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Research article

Topsoil depth substantially influences the responses to drought of the foliar metabolomes of Mediterranean forests

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ABSTRACT

The upper soil provides support, water, and nutrients to terrestrial plants and is therefore crucial for forest dynamics. We hypothesised that a tree's metabolic activity (and therefore its metabolome; the total set of metabolites) would be affected by both the depth of upper soil layers and water availability. We sampled leaves for stoichiometric and metabolomic analyses once per season from differently sized *Quercus ilex* trees under natural and experimental drought conditions representing the likely conditions in the coming decades). Although the metabolomes varied according to tree size, smaller trees did not show higher concentrations of biomarker metabolites related to drought stress. However, the effect of the drought treatment on the metabolomes was greatest for small trees growing in shallow soils. Our results suggest that tree size is more dependent on the depth of the upper soil, which indirectly affects a tree's metabolome, rather than on the moisture content in the upper soil. Metabolomic profiling of *Q. ilex* supports our finding that water availability in the upper soil is not necessarily correlated with tree size. The higher impact of drought on trees growing in shallower soils nevertheless indicates that any increase in the frequency, intensity, and duration of drought – as has been projected for the Mediterranean Basin and other areas – would affect small trees most. Metabolomics has proved to be a useful means for investigating the links between plant metabolism and environmental conditions.

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1. Introduction

Soil provides a physical support system and a reservoir of water for terrestrial primary producers (Montheith, 1981). A scarcity of soil resources, particularly water, is often associated with restricted development of plant-soil systems and reduced biomass (Huxman et al., 2004; Knapp and Smith, 2001; Orwig and Abrams, 1997). Soil biological activity and tree growth can be limited by several factors such as nutrients (Bowman et al., 1993; Sardans and Peñuelas, 2015; Sardans et al., 2012a, b), light (Poorter, 1999), temperature (Epstein et al., 1997), or water (Huxman et al., 2004; Rosenzweig, 1968; Sala et al., 1988). Topographic factors such as slope variation and/or soil texture also play important roles in the retention and storage of soil water (Farahani et al., 1998; Fernandez-Illescas et al., 2001) and can influence soil enzymatic activity (Bastida et al., 2008) and erosion (Kinnell and Cummings, 1993). Soil depth is tightly linked with the physiology and respiration rates of tree roots (Pregitzer et al., 1998), the composition of microbial communities (Fierer et al., 2003), and even plant biodiversity (Fuhlendorf and Smeins, 1998). Most of the biological activity and nutrient recycling in soil occurs in the upper topsoil layers, so water availability in these layers is crucial to forests (Jobbagy and Jackson, 2000; Wardle et al., 2004). Hydraulic lift, mainly the transport of water from deep to shallower soil layers through roots to maintain physiological activity (Canadell et al., 1996; Nepstad et al., 1994; Schulze

Abbreviations: H-Forest, Forest with high canopies; L-Forest, Forest with low canopies; VS, Vegetation structure; DUSL, Depth of upper soil layers; D50, Diameter at 50 cm from soil; SEM, Structural Equation Model; LC–MS, Liquid chromatography coupled to mass spectrometry.

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et al., 1996), is common in several plant species (Caldwell and Richards, 1989; Caldwell et al., 1998; Peñuelas and Filella, 2003; Prieto et al., 2012; Wan et al., 2000). Plants also intensely influence soil; interdependence between changes in plant communities and in soil properties, such as fertility, has been observed in the Mediterranean Basin, especially in dry areas (Ruiz-Sinoga et al., 2011). For example, increased plant cover had a direct effect on soil porosity by increasing water infiltration and decreasing runoff (Garcia-Estringana et al., 2010; Goberna et al., 2007; Johnson-Maynard et al., 2002). The enhancement of soil quality by plant cover thus improves fertility (Gallardo et al., 2000) and enzymatic activity (Garcia et al., 2002), which in turn have positive effects on plant metabolism and growth (Ruiz-Sinoga et al., 2011; Sadaka and Ponge, 2003). The depth and texture of the topsoil, amongst other topographic factors, and the link between vegetation and soil are thus fundamental to the availability of water and nutrients for plants and may further play crucial roles in determining plant cover, habitat fragmentation, landscape patchiness, and changes in biodiversity (Fahrig, 2003; Sardans and Peñuelas, 2014; Allen and Breshears, 1998).

Mediterranean and other arid and semi-arid ecosystems have marked seasonality, with hot and dry summers (Aschmann, 1973; Schwinning et al., 2004). Soil depth can play a critical role in these ecosystems, because summer drought is an important factor limiting the growth of plants (Ogaya et al., 2003) and determining tree mortality (Barbeta et al., 2013). High temperatures and the absence of precipitation during summer deplete moisture in the upper soil layers, so plant activity can only be sustained where enough moisture is available in deep soil layers (Barbeta et al., 2015). Mortality and crown damage caused by extreme droughts are usually minimised where soil is deep (Lloret et al., 2012). The most recent climatic models project more frequent and severe droughts in Mediterranean ecosystems (IPCC, 2007). The effects of the increased drought conditions have already been observed during the last two decades affecting forest communities by increasing crown defoliation and tree mortality (Allen et al., 2010; Bigler et al., 2006; Carnicer et al., 2011; Galiano et al., 2011, 2010; McDowell et al., 2008; Poyatos et al., 2013; Rebetez and Dobbertin, 2004). The effects of drought on plant fitness and performance may also trigger important cascade effects through various trophic levels (Harrison, 2001; Kuske et al., 2003), thus producing important changes to entire ecosystems. A future exacerbation of drought in Mediterranean and other arid and semi-arid ecosystems may thus potentially affect, in a more intense or different way, the metabolism of trees growing in shallow soils with low capacities to store water, thereby leading to landscape patchiness and desertification (Sardans and Peñuelas, 2014). Forest decline and/or large changes in the structure of the vegetation and habitat are thus more likely in areas with shallower soil that are more susceptible to extreme drought (Galiano et al., 2012; Lloret et al., 2004).

Plants can adjust their metabolisms to maintain homeostasis under marked seasonality (Bertram et al., 2010; Falasca et al., 2013; Rivas-Ubach et al., 2014, 2012). Extreme droughts can cause drastic vegetation shifts, especially in Mediterranean, semi-arid, and arid ecosystems (Allen and Breshears, 1998; Hanson and Weltzin, 2000; Mueller et al., 2005), so the study of plant metabolomes can contribute to our understanding of how plants can metabolically cope with intense drought stress. Plants under drought conditions can adjust their chemistry to maintain physiological functions by, for example, increasing foliar concentrations of K (Cakmak and Engels, 1999; Sardans and Peñuelas, 2015), proline (Tymms and Gaff, 1979; Yamada et al., 2005), antioxidants (Rivas-Ubach et al., 2014), sugars (Ingram and Bartels, 1996; Porcel and Ruiz-Lozano, 2004; Rivas-Ubach et al., 2014), and/or other species-specific compounds (Sardans et al., 2011).

The metabolome, the chemical phenotype of an organism, is the total set of low molecular weight metabolites (typically <1200 Da) present in an organism at a particular moment (Fiehn, 2002). The metabolome, includes thus amino acids, sugars, and nucleotides from primary plant metabolism and many secondary metabolites such as phenolics and terpenes representing the diverse physiological processes in an organism for maintaining internal homeostasis and function. The first functional responses of an organism facing abiotic and biotic stressors are typically at the metabolomic level (Peñuelas and Sardans, 2009). Metabolomics represents thus a powerful tool for ecological studies (ecometabolomics) to identify the main changes in organisms directly associated with metabolism and performance (Sardans et al., 2011). Metabolomics allows us to understand the metabolic variation of organisms under stressful environmental conditions, including the complete set of metabolites and not just single compounds or families of metabolites (Fiehn, 2002; Bundy et al., 2008; Sardans et al., 2011). The study of the metabolomic changes of wild plant species helps to comprehend the mechanisms behind plant physiological responses to natural or experimental stressors. Metabolomic techniques can also assess the plasticity of specific metabolomes and detect and quantify the metabolic biomarkers linked with specific environmental stressors (Bundy et al., 2008; Sardans et al., 2011; Rivas-Ubach et al., 2016a). Ecometabolomics has advanced our understanding of the natural variability and flexibility of the metabolomes of wild organisms under climatic stressors (Gargallo-Garriga et al., 2014; Rivas-Ubach et al., 2014), amongst seasons (Rivas-Ubach et al., 2012), and under attack from folivorous insects (Rivas-Ubach et al., 2016b). Ecometabolomics is thus valuable for exploring the organism-environment interaction by detecting and quantifying the final phenotypic response of an organism to environmental changes.

The physiological response to drought of *Quercus ilex* L., an evergreen sclerophyllous tree species widely distributed in the Mediterranean Basin (Barbero et al., 1992), has been extensively studied (Filella et al., 1998; Nardini et al., 2000; Ogaya and Peñuelas, 2003; Peñuelas et al., 2000; Sala and Tenhunen, 1996). *Q. ilex* is a keystone species in many Mediterranean ecosystems and is currently expanding its dominance by recolonising abandoned cropland and pastures and by out-competing Mediterranean conifers that are more sensitive to rising temperatures (Carnicer et al., 2013). Drought-induced declines in *Q. ilex* forests have been reported (Galiano et al., 2012; Camarero et al., 2015), but this tree possesses an array of functional and morphological traits (such as an extensive root system) for surviving periods of drought. Ecometabolomic studies of *Q. ilex* could thus identify the key metabolites involved in drought tolerance and resistance as well as measure how flexible are the individual metabolomes under stress conditions. We sampled once per season the leaves of differently sized mature *Q. ilex* trees of the same age from a forest exposed to a moderate experimental drought and analysed the elemental stoichiometries and metabolomes. We discuss three important issues of *Q. ilex* metabolic responses to environmental variables or factors by multivariate approximations: (i) we hypothesise that the depth of the upper soil layers (DUSL, typically the A + B horizons), where most of the biological activity and water uptake occur, may determine vegetation structure (VS) and overall metabolomic composition; ii) we evaluate how different VSs, with special attention to trees growing in shallower soils, respond to the marked seasonality of the Mediterranean Basin and to experimental drought stress; and iii) we apply the results of this study to illustrate the crucial necessity in ecometabolomic studies of controlling the factors potentially able to produce large metabolomic shifts in plants.