ARTICLE IN PRESS

STOTEN-22200; No of Pages 10

Science of the Total Environment xxx (2017) xxx–xxx

Contents lists available at ScienceDirect

Science of the Total Environment

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

Additive or non-additive effect of mixing oak in pine stands on soil properties depends on the tree species in Mediterranean forests

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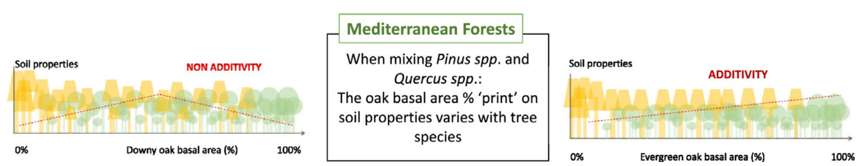
HIGHLIGHTS

- In litter, aromatic compounds decrease with high OBA%, promoting microbial growth.
- Oak abundance favored microbial diversity and biomass in topsoils for both stands.
- The holm oak print was linked to cutin.
- Non-additive effect on soil properties was found under *Q. pubescens* and *P. sylvestris*.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 6 January 2017
Received in revised form 2 March 2017
Accepted 3 March 2017
Available online xxxx

Editor: P Elena PAOLETTI

Keywords:
Mediterranean forests
Mixed forests
Non-additive effects
Organic matter quality
Soil horizons

ABSTRACT

This study investigated how oak abundance in pine stands (using relative Oak Basal Area %, OBA%) may modulate soil microbial functioning. Forests were composed of sclerophyllous species i.e. *Quercus ilex* mixed with *Pinus halepensis* Miller or of *Q. pubescens* mixed with *P. sylvestris*. We used a series of plots with OBA% ranging from 0 to 100% in the two types of stand ($n = 60$) and both OLF and A-horizon compartments were analysed. Relations between OBA% and either soil chemical (C and N contents, quality of organic matter via solid-state NMR, pH, $CaCO_3$) or microbial (enzyme activities, basal respiration, biomass and catabolic diversity via BIOLOG) characteristics were described. OBA% increase led to a decrease in the recalcitrant fraction of organic matter (OM) in OLF and promoted microbial growth. Catabolic profiles of microbial communities from A-horizon were significantly modulated in *Q. ilex* and *P. halepensis* stand by OBA% and alkyl C to carboxyl C ratio (characteristic of cutin from *Q. ilex* tissues) and in *Q. pubescens* and *P. sylvestris* stands, by OBA% and pH. In A-horizon under *Q. ilex* and *P. halepensis* stands, linear regressions were found between catabolic diversity, microbial biomass and OBA% suggesting an additive effect. Conversely, in A-horizon *Q. pubescens* and *P. sylvestris* stands, the relationship between OBA% and either cellulase activities, polysaccharides or ammonium contents, suggested a non-additive effect of *Q. pubescens* and *P. sylvestris*, enhancing mineralization of the OM labile fraction for plots characterized by an OBA% ranging from 40% to 60%. Mixing oak with pine thus favored microbial dynamics in both type of stands though OBA% print varied with tree species and consequently sustainable soil functioning depend strongly on the composition of mixed stands. Our study indeed revealed that, when evaluating the benefits of forest mixed stand on soil microbial functioning and OM turnover, the identity of tree species has to be considered.

1. Introduction

The dynamics of Mediterranean forest stands is subject to various environmental drivers acting at different spatio-temporal scales. Land use legacy and specific edaphic and climate conditions (geological

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http://dx.doi.org/10.1016/j.scitotenv.2017.03.023
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Please cite this article as: Brunel, C., et al., Additive or non-additive effect of mixing oak in pine stands on soil properties depends on the tree species in Mediterranean fores..., Sci Total Environ (2017), http://dx.doi.org/10.1016/j.scitotenv.2017.03.023

substratum, soils poor in organic matter, intense summer drought) play an important role. The surface and composition of European Mediterranean forests were strongly structured by wildfires and human activities leading to agro-sylvo-pastoral systems during the last century (Quézel and Médail, 2004). From the 1930s on, abandonment of land previously devoted to agriculture and increasingly frequent wildfires promoted massive colonization of natural spaces by resinous species such as *Pinus halepensis* Mill. and *Pinus sylvestris* (Tatoni and Roche, 1994), resulting in pure stands. Yet mixed forests have long been considered essential to sustainable forestry management (Gartner and Cardon, 2004; Rodriguez-Loinaz et al., 2008). Biodiversity creates more varied habitats (Lust et al., 1998), produces more above-ground biomass (Vilà et al., 2007) and enhances resilience to stress and to disturbances like diseases (Pautasso et al., 2005), fires (Wirth, 2005) or extreme weather events (Dhôte, 2005). However, in Mediterranean areas, few studies have explored the relationship between above-ground tree species composition and both soil chemical and microbial characteristics (Lucas-Borja et al., 2011).

Mixed forests are heterogeneous ecosystems composed of patches varying in their relative proportion of tree species. Broadleaved species can be considered as ameliorative species, increasing the easily-degradable nutrients in conifer stands and thus enhancing microbial metabolism (Polyakova and Billor, 2007). By contrast, the chemical identities of pine litters and root exudates induce lower rates of decomposition because of their lack of nutrients (Khiewtam and Ramakrishnan, 1993), high recalcitrant organic matter content (Van Wesemael and Veer, 1992), allelopathic compounds and soil acidification (Rousk et al., 2010). Moreover, mixed stand 'print' has been shown to vary according to the functional traits of the tree-species constituting the mixture (Prescott and Grayston, 2013). Changes in tree species identity can actually modify both the biomass and the structure of soil microbial communities (Laganière et al., 2009; Matos et al., 2010). Thus, in mixed stands, these subtle variations in organic matter (OM) input can lead to additive or non-additive (synergistic or antagonistic) effects, modifying C and N dynamics (Scheibe et al., 2015). Overall, although the positive effect of mixed stands on the dynamics of OM decomposition has been reported (Bonanomi et al., 2014; Cuchietti et al., 2014; Sariyildiz et al., 2005), no simple and predictable patterns are available for forest management (Poca et al., 2015; Tardif et al., 2014).

In this study, we investigated the influence of oak occurrence on soil microbial functioning in pine stands, using an oak abundance gradient (assessed via Oak Basal Area percentage, OBA %). We hypothesized that, in Mediterranean forests, oak abundance favors soil carbon dynamics via more labile and more diversified organic matter input. We focused on two types of mixed stands: *Pinus halepensis* Mill. and *Quercus ilex* and *P. sylvestris* and *Q. pubescens*. We assumed that the influence of the 'oak print' would vary with the *Quercus* species considered, since the evergreen *Q. ilex* and the deciduous *Q. pubescens* have species-specific chemical signatures and functional traits. Moreover, we investigated the effect of tree species relative composition on two soil compartments (OLF and A-horizon).

2. Material and methods

2.1. Sampling strategy of A-horizon and across the gradient of admixture in the two forests studied

The study was conducted in South Eastern France (Provence-Alpes Côte d'Azur), a region characterized by intense summer droughts and wet and temperate winters typical of Mediterranean climate. The Provence's soil is characterized by carbonatic pedofeatures, such as a fine calcareous silty clay loam. We conducted our study in two distinct zones in sub-humid (mean altitude: 400 m, mean precipitations: 755 mm per year and mean air temperatures: 13.01 °C, www.worldclim.org) and humid (mean altitude: 1000 m, mean annual precipitations: 842 mm and air temperatures: 9.6 °C, www.worldclim.org) bioclimates. Sclerophyllous forests of *Quercus ilex* mixed with *Pinus halepensis* are characteristics of the sub-humid climate while *Quercus pubescens* and *Pinus sylvestris* forests are found in the humid climate.

Sampling plots were selected in a 70 km^2 area in either the Massif de la Sainte Baume (43°21′ N, 5°43′E) for the *Q. ilex* and *P. halepensis* stands (in the sub-humid climate) or in the Réserve Géologique of Digne les Bains (44°12′ N, 5°59′ E) for the *Q. pubescens* and *P. sylvestris* stands (in the humid climate). For each area, a total of 30 circular plots of 0.02 ha with different relative basal areas of pine and oak (ranging from 0 to 100% of oaks in pine stands) were selected. The plots were chosen using the database of the Regional Center of Forest Property: mixed forest stands of 60 ± 10-year old, with similar north-facing slopes and soil type (calcaric Cambisol) and no sylvicultural practices for 30 years. For each plot, the numbers of stems found, stem density and basal area were measured and averages for each species are presented in Table 1.

The basal area of *Quercus* spp. and *Pinus spp.* (OBA and PBA respectively) of each sampling plot ($m^2 \cdot ha^{-1}$) was determined from each tree diameter measured at 1.20 m from the ground. The relative Oak Basal Area % (OBA%) was calculated as follows: $OBA\% = \frac{\sum(OBA)}{\sum(OBA+PBA)} \times 1$ 00

Sampling was performed in the first two weeks of October 2013. Within each plot, two sets of ten samples were collected in two soil compartments: the forest floor (OLF) and the surface mineral soil horizon from 0 to 15 cm depth (A-horizon). In each plot, set of 10 randomly chosen samples were pooled per horizons and each set of samples were pooled and sieved (2 cm and 2 mm mesh respectively for OLF and A-horizon). Soil samples were stored at 4 °C before the determination of microbial activities.

2.2. Microbial community structure, respiration and enzymatic activities

The microbial communities' catabolic profiles were compared with BIOLOG® Ecoplates (BIOLOG Inc., Hayward, CA) according to (Garland and Mills, 1991). 3 g of OLF or 5 g of A-horizon sample (dry weight equivalent) were shaken in 100 ml sterile 0.1% sodium pyrophosphate (pH 7.0) for 2 h. The bacterial suspension was centrifuged at low-speed run (500 *g*) for 6 min. After further dilution in 0.85% NaCl, a 1/50 dilution was used to inoculate a series of 32 wells (125 l per well). The plates were incubated for up to 5 days at 25 °C and the color development was recorded using a microplate reader (Infinite Tecan) at 590 nm twice a day. Microbial response in each microplate that expressed average well-color development (AWCD) was determined as follows: $AWCD = \frac{\sum ODi}{31}$ where OD_i is optical density value from each well, corrected subtracting the blank well (inoculated, but without a carbon source) values from each plate well. The Shannon-Weaver index was calculated as follows: $H = -\sum pi(lnpi)$ where p_i is the ratio of the OD on each substrate (OD_i) to the sum of OD on all substrates $\sum OD_i$.

Microbial biomass (MB) was estimated using Substrate-Induced Respiration (SIR) according to Anderson and Domsch, 1978. Ten grams (dry weight equivalent) of standardized samples at 60% of WHC were placed in 117 ml air flushed glass jars and amended with a mix powder of talc and glucose (1000 μg C g^{-1} soil). After 90 min, 1 ml of air was sampled with a syringe and injected into a gas chromatograph (Chrompack CHROM 3 – CP 9001) to determine CO_2 production. The CO_2 concentration of flushed air was subtracted from CO_2 concentrations of each samples and resulting values were adjusted to 22 °C according to Ideal Gas Laws using a $Q_{10} = 2$. SIR rates were converted into MB using equations given by (Beare et al., 1990). Basal respiration was estimated using the same method to calculate the metabolic quotient qCO_2 (the ratio of basal respiration to microbial biomass), which is a sensitive ecophysiological indicator of soil stress induced by environmental conditions (Anderson, 2003).