alone. More concentrated samples also exhibited increased MED values with heating. There was a gradual increase in MED from 2% BM up to the highest value of 7.05 *M* at 10% BM. EM and AF exhibited smaller increases (4.3 and 5.8 *M* for EM; 3.6 and 4.3 *M* at 2 and 10%, respectively).

Experiment 3 This experiment examined the effect of heating temperature (30–200 °C) for samples with 4% plant powder mixed with AWS. At 30, 50 and 75 °C, all plant powders exhibited a significant increase in MED values compared to samples without heating (Fig. 2). This trend continued to increase slightly until all species reached their most severe WR at 120 °C. BM had the highest MED at 6.1 *M*, followed by EM (5.3 *M*), AF (4.6 *M*) and XP (4.2 *M*). A decrease in the severity of WR was observed in BM (5.4 *M*), EM (4.6 *M*) and XP (2.9 *M*) at 200 °C, but the WR of AF samples did not change when heated beyond 200 °C (Fig. 2).

Experiment 4 Leaf powder from all species exhibited a marked increase in WR as a function of heating time (105 °C) compared to samples without heating (Fig. 3). After one hour of heating, BM had the highest MED values (5.1 *M*), followed by EM (4.1 *M*),

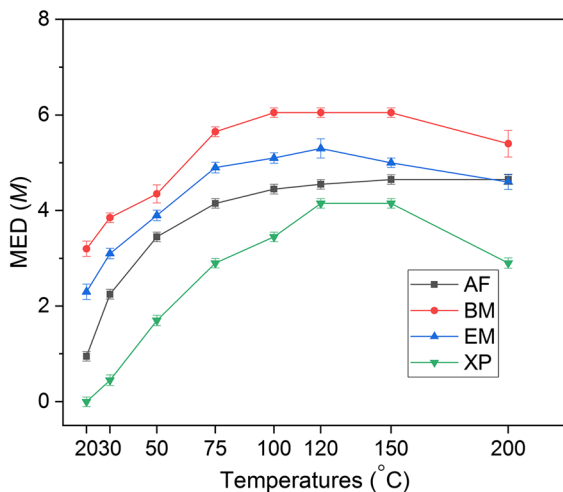


Fig. 2 MED values (means $\pm$ standard deviation, $n=4$) as a function of heating temperatures for mixtures of AWS and leaf powder from *Allocasuarina fraseriana* (AF), *Banksia menziesii* (BM), *Eucalyptus marginata* (EM) and *Xanthorrea preissii* (XP)

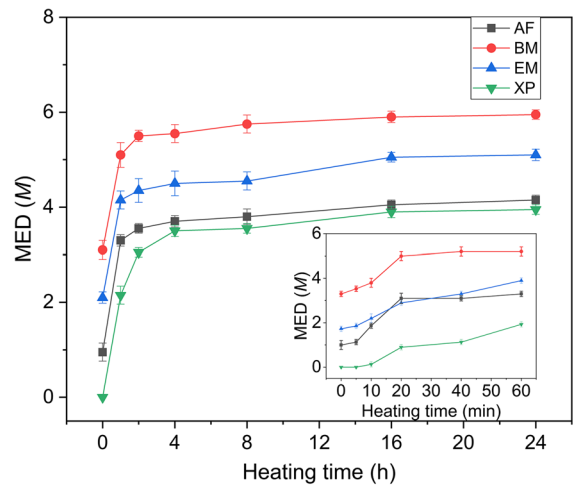


Fig. 3 MED values (means $\pm$ standard deviation, $n=4$) as a function of heating duration for mixtures of AWS and powdered leaf material (4% w/w) from *Allocasuarina fraseriana* (AF), *Banksia menziesii* (BM), *Eucalyptus marginata* (EM) and *Xanthorrea preissii* (XP)

AF (3.3 *M*) and XP (2.3 *M*). From 2 to 8 h, all species showed a slight increase in MED values (Fig. 3). Notably, increasing the heating time from 16 to 24 h did not affect the severity of WR for any of the species investigated, being around 5.9 *M* for BM, 5.1 *M* for EM, 4.1 *M* for AF and 3.9 *M* for XP.

To further analyse the change in WR with heating time, mixtures of 4% of leaf powder and AWS were heated at 105 °C for 5, 10, 20, 40 and 60 min. After the initial 10 min of heating, MED values increased slightly in all species. The severity of WR then increased from 10 to 20 min, up to 5 *M* (BM), 2.9 *M* (EM), 3.1 *M* (AF), and 0.9 *M* (XP). After 20 min, there was a gradual increase (EM and XP) or the MED values remained constant (AF and BM). After one hour, none of the leaf powders reached the WR values achieved for longer periods of heating (Fig. 3).

Organic compounds in leaves associated with WR

Long chain *n*-alkanes, fatty acids and *n*-alcohols were detected in the dichloromethane/MeOH (9:1, v/v) extracts of leaf powders from AF, BM, EM and XP (Fig. 4). Odd-number *n*-alkanes (C_{25} – C_{31}) were observed in all species (Fig. 5a). C_{27} and C_{29} *n*-alkanes dominated in leaves of all species except

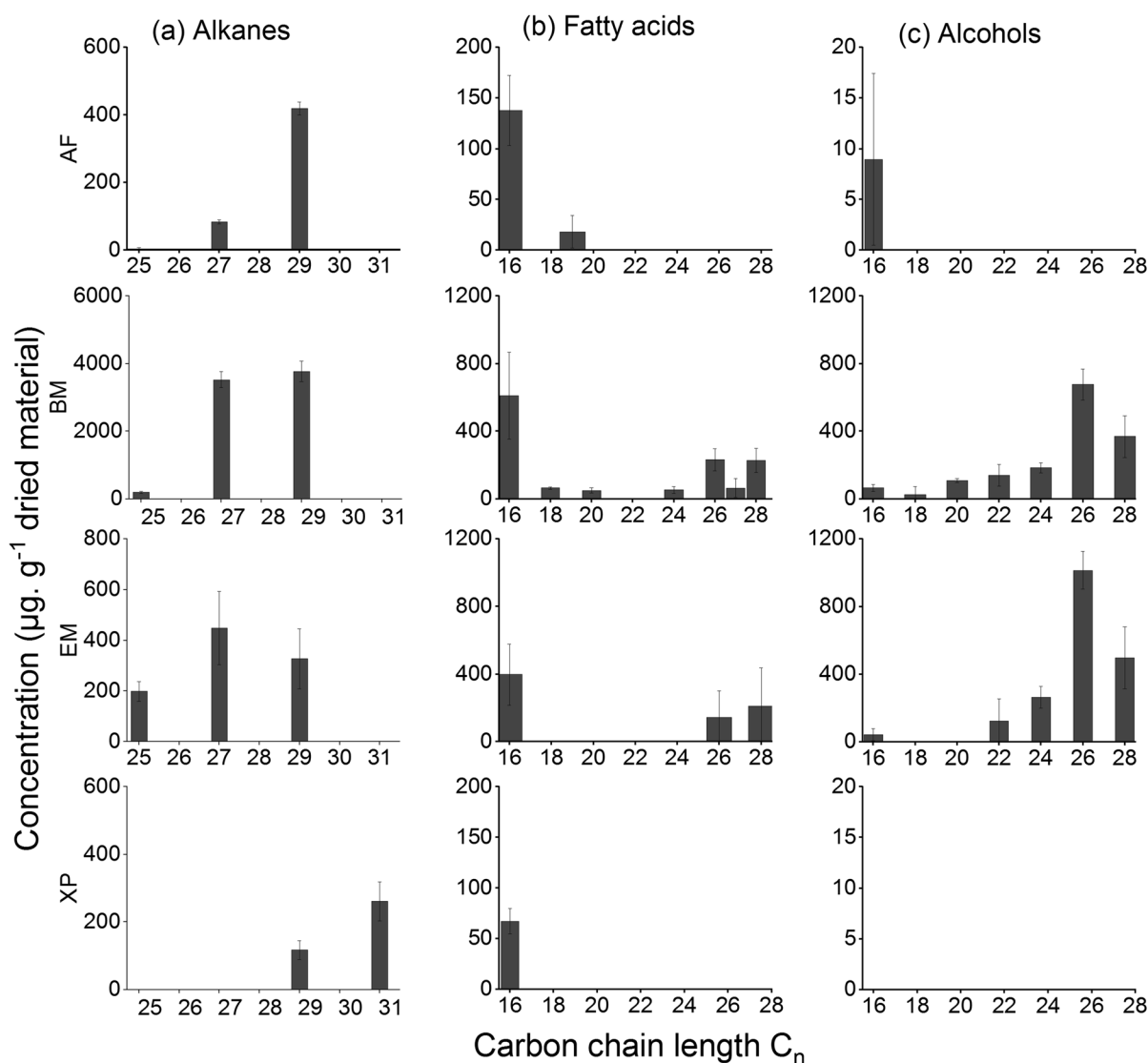


Fig. 4 Concentrations of (a) *n*-alkanes, (b) fatty acids and (c) *n*-alcohols from dichloromethane/MeOH extraction of *Allo-casuarina fraseriana* (AF), *Banksia menziesii* (BM), *Eucalyptus marginata* (EM) and *Xanthorrea preissii* (XP) leaf powder. Note that the y axis scale differs between figures

XP. BM had concentrations of C_{27} and C_{29} alkanes at $3526.5 \mu\text{g g}^{-1}$ (standard deviation = 234.7) and $3775.2 \mu\text{g g}^{-1}$ (standard deviation = 304.6), respectively. The C_{27} and C_{29} alkane concentrations of BM were extremely high compared to that of EM (449 and $327 \mu\text{g g}^{-1}$) and AF leaves (83 and $419 \mu\text{g g}^{-1}$), respectively. C_{31} alkane was only found in XP with a concentration of $260 \mu\text{g g}^{-1}$.

Even-over-odd long chain fatty acids ranging from C_{16} to C_{28} were observed (Fig. 4b). BM showed the

most diverse kinds of even chain fatty acids ranging between C_{16} and C_{28} . For XP, C_{16} was the only fatty acid, while AF contained C_{16} and C_{19} fatty acids and EM had C_{16} , C_{26} and C_{28} fatty acids. Interestingly, C_{16} fatty acid was the most abundant fatty acid in leaves of all species, of which BM had the highest concentration ($610 \mu\text{g g}^{-1}$), followed by EM, AF and XP (397 ; 137 and $67 \mu\text{g g}^{-1}$, respectively).

Similar to fatty acids, even-numbered alcohols ranging from C_{16} to C_{28} were found in leaves of BM

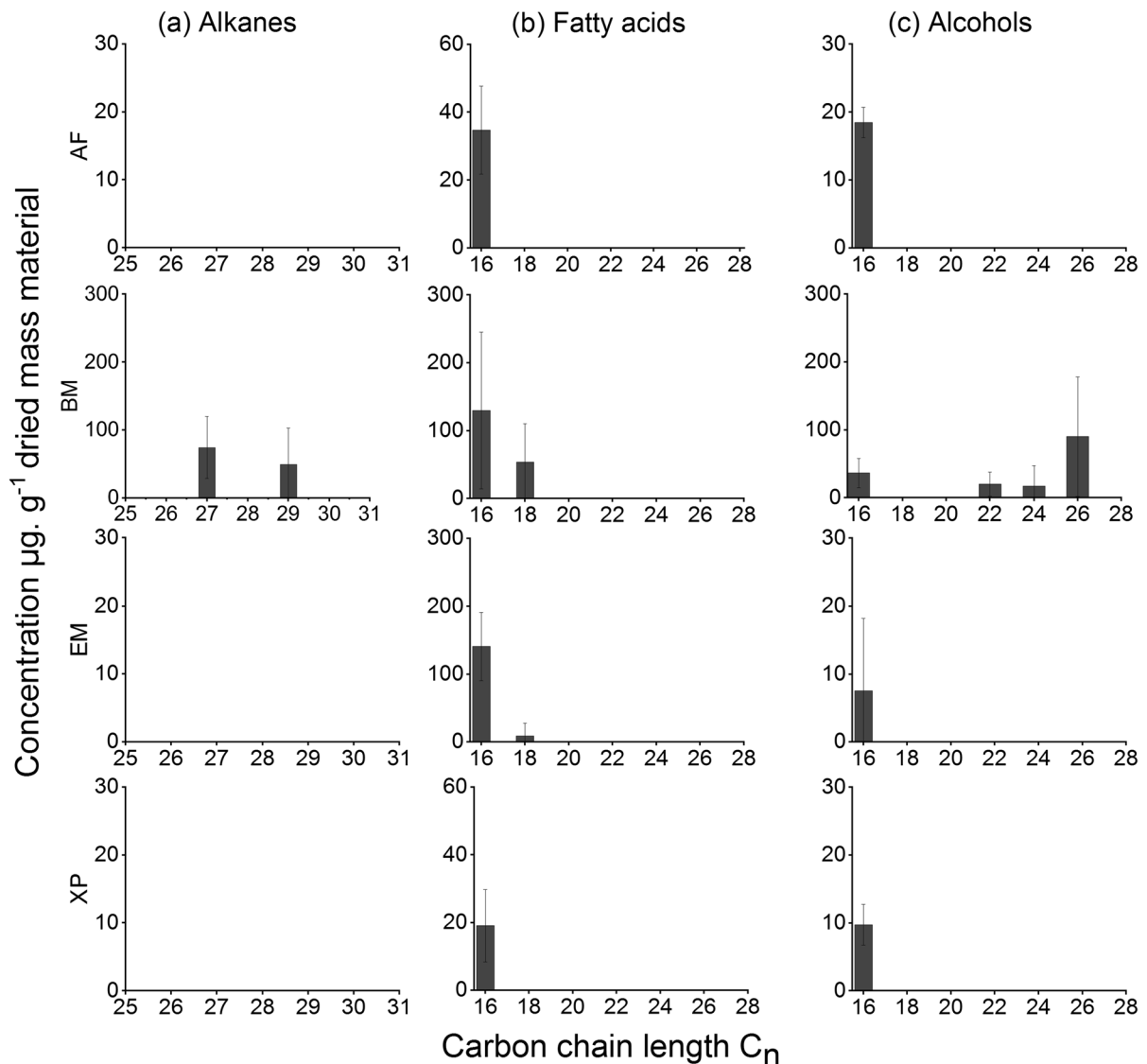


Fig. 5 Concentrations of (a) *n*-alkanes, (b) fatty acids and (c) *n*-alcohols from isopropanol/NH₃ (7:3) extraction of *Allocastraria fraseriana* (AF), *Banksia menziesii* (BM), *Eucalyptus*

marginata (EM) and *Xanthorrea preissii* (XP) leaf powder. Note that the y axis scale differs between figures

and EM with average concentrations 1587 μg and 1941 $\mu\text{g g}^{-1}$, respectively (Fig. 4c). C₂₆ alcohol was the most abundant in both BM and EM leaves, with concentrations of 676 and 1014 $\mu\text{g g}^{-1}$ dried leaf material, respectively (Fig. 4c). In contrast, C₁₆ alcohol was the only alcohol observed in AF leaves with a small concentration of 9 $\mu\text{g g}^{-1}$ dried material. XP leaves had no detectable alcohols.

Following isopropanol/NH₃ (7:3, v/v) extraction of leaf powder, the dried extract was diluted in

dichloromethane/MeOH to dissolve the compounds (Llewellyn et al. 2004). The total fatty acids were calculated as the total of both fatty acids and amides. C₁₆ acid was the only fatty acid found in AF, XP (Fig. 5b). In comparison, BM and EM had both C₁₆ and C₁₈ fatty acids. BM and EM had similar concentrations of C₁₆ fatty acid at 129 and 140 $\mu\text{g g}^{-1}$, respectively. C₁₈ fatty acids in EM was lower than in BM. AF and XP had much smaller concentrations of C₁₆ fatty acid, with 34.7 and 19 $\mu\text{g g}^{-1}$, respectively.

Long chain alcohols were also detected from the isopropanol/NH₃ extraction of leaf powder for all four species. BM contained a range of alcohols between C₁₆ and C₂₆ with C₂₆ alcohol present in the highest concentration (90 µg g⁻¹). Small amounts of C₁₆ alcohol were found in the isopropanol/NH₃ extracts of AF (18.5 µg g⁻¹), EM (7.5 µg g⁻¹) and XP (3 µg g⁻¹) (Fig. 5c). *n*-alkanes were only extracted from BM using isopropanol/NH₃ (Fig. 5a).

Discussion

Immediate effects of different plant species on WR

There was a clear relationship between the severity of WR and the type of vegetation, with WR varying in the plant powder/AWS mixtures from wettable XP (MED=0 M) to severely water repellent BM (MED=4.9 M). To our knowledge, few studies have investigated the direct effects of adding dried plant powder on WR, either in soils or AWS (McGhie and Posner 1981; Miller et al. 2019). McGhie and Posner (1981) added 2 and 5% by weight of plant powder to fired sand and found that the most severe WR was derived from legume pasture (*Ornithopus sativus*), and a native woody shrub *Allocasuarina heugeliana*. In our study XP gave the least WR compared to the woody plants (BM, EM and AF). Interestingly, King (1981) suggested that severe WR in some South Australian soils was associated with an *Xanthorrhoea* spp. McGhie and Posner (1981) used scanning electron microscopy as a method to investigate morphological differences in plant material in soil. However, those differences were not always identified. Miller et al. (2019) measured WR of ground plant materials of major agricultural species directly rather than mixing with AWS. The authors presented SEM photos of leaf surface morphology of the highest and lowest WR species but the chemical composition of those species was not described.

Although a link between soil WR and soil OM content has been established (Mataix-Solera et al. 2007; McKissock et al. 1998; Täumer et al. 2005), it has been difficult to establish the importance of both the composition of that OM and of the distribution of the OM within the soil matrix on soil WR. Previous studies have shown that the addition of selected compounds to AWS induced WR (Mainwaring et al.

2013; Uddin et al. 2017). However, interstitial OM is also believed to be important for the induction of WR (Smettem et al. 2021). The increase in WR following the addition of plant powder for three species BM, EM and AF to AWS with only physical mixing (Fig. 1a) supports the hypothesis that interstitial matter can play a role in inducing WR.

In addition, increases in WR were observed as the amount of added OM increased. For most species 2–4% OM, followed by heating was sufficient to coat soil particles with WR inducing compounds. These findings are in accordance with other studies on the positive relationship of OM content and soil WR (Mataix-Solera et al. 2007; McKissock et al. 1998; Täumer et al. 2005). However, physical mixing of XP leaf powder did not induce WR at any of the concentrations considered.

Plant powder from BM consistently induced the highest MED values compared to other species. This suggests that the chemical composition of the waxes and resins in BM consists of more types of hydrophobic compounds than powder of the other species (EM, AF, XP) or that the compounds are more easily released from BM plant powder during physical mixing.

Alkanes were present in leaves of all four species in the GC/MS analysis. The C₂₇ and C₂₉ alkanes were the predominant alkanes in leaves of AF, BM, EM, these being typical alkanes of deciduous trees (Schäfer et al. 2016; Zech et al. 2010). C₃₁ alkane dominated in XP, and this is a typical alkane of grass (Zech et al. 2010). However, the concentrations of *n*-alkanes in leaves varied widely among species with that of the C₂₇ and C₂₉ alkanes in BM leaves about 10 times higher than that in AF, EM and XP leaves. It has previously been shown that when alkanes alone were added to AWS, the material remained wettable. However, adding a combination of alkanes and fatty acids to AWS resulted in extreme WR (Mainwaring et al. 2013; Uddin et al. 2019), suggesting that very high concentrations of *n*-alkanes in BM leaves might combine with carboxylic compounds and/or alcohols in inducing extreme WR. Although alkanes were present in all species, the variations in concentration were insufficient to explain the differences in WR.

Carboxylic acids and derivatives are known to induce WR in soils (Atanassova and Doerr 2010; Mao et al. 2014; Morley et al. 2005). Loading C₁₆ or C₁₈ acid alone on acid washed sand induced

water repellency (Daniel et al. 2019; Mainwaring et al. 2013; Uddin et al. 2017). Extreme water repellency was induced when fatty acid (C_{18} or C_{22}) was combined with alkane (Mainwaring et al. 2013). However, the mixture of C_{14} acid/alkane on AWS remained wettable (Mainwaring et al. 2013). However, comparing the presence of various fatty acids and alkane concentrations together among species might be useful in explain the degree of WR. BM had the widest range and the highest concentrations of fatty acids and major alkanes from both the dichloromethane/MeOH and isopropanol/ NH_3 extractions, suggesting that the extreme WR of BM might be a result of the natural combination of alkanes and fatty acids in leaf powder. Extreme WR in the EM samples can be explained by the presence of fatty acids in leaves similar to BM. The lower concentrations and less diversity of fatty acids (C_{16} and C_{19}) found in AF leaves is consistent with the less severe WR induced by plant powder of this species compared to BM and EM. The smallest WR was observed from XP plant powder mixture in which only C_{16} fatty acid was identified with the smallest concentration in both extractions. These results are consistent with (Atanassova and Doerr 2010; Morley et al. 2005) who suggested that high WR in soil requires the presence of the long chain ($> C_{18}$) fatty acids in which those fatty acids were not detected in XP leaves.

Long chain alcohols are also known to contribute to WR (Atanassova and Doerr 2010; Daniel et al. 2019; Hansel et al. 2008). Mao et al. (2015) suggested that alcohols were the only class that can reliably be used to predict WR in sub-soil. Various types of alcohols were observed from leaves of BM and EM in higher concentrations compared to other species, from both the dichloromethane/MeOH (9:1) and isopropanol/ NH_3 (7:3) extractions and their presence is consistent with the higher WR in BM and EM samples. The smaller WR in samples prepared with AF and XP leaf powder correlated with only C_{16} alcohol being detected from leaves of these species at very low concentration compared to BM and EM. These results are also consistent with Atanassova and Doerr (2010) who reported that the soil with the smallest WR had the lowest concentration of long chain alcohols C_{26} , C_{28} and C_{30} . The lack of those long chain alcohols in AF and XP leaves can help explain the lower WR of leaf material of those species.

It seems reasonable to suggest that the degree of WR induced by the leaf powder of each species can be related to the composition of organic compounds present in the leaves. In particular, the greater WR in the BM and EM mixtures was correlated with higher concentrations and the presence of a broad range of alcohols and/or fatty acids in combination with high concentrations of long-chain alkanes in leaves. The smaller WR from XP, with only C_{16} fatty acid and C_{16} alcohol, was associated with the fewest types of hydrophobic compounds compared to other species. That is why the mixture of AWS and XP leaf powder remained wettable at all concentrations with physical mixing, and induced WR after heating. These results are consistent with Atanassova and Doerr (2010) who observed the soil with the lowest WR also had the lowest concentrations of long chain alkanols and lacked long chain fatty acids ($> C_{18}$). These results are also consistent with Daniel et al. (2019) who reported that applying C_{16} alcohol to AWS induced WR only at loadings $\geq 1 \times 10^{-6}$ mol g^{-1} at 20 °C and WR became severe after heating. However, it is unclear whether WR is the result of the occurrence of various *n*-fatty acids/ *n*-alcohols alone or the combination of *n*-fatty acids/ *n*-alcohols with *n*-alkanes or the combination of fatty acids with alcohols.

Immediate effects of heating on changes of WR in different species

Heat is a significant environmental factor within Mediterranean climates in terms of extremes of temperature, the long periods of exposure to elevated temperature and fire. We analysed the possible correlation between temperatures and induction of WR from leaf matter (Fig. 2). For all species physical mixing (2–10%) of plant powder followed by heating at 105 °C for 24 hours was sufficient to increase water repellency compared to physical mixing alone (Fig. 1). The temperatures and time chosen for this experiment were consistent with conditions reported in some studies for drying of soil field samples (Dekker et al. 1998; Moral García et al. 2005).

The temperature dependence of WR induction by leaf matter was investigated in the range of 30–200 °C. Even at moderate temperatures of 30, 50 and 75 °C, WR increased. The increases in WR were positively correlated with temperature up to 120 °C for all species (Fig. 2). Beyond this temperature, there

was no further increase in MED except for AF samples, which continued to show a slight increase at 150 °C then remained constant up to 200 °C. A possible explanation for this is that organic compounds in plant powder may experience a transition phase in temperatures close to their melting points (Koch and Ensikat 2008), and be re-organized into hydrophobic layers. Therefore, the observed increases in WR might be due to the redistribution of compounds in the mixtures when temperatures are close to their melting points. The different slopes of the curves in the region 0–120 °C implies that the kinetics for WR induction differs for the leaf matter of different species. The increase in MED values after heating to higher temperatures can be also explained by the dispersal of organic compounds into soil (Savage et al. 1972). It has also been suggested that hydrophobic substances from soil OM might not only be volatilized during heating but also diffuse downward into the soil (DeBano et al. 1970; Doerr et al. 2000; Franco et al. 1995). In this study, the mixture of AWS and AF leaf powder did not reduce WR at 200 °C. A possible reason for this is that organic compounds responsible for WR derived from AF leaves are unusually heat- and oxidation- resistant or stable up to 200 °C.

Many authors have reported that heating temperature from 50 to 150 °C can result in greater WR in field samples (DeBano 2000; Doerr et al. 2005b). Additional studies involving higher temperatures reported that the expression of WR intensified at 175–200 °C (DeBano et al. 1976; Dlapa et al. 2008; Doerr et al. 2004; Doerr et al. 2005a). The differences in WR responses of the current samples (Fig. 2) compared with previous studies for field samples are likely due to the differences in soil matrices and composition of the leaves and soil OM.

The observed increases in WR with heating implies that heat promotes the release of WR inducing organic compounds from plant matter. The mechanism for this process most likely includes the combined effect of softening the particles of plant powder and providing energy for the compounds to diffuse away from the leaf particles onto soil particles. The smaller relative increase in WR with plant powder concentration suggests that the samples were approaching a steady state or equilibrium distribution of compounds between the plant powder particles and sand particle coating. In this context,

the high temperature used in this experiment can be viewed as a mechanism to accelerate the kinetics of this progression to this steady state.

BM, EM and XP samples exhibited a decline in MED when heated above 120 °C or 150 °C. WR has been noted to decline in field samples at 250 °C and be eliminated at 300 °C (Doerr et al. 2005a). The reduction and its elimination of WR in soil at high temperatures (>250 °C) is likely associated with volatilisation and oxidation (DeBano et al. 1976). It is also possible that the molecular arrangement of the organic compounds achieved at lower temperatures that induce soil WR may be disrupted at higher temperatures, leading to the observed decrease in MED. However, it remains unclear which factors determine the temperature above which WR starts reducing in each species. The experiments were performed in the chosen temperature range to prevent combustion of the leaf matter, which is beyond the scope of the present study. It is important to note that the aim of this study was not to determine the temperature thresholds where WR was reduced or eliminated for the different species.

Heating duration is also an important factor inducing or intensifying soil WR. For all samples heated at 105 °C, there was a significant increase in MED during the first 2 hours. Samples heated for longer than 2 hours exhibited only a slight further increase in WR (Fig. 3). This suggests that redistribution or reorganisation of OM from plant powder is achieved within 2 hours at 105 °C. To more narrowly bracket the time required for this redistribution of OM, shorter heating times were investigated (Fig. 3). There was an increase in the severity of WR in samples of all species after 10 min of heating. This result suggests that even a short duration of heating would lead to the redistribution or reorganisation of organic compounds that lead to higher WR. These results are consistent with previous studies of field soil samples that reported WR increased gradually for heating durations from 5 to 30 min at 125 °C (Dlapa et al. 2008). Another study reported that maximum WR could be reached in 5 to 10 min if heating temperatures ranged between 250 and 280 °C (Doerr et al. 2004). A gradual or a sharp increase in MED values in samples heated for 1 hour again indicates that there might be different rates of organic compound diffusion from OM into AWS.

Conclusions

The approach of adding plant material to acid washed sand allows the direct investigation of the contribution of leaf matter from different species on water repellency, in the absence of complicating factors seen in field studies, such as the contribution of multiple plant species, soil microbial processes, and soil physical properties such as clay content. Indeed, in this study there were pronounced differences in WR induced by leaf powder as a function of species and concentration. BM plant powder consistently induced the highest WR at concentrations from 2 to 10%. EM and AF plant powder induced water repellency for concentrations >2%, whereas XP plant powder only induced WR after heating. Following heating, all species exhibited a marked increase in WR, and this was consistent with the movement of WR inducing compounds from the plant material into the AWS matrix. This WR was induced relatively quickly after heating at a range of temperatures, but the order of WR was consistent with that observed in un-heated samples.

The higher concentration and diverse array of fatty acids and alcohols (C_{16} – C_{28}) and extremely high concentration of alkanes in BM leaves may explain the greater WR of samples prepared from BM powder mixed with AWS. The lower WR in EM and AF mixtures may be related to the lower concentration and less diversity of *n*-fatty acids, *n*-alcohols and lower concentrations of alkanes in their leaves. In XP, only C_{16} alcohol and C_{16} acid were recorded from both extractions. Therefore, the concentration of *n*-alcohols and *n*-fatty acids in combination with alkanes correlates with the severity of WR associated with each species. However, the degree to which these compounds are released from the plant powder to coat the sand grains is also a factor contributing to induction of WR. The observed temperature effects on WR for the different species provide insights into the kinetics of the release and dispersal of organic compounds from leaf matter of these species. This will also have implications for the effects of fire and climate induced soil heating on soil WR.

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Data availability The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

Declarations

Competing interests The authors declare that there are no competing interests.

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