



## 1. Introduction

Forests not only supply essential harvestable product but also regulate climate and water cycle, mineral cycling or prevent soil erosion and flooding (Kozłowski, 2002). Unfortunately, a wide range of abiotic and biotic stresses (i.e. climate change, excessive harvesting, pests and diseases, drought, forest fires and soil compaction) threatens the goods and services that forest can supply (Ferguson et al., 1996). On this context, sustainable management of forest ecosystems requires that primary production, carbon storage capacity and biological functions, that is, multiple ecosystem functions and services, be preserved over time (Bengtsson et al., 2000).

Vegetation affects soil properties, which in turn define interspecific competition and therefore plant growth, setting plant–soil feedback systems up (Miki, 2012). Several physico-chemical properties of the soil as C/N ratio, pH or total organic carbon are deeply driven by plant type and coverage, which in turn could be altered by changes in these mentioned variables (Sardans and Peñuelas, 2013). Soil exerts an essential function in the forest ecosystems fertility and stability by highlighting microorganisms, which manage reactions to liberate soil nutrients for plant development (Hannam et al., 2006).

Biogeochemical soil cycles are influenced by the microbial community of the soil, since microorganisms control soil mineralization and humification processes. In this manner, the combination of plants, soil structure and soil function create extensive relationships with numerous feedbacks, being biotical integrals important to soil-plant system elements (Sardans and Peñuelas, 2013; Bastida et al., 2007). Moreover, microbial communities play key roles in maintaining multiple ecosystem functions and services simultaneously, including nutrient cycling, primary production, litter decomposition and climate regulation (Delgado-Baquerizo et al., 2015).

Several soil properties like microbial biomass carbon, soil respiration, total organic carbon, enzymatic activities or nutrient content availability are extremely related to soil organic matter (Doran and Parkin, 1994; Larson and Pierce, 1994; Entry and Emmingham, 1998; Bastida et al., 2007; Hedo et al., 2015a). Moreover, different authors exposed that soil enzyme activities can be an excellent approximation of microbial activity, and in turn of soils health (Dick et al., 1996; Hedo et al., 2015b). Forest soil organic matter derives from litterfall and root inputs, while losses come from the microbial degradation of organic matter, erosion, solution losses, and eluviations, achieving a balance between soil organic matter loss and soil organic accumulation (Entry and Emmingham, 1998). The mentioned equilibrium could be altered by plant cover or tree age, which in consequence may modify soil status. Thus, many authors have proposed the combined use of several variables as early indicators of soil functionality (Nannipieri et al., 1990; Lucas-Borja et al., 2012). Specific indicators of soil microbial activity have been proposed to assess soil quality (Bastida et al., 2008; Hedo et al., 2015c; Lucas-Borja et al., 2011), being worthy to mention a few enzyme activities, specifically related to the cycles of S, C, P and N (arylsulfatase,  $\beta$ -glucosidase, alkaline and acid phosphatase and urease, respectively) and a few others general microbial indicators such as soil respiration and dehydrogenase activity. pH (Lucas-Borja et al., 2011), carbon nitrogen ratio (C/N ratio) (Lucas-Borja et al., 2011; Hedo et al., 2015b), soil texture (Fterich et al., 2014), nutrients status (Kutiel and Naveh, 1987; Burgess and Wetzel, 2000) or microbiological communities. All these soil properties are commonly used as have been used as helpful and practical indicators of soil functionality (Wu et al., 2013).

The microbiological soil properties and nutrient ecological stoichiometry of litter and soil are important for the growth and dynamics of species, but the stoichiometric relationships among leaf, litter, and soil remain poorly understood. As different studies have demonstrated, tree stand composition and forest structure may affect physico-chemical and microbiological soil properties or modify nutrient storage (Entry and Emmingham, 1998; Hedo et al., 2015c; Lucas-Borja et al.,

2011; Mund and Schulze, 2006; Jandl et al., 2007). Nevertheless, the connection among soil quality and aboveground trees age species in Mediterranean forests remains still not fully understood (Gleixner et al., 2005; Grayston and Prescott, 2005). Particularly, the relation between multiple forest functions (including nutrient cycling, primary production, litter decomposition, water and climate regulation) derived from different forest age stands or different forest structures is especially unclear in Mediterranean forest sites. Moreover, it is difficult to explain the qualitative and quantitative relationship between the litter and soil for the diverse field conditions (Ge et al., 2013). The aim of the present work was to evaluate stand age-related effects on nutrient content and microbiological properties in litter and soil. The collected information in this study could help to design proper forest management guidelines to increase the health of this forest ecosystems and their functions.

## 2. Materials and methods

### 2.1. Study area

This study was conducted in “Los Palancares y Agregados” forest (40°01'50"N; 1°59'10"W; Spain). A natural forest of Spanish black pine (*Pinus nigra* Ar. ssp. *Salzmannii*), Holm oak (*Quercus ilex* L.), and Spanish juniper (*Juniperus Thurifera* L.) trees dominates the current vegetation of the study area. The herbaceous vegetation is mainly represented by *Eryngium campestre* L., *Geranium selvaticum* L., *Centaurea paniculata* L., and *Plantago media* L. At the end of the 19th century, Spanish black pine forest stands were managed under different systems in this region. Forest under age-class management is one of the most used methods in Spanish black pine forest management, more specifically using a shelterwood system with a 20–30 year regeneration period in a 100–120 year rotation (Lucas-Borja et al., 2012), generation different sections with defined forest age and structure. The climate is classified as Mediterranean humid (Allué, 1990), with a mean annual temperature of 11.9 °C (the mean lowest temperature of the coldest month is –0.5 °C and the mean highest temperature of the hottest month is 30.5 °C) and mean annual precipitation of 595 mm (99 mm in summer). The average elevation of the study area is 1200 m.a.s.l. The soil is represented by the Entisol class, according to the USDA (Soil Survey Staff, 1999). The soil texture was characterized as sandy-loam (Table 1). Other soil characteristics can be seen in Table 2.

### 2.2. Experimental design

The study began in October 2014, taking the advantage of the previously designed experimental design (Lucas-Borja et al., 2016). Twenty-five forest sections of about 50 ha were arbitrarily selected at the “Palancares y Agregados” forest area, breaking down forest stands into five compartments, each one with an age-class: compartments I (TI: age 100–80 years), five compartments II (TII: age 79–60 years), five compartments III (TIII: age 59–40 years), five compartments VI (TIV: age 39–20 years), five compartments V (TV: age 19–1 years). Moreover, a forest compartment without any anthropic influence and ranging 80–120 years old was chosen as control (T0). The forest under age-class management is outlined by a sequence of relatively homogenous, even-aged stands once the rotation period (100 years) is achieved. Table 1 summarized the main characteristics of the compartments in terms of forest structure. Litterfall was estimated using twelve litter traps randomly placed at each compartment. Six samples (500 g each) were randomly collected in undisturbed and unaltered forest floor areas at each of these compartments (TI, TII, TIII, TIV, TV and control). Sampled points were separated from each other by >500 m, considering them as an independent and in order to avoid pseudoreplications problems. Similar topography and altitude were presented in the whole experimental area. On the other hand, the sampled points also had similar conditions such as slope and aspect. Soil samples were collected from

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