Geoderma 207–208 (2013) 212–220

Contents lists available at SciVerse ScienceDirect

Geoderma

journal homepage: www.elsevier.com/locate/geoderma

Biological and chemical factors controlling the patchy distribution of soil water repellency among plant species in a Mediterranean semiarid forest

E. Lozano [a,*], P. Jiménez-Pinilla [a], J. Mataix-Solera [a], V. Arcenegui [a], G.M. Bárcenas [b], J.A. González-Pérez [c], F. García-Orenes [a], M.P. Torres [d], J. Mataix-Beneyto [a]

[a] GEA (Grupo de Edafología Ambiental), Departamento de Agroquímica y Medio Ambiente, Universidad Miguel Hernández, Avenida de la Universidad s/n, 03202 Elche, Alicante, Spain
[b] MED_Soil Research Group, Departamento de Cristalografía, Mineralogía y Química Agrícola, Facultad de Química, Universidad de Sevilla, C/Profesor García González, 1, 41012 Sevilla, Spain
[c] Instituto de Recursos Naturales y Agrobiología de Sevilla, IRNAS-CSIC, Av. Reina Mercedes, 10, 4012 Sevilla, Spain
[d] Área de Botánica, Departamento de Biología Aplicada, Universidad Miguel Hernández, Avenida de la Universidad s/n, 03202 Elche, Alicante, Spain

ARTICLE INFO

Article history:
Received 27 February 2013
Received in revised form 20 May 2013
Accepted 21 May 2013
Available online 15 June 2013

Keywords:
Water repellency
Organic matter content
Lipid fraction
Glomalin related soil protein
Ergosterol

ABSTRACT

Natural soil water repellency is a property that has already been observed in forest soils and is characterized by its patchy distribution. There are many factors involved in its development. In this work, we have studied a large number of chemical and biological factors under the influence of different plant species (*Pinus halepensis*, *Quercus rotundifolia*, *Cistus albidus* and *Rosmarinus officinalis*) to learn which has the greatest responsibility for its presence and persistence in the top-soil layer. We observed strong and significant correlations between ergosterol, glomalin related soil protein (GRSP), extractable lipids, soil organic matter (SOM) content and water repellency (WR). Our results suggested lipid fraction as the principal factor. Moreover, apart from *Pinus*, fungal biomass seems to be also related to the SOM content. Soil WR found under *Pinus* appears to be the most influenced by fungi. Quality of SOM, to be precise, lipid fraction could be responsible for WR and its relationship with fungal activity.

1. Introduction

Soil water repellency (WR) has been observed in forest soils under different climatic conditions, soil types and vegetation covers (Doerr et al., 2000). Soil WR is normally characterized by a high spatial variability in persistence, with wettable and water repellent patches next to each other. This phenomenon is of special interest in semiarid areas, such as Mediterranean ecosystems, where water is considered to be one of the fundamental controls affecting the structure, function, and diversity of ecosystems (Rodriguez-Iturbe, 2000). In ecosystems where water resources are limited, even slight WR may play an important role in the infiltration patterns and the spatial distribution of water in the soil (Mataix-Solera et al., 2007). WR has hydrological impacts, but also ecological consequences, with repercussions on plant growth (Doerr et al., 2000). This could be the reason why several studies single out the production of hydrophobins by plants, as a possible ecological strategy (Mataix-Solera et al., 2007). It is thought to be a mechanism for improving water conservation by channeling water deep into the soil profile following preferential flow pathways (Moore and Blackwell, 1998; Robinson et al., 2010), while at the same time reducing evaporation due to the spatial dryness of the surface layer (Doerr et al., 2000).

It has been proposed that the origin of natural WR is caused by organic compounds released from different plant species and sources, due to resins, waxes and other organic substances in their tissues. In the Mediterranean areas different evergreen trees (such as *Pinus* and *Quercus*) and shrubs are usually associated with soil WR under natural conditions (Arcenegui et al., 2008; Jordán et al., 2008; Mataix-Solera et al., 2007; Verheijen and Cammeraat, 2007). There is a large quantity of research publications that associate soil WR with the SOM content (Doerr et al., 2000; Mataix-Solera and Doerr, 2004; Zavala et al., 2009). Despite this, many of them suggest that this relationship could be due to the quality of SOM (Mataix-Solera et al., 2007; Rumpel et al., 2004). In fact, literature has emphasized the importance of lipid fractions released to soil by plants or microorganisms (fungi) (Franco et al., 2000; Hudson et al., 1994; Ma'shum et al., 1988), as well as the behavior of specific characteristics of the organic matter, in general associated with moisture regimes, e.g., temporarily waterlogged soils (Fridland, 1982). In particular, considerable experimental effort has been carried out in the last decade to identify specific substances with a potential relevance on WR (De Blas et al., 2010; Doerr et al., 2005b; Franco et al., 1994, 1995; Hudson et al., 1994; McIntosh and Horne, 1994; Wallis et al., 1993).

On the other hand, research highlights the point that the relationship between WR and plants may not always be direct: a group of fungi and microorganisms, which might be associated with specific plants, could also contribute to soil hydrophobicity through their

* Corresponding author. Tel.: +34 966658337; fax: +34 966658340.
E-mail address: elozano@umh.es (E. Lozano).

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http://dx.doi.org/10.1016/j.geoderma.2013.05.021

products or by processing organic material (Feeney et al., 2004; Hallett and Young, 1999; Morales et al., 2010; White et al., 2000). In concrete, fungal hyphae, glomalin related soil protein and more recently ergosterol are being studied to understand their influence on the development of soil WR (Rillig, 2005; Rillig et al., 2010; Young et al., 2012). GRSP is a glycoprotein produced primarily by arbuscular mycorrhizae (AM) (Buyer et al., 2011; Treseder and Turner, 2007). Glomalin is not exuded by AM hyphae, but is instead contained within hyphal walls (Driver et al., 2005). When the AM hyphae die and decompose, they are thought to leave a residue of glomalin in the soil (Treseder and Allen, 2000). The importance of the presence of GRSP relates to its supposed hydrophobic properties. Results on the influence of GRSP differ, so that the question is still unclear (Feeney et al., 2004; Young et al., 2012). Ergosterol is a specific component of fungal membranes and the major sterol in most filamentous fungi (Van den Bossche, 1990). It is recognized as being an important biomolecule through which reduced permeability may occur in a wide variety of biological surfaces/membranes (Young et al., 2012). Its content is considered as a marker for living fungi and a good estimate of metabolically active fungal mycelium in soil (Montgomery et al., 2000).

In this research we have studied at the same time chemical and biological factors involved in the occurrence of superficial soil WR under different plant cover. Our aim here is to find out about which factors are the most relevant in the development of soil WR and possible relationships between them. This research could be a contribution to better understanding of why this phenomenon occurs in the semi-arid Mediterranean context under natural conditions.

2. Materials and methods

2.1. Study area

The study area is located in the 'Sierra de la Taja' (38°23′N; 0°59′W) near Pinoso, in the province of Alicante (SE of Spain). The region has a semi-arid Mediterranean climate with a mean annual precipitation of 277.5 mm and a mean annual temperature of 15.8 °C ranging from 7.8 °C in January to 24.1 °C in August (average 1980–2010). The whole area of the 'Sierra de la Taja' is approximately 500 ha. The samples were taken under similar conditions with respect to soil type, geology, plant distribution and slope. The soil is a Lithic Xerorthent (Soil Survey Staff, 1998), developed over Jurassic limestone. The soil texture in the area is loam, with a 36% of sand, a 49% of silt and a 15% of clay.

The tree stratus of the area is formed by *Pinus halepensis* Miller of approximately 40 years and *Quercus rotundifolia* is also present. Shrub vegetation comprises mainly *Quercus coccifera* L., *Rosmarinus officinalis* L., *Juniperus oxycedrus* L., *Cistus albidus* L., *Brachypodium retusum* Pers. (Beauv.), *Stipa tenacissima* L., and *Pistacia lentiscus* L. Tree and shrub species are mixed in the study area, but as a consequence of the relatively low density of vegetation, it was possible to carry out the sampling in microsites per stem of each species, avoiding interference between them.

2.2. Soil sampling

Samples were taken in September 2011, when the soil WR is expected to be at its strongest after the typical Mediterranean summer drought (DeBano, 1981; Dekker and Ritsema, 1994; Doerr et al., 2000). Soil samples were collected from the first 2.5 cm of the mineral A horizon at microsites beneath each of the four most representative species (*P. halepensis*, *R. officinalis*, *Q. rotundifolia* and *C. albidus*; n = 15 per species) and 5 samples from bare soil with no influence from any species. The sampling was done by selecting stems randomly, and taking two samples per stem. Half of the samples were preserved and frozen at −5 °C and the other half were preserved at 25 °C. The distance between the stems sampled was around 10 m.

2.3. Laboratory methods

Soil samples (not frozen) were dried at room temperature (20–25 °C) to a constant weight and sieved (2 mm) to eliminate coarse soil particles before soil analysis. Soil pH was measured in aqueous soil extract in de-ionized water (1:2.5 w:s) at 25 °C. SOM content was analyzed by rapid dichromate oxidation of organic carbon (Walkley and Black, 1934).

For measuring WR, approximately 15 g of soil per sample was placed on separate 50-mm diameter plastic dishes and exposed to a controlled laboratory atmosphere (20 °C, ~50% relative humidity) for one week to eliminate potential effects of any variations in preceding atmospheric humidity on soil WR and in accordance with the findings of Doerr et al. (2005a). The persistence of WR was measured by the Water Drop Penetration Time (WDPT) test (Wessel, 1988). This involved placing 3 drops of distilled water (~0.05 ml) onto the sample surface and recording the times required for their complete penetration. The average time for triplicate drops has been taken as the WDPT value of a sample. Penetration times were classified in intervals and in classes according to Bisdom et al. (1993), with WDPT ≤5 s representing wettable and WDPT >5 s water repellent conditions. The logarithm of the WDPT value in seconds has been used; being water repellent if the value of log (WDPT) is >0.7. The water repellency classes used are indicated in Table 1.

Extractable lipids were Soxhlet-extracted from soil samples (10 g) with a dichloromethane–methanol (3:1 v/v) for 16 h at 70 °C (González-Vila et al., 2003; Van Bergen et al., 1997). Extracts were filtered and dried and then total lipid content was gravimetrically determined and referred to as percentages of g soil.

To determine the possible relationship of fungal activity and its presence in soil with WR, three different fungal parameters were measured; GRSP, mycelium length and ergosterol. GRSP measured was the Easily Extractable Glomalin, which corresponded with the fraction of protein most recently deposited into the soil. GRSP was extracted from 0.25 g subsamples with 2 ml citric acid buffer, pH 7.0 at 121 °C for 30 min. After extractions, samples were centrifuged at 3000× during 15 min to remove soil particles. Protein in the supernatant was determined by a Bradford assay (Wright and Upadhyaya, 1996). Concentrations of glomalin were extrapolated to μg/g by correcting for the dry weight of coarse fragments (>0.25 g) included in the extraction of soil.

For the measurement of mycelium length, hyphae were extracted from a 10 g soil subsample by an aqueous extraction and membrane filter technique (Bååth and Söderström, 1980; Bardgett, 1991; Hanssen et al., 1974). Soil samples were mixed and suspended in 100 ml of deionized water. Suspensions diluted (10^{-2} ml) for measuring the mycelium length were stained with 0.05% Trypan Blue, and filtered

Table 1
WDPT classes and class increments used in the present study.
After Bisdom et al. (1993).

Repellency rating	Wettable	Water repellency								
		Slight			Strong			Severe		Extreme
WDPT classes	≤5	10	30	60	180	300	600	900	3600	>3600
WDPT interval (s)	≤5	6–10	11–30	31–60	61–180	181–300	301–600	601–900	901–3600	>3600
Log WDPT interval	≤0.7	0.7–1.0	1.0–1.5	1.5–1.8	1.8–2.3	2.3–2.5	2.5–2.8	2.8–3.0	3.0–3.6	>3.6