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Influence of management and stand composition on ecosystem multifunctionality of Mediterranean tree forests

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ABSTRACT

In semi-arid ecosystems, forests are critical sites for supporting multifunctionality, which is endangered by multiple environmental stresses. In this regard, understanding how ecosystem multifunctionality (EMF) develops in semi-arid forests is important to setup actions preserving these delicate environments. Changes in species composition and management operations can have heavy effects on the Mediterranean forest ecosystem. In order to better understand the influence of these drivers on EMF of Mediterranean forests, this study compares ecosystem structure, properties and functions as well as the resulting EMF in four types of forests in Central Eastern Spain: (1) a pure and unmanaged stand of Spanish black pine, assumed as control; (2) a pure but managed stand of Spanish black pine; (3) two mixed and unmanaged stands of Spanish black pine and (3.a) Spanish juniper and (3.b) holm oak. Regarding the ecosystem structure, both forest management and stand composition altered plant diversity but not soil covers (except for vegetation). About the ecosystem properties, soil characteristics significantly changed between pairs of stands (especially texture, pH and bulk density). Concerning the ecosystem functions, forest stand structure was a significant driver of waste decomposition but not of wood production, while its effect on nutrient cycling, belowground carbon stocks and water cycle was different according to the specific tree species. The impacts of forest management on the ecosystem functions were in general significant compared to the unmanaged stand in terms of wood production, belowground carbon stocks, nutrient cycling, but not of water cycle and waste decomposition. Overall, this study demonstrates that the average EMF is primarily affected by forest management (with a decrease in EMF in managed stands compared to the unmanaged forest), and by stand composition only in the case of one mixed stand. As such, the forest management actions must be carefully adopted, in order to avoid EMF degradation.

KEYWORDS: Mixed forest stands; pure forest stands; ecosystem properties; ecosystem structure; ecosystem functions.

KEY MESSAGE: The multiple functions of Mediterranean forest ecosystems primarily decrease with management operations, and secondarily with tree composition. This finding emphasises the importance of a suitable management for maintaining ecosystem functioning in Mediterranean forests.

1. INTRODUCTION

Ecosystem multifunctionality (hereafter "EMF") is "the simultaneous provision of multiple services and functions by landscape to society" (Maestre et al. 2012; Byrnes et al. 2014). In forests, these functions or services include nutrient cycling (e.g., nutrient availability and mineralization), carbon sequestration, climate and water regulation, organic matter decomposition (e.g., lignin degradation) and wood production (Ushio et al. 2010; Aponte et al. 2013; Byrnes et al. 2014). Forests are critical sites for supporting EMF, especially in semi-arid ecosystems, such as in the Mediterranean region. Here, multiple environmental stresses (i.e., climate change, excessive harvesting, pests and diseases, drought, forest fires and low contents of organic matter and nutrients) are great challenges for the sustainability and productivity of forest ecosystems. Therefore, understanding how EMF develops in Mediterranean forests is important for landscape planners to safeguard these delicate ecosystems.

Different environmental stressors (e.g., droughts or wildfires) and inadequate management strategies of forests, such as some silvicultural operations that promote specific stand composition, may significantly affect ecosystem functions or services (Benz et al. 2020). For instance, Pohjanmies et al. (2021) showed that management strategies promoting monospecific forest (e.g., monocultures) negatively affects ecosystem multifunctionality, also hindering its recovery in comparison to mixed forests. Moreover, in monospecific forest ecosystems, resource extraction can fundamentally change its structure and functioning (Edwards et al. 2014). In contrast, the effects of stand composition in mixed forests are usually considered beneficial for the ecosystem, because the different residues of plants can improve soil health, increasing biodiversity, contents of nutrients, and waste decomposition. As a result of complementarity (e.g., positive interactions between forest species or more efficient use of light or soil resources) or selection (e.g., increase in the presence of species with higher overall ecosystem service potential in comparison to monocultures) effects, tree species diversity may increase ecosystem productivity in comparison to monospecific forest stands (Zhang et al. 2012; Huuskonen et al. 2021). In this context, we are still lacking to know the associations between tree stand composition and EMF in pure or mixed Mediterranean forest species, limiting our capacity to predict how future forest management plans and restoration efforts may help to promote EMF and mitigate climate change (Gleixner et al. 2005; Grayston and Prescott 2005; De Cáceres et al. 2021).

However, the different types of plant may cause contrasting influences on EMF, since Mediterranean forests include a wide variety of dominant tree species. In more detail, the specific composition of tree species and other plant types may change the equilibrium of some components in forest ecosystem, such as soils and plants. Soils can undergo both accumulation and loss of organic matter in the forest floor, which thereby can alter the physico-chemical and microbiological properties of soils (Entry and Emmingham 1998). The quality and quantity of soil organic matter are important drivers of the soil component in forest ecosystems (and thus of EMF), since organic compounds support productivity, biodiversity, and other ecosystem services (van Leeuwen et al. 2014; Zornoza et al. 2015; Lozano-García et al. 2016). Moreover, the different plant species may differently influence the enzymatic activities (associated to the dynamics of soil organic matter and nutrients in forest soils), which are considered among the best proxies of soil health and activity (Dick et al. 1997). This means that both the dynamics of organic matter and nutrients, and the variability of enzymatic activities need to be considered concurrently to understand the changes of EMF in different forest stands.

The relations between forest management and stand composition on one side, and EMF on the other side are still vague. This insufficient knowledge requires investigations that should explore the changes in EMF in relation to both forest operations and presence of different tree species, since the potential trade-offs between ecosystem functions and these factors are virtually unknown in Mediterranean forests. Improving our understanding of the changes in EMF as a response to forest management and stand composition is of paramount importance for conservation of Mediterranean forests and for a proper restoration of the threatened forest ecosystems worldwide (Ferguson 1996). At least to the authors' best knowledge, no previous assessments of EMF and its main drivers have been carried out in semi-arid Mediterranean forests, either in pure or mixed stands or between managed and unmanaged sites, and this is the main novelty of this study.

To fill this gap, this study aims at evaluating how tree stand composition and management influence ecosystem multi-functionality (EMF) in Mediterranean forests. More specifically, EMF was compared in four forest stands of Central Eastern Spain: (1) a pure and unmanaged stand of Spanish black pine, assumed as control; (2) a pure but managed stand of Spanish black pine; and (3) two mixed but unmanaged stands of Spanish black pine and (3.a) Spanish juniper and (3.b) holm oak. The research questions supporting this study are two: (i) does forest management or stand composition noticeably affect EMF in Spanish black pine forests under semi-arid conditions? And (ii) which of multiple forest functions (nutrient cycling, wood production, waste decomposition, water, and belowground carbon stocks) mostly influences EMF of managed vs. unmanaged pure stands or pure vs. mixed stands of Spanish black pine? The replies to these questions may improve our understanding of the changes in EMF as a response to forest management and stand composition, and this knowledge may support the selection of the most suitable forest management actions, in order to avoid EMF degradation.

2. MATERIAL AND METHODS

2.1. Study area

The study was conducted in “Los Palancares y Tierra Muerta” forest (geographical coordinates: 40º01´50´´N; 1º59´10´´W; average elevation: 1200 m a.s.l.) (Figure 1), which, covering 18078 ha, is the largest element among the Natural Monuments in Castilla La Mancha (Central-Eastern Spain). Moreover, this natural ecosystem is included in the endangered habitats of European Union, since it belongs to a natural habitat requiring specific conservation measures (Resolution 4/1996 by the Convention on the Conservation of European Wildlife and Natural Habitats). The study region has a Mediterranean climate with hot dry summers and humid winters (de Zulueta 1990). Mean annual temperature is 11.9 ^{o}C, ranging from -0.5 ^{o}C in January to 30.5 ^{o}C in July. Mean annual precipitation is 595 mm, 99 mm of which occurring on average in summer. Regarding vegetation, the tree layer is dominated by natural Spanish black pine (*Pinus nigra* Arn. ssp *salzmannii*), holm oak (*Quercus ilex* L.) and Spanish Juniper (*Juniperus Thurifera* L.) trees. Herbaceous vegetation mainly consists of *Eryngium campestre* L., *Geranium selvaticum* L., *Centaurea paniculata* L. and *Plantago media* L. Soils were *Leptosols* (according to the Soil Atlas of Europe, 2005) with sandy-loam or loamy-sand texture (USDA classification) (Table 1).

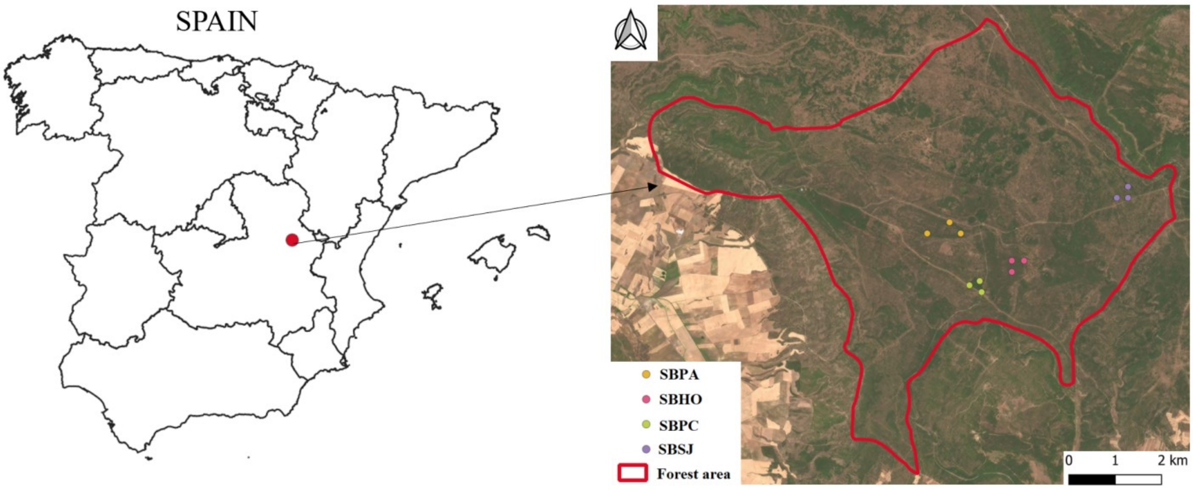

Figure 1 - Geographical location (a) and aerial map (b) of the study area ("Los Palancares y Tierra Muerta" forest, Castilla La Mancha, Central-Eastern Spain). *Legend: SBPA = pure and managed stand of Spanish black pine; SBSJ = mixed stand of Spanish black pine and Spanish juniper; SBHO = mixed stand of Spanish black pine and holm oak; SBPC = pure and unmanaged stand of Spanish black pine.*

Table 1 – Main vegetal characteristics of the four forest stands in the study area ("Los Palancares y Tierra Muerta" forest, Castilla La Mancha, Central-Eastern Spain).

Forest stand	**Tree composition***	**Main herbaceous composition**	**Soil type and texture**
SBSJ	*Pinus nigra* Arn. ssp *salzmannii* (65 %) *Juniperus thurifera* (35 %)	*Eryngium campestre L., Geranium selvaticum L., Plantago media L., Festuca rubra L., Centaurea paniculata L. and Achillea odorata L*	leptosol loamy-sand
SBHO	*Pinus nigra* Arn. ssp *salzmannii* (80 %) *Quercus ilex* (20 %)	*Eryngium campestre L., Geranium selvaticum L., Plantago media L., Festuca rubra L., Centaurea paniculata L. and Achillea odorata L*	leptosol sandy-loam
SBPA	*Pinus nigra* Arn. ssp *salzmannii* (100 %)	*Eryngium campestre L., Geranium selvaticum L., Plantago media L., Festuca rubra L., Centaurea paniculata L. and Achillea odorata L*	leptosol sandy-loam
SBPC	*Pinus nigra* Arn. ssp *salzmannii* (100 %)	*Geranium selvaticum L., Achillea odorata L., Lavandula latifolia L., Festuca rubra L., Cardus-cellus hispanicus L., Trifolium montanum L*	leptosol sandy-loam

*Notes: * based on tree density; soil type information derived from Soil Atlas of Europe (2005); soil texture identified according to the USDA classification; SBPA = pure and managed stand of Spanish black pine; SBSJ = mixed stand of Spanish black pine and Spanish juniper; SBHO = mixed stand of Spanish black pine and holm oak; SBPC = pure and unmanaged stand of Spanish black pine.*

Since the end of the 19th century, Spanish black pine stands have been managed under different systems. A shelterwood system with a 100-to-120-year rotation and a 20-to-30-year regeneration period is the traditional method. Initial seedling recruitment has always been difficult, due to factors such as summer drought, soil compaction, masting conditions, seed predation, inadequate overstorey density, attacks by European pine shoot moth on seedlings, and damage from grazing animals, which is often the major problem. During the last century, forest managers tried to enhance regeneration by soil treatments, planting, and introduction of new varieties of species, but the results have been disappointing. Protecting areas of natural regeneration from browsing by large mammals has also been necessary, since this damage is a major problem for the regeneration of forest stands.

2.2. Experimental design

2.2.1. Selection of forest stands and plot installation

In the study area, four forest stands were considered to explore the ecosystem multifunctionality. Specifically, an unmanaged and pure stand of Spanish Black Pine (hereafter indicated by "SBPC") was assumed as reference stand. This stand, which was about 180-200 years old, represents a natural land evolution in the study area. Three other stands were selected as pure but managed, or mixed but unmanaged stands: (1) a pure stand of Spanish black pine, managed for timber production according to traditional silvicultural practices in Cuenca Mountains (remained the same since the end of the 19th century) (hereafter "SBPA"); and (2) two mixed stands of Spanish Black Pine and (2.a) Spanish Junipers tree ("SBSJ") or (2.b) holm oak ("SBHO"). These two mixed stands, with tree age over 100 years, were near-natural without distinct anthropogenic impact on their structure in the past several decades. Since mid-last century, holm oak and Spanish juniper species have been excluded from silvicultural treatments, thus promoting the coexistence of mixed forest with these species and Spanish black pine (Table 1). The regeneration method used in both mixed and pure Spanish black pine stands created a uniform opening of the canopy without soil preparation. The main goal for forest management was the increase of forest standing stock and transformation of age-heterogeneous stands into even-aged stands.

In September 2017, twelve plots (each of 10 m x 10 m, covering 100 m^2) were established in four groups (one group per forest stand, each with three replicates). The reciprocal distance among plots was always higher than 300 m. The selection of locations for the studied stands was a compromise between the double objective to have (i) replicated plots at a suitable reciprocal distance (which avoided the risk of pseudo-replications and thus took into account the spatial heterogeneity of the ecosystem features), and (ii) sites with homogenous ecosystem properties (referred to soils and plants), being, in spite of this reciprocal distance, the topography (aspect, all exposures, and slope, 2 to 5%), altitude (1203 to 1237 m), climatic conditions, and soil type and texture similar (Lucas-Borja et al. 2010) (Table 1). The selected plot size appears to be suitable for the specific study, since this size allowed the identification of homogeneous sites with minor differences in the indicators used to evaluate the ecosystem features and the resulting EMF in the selected stands. Moreover, although the selected stands are of different age, the difference between the selected old and managed stands seems to be appropriate, also considering that Spanish Black Pine is one of the long-living tree species in Spain, with some trees reaching 800 years old.

The same approach (with different purposes) as in this study was used in Zema et al. (2021a), Zhou et al. (2022a; 2022b) and Carmona-Yáñez et al. (2023).

2.2.2. Analysis of plant cover and diversity

In September 2017, a species survey was carried out in the plots, to define the structure of the plant communities for each forest stand. Three 10-m long transects were set up in the centre, and in the right and left sides of each plot. Ground cover and species richness were calculated at each transect following (Elzinga et al. 2001). The ground cover was calculated for each plot as the average of the three transects, considering only trees with height > 1.5 m. At each plot of the forest stands, the tree basal area was calculated as the sum of each tree cross-sectional area at breast height (1.3 m). Tree density was calculated as the mean value of the sum of all trees divided by plot surface. Tree age was calculated by coring and counting tree rings obtained from five dominant trees. Finally, tree height was calculated by measuring the tree height of five dominant trees using a Suunto Clinometer PM-5 Series®.

2.2.3. Soil sampling and analysis

At the same date as the vegetation survey, soil samples (diameter of 30 cm) were manually collected at each plot. Sampling was done, after removing litter, on the surface horizon (up to -5 cm), where the largest portion of microbial biomass and activity occurs, and the effects of tree species (through litter chemistry) are stronger compared to deeper horizons. Each soil sample was composed by six sub-samples (200 g), randomly collected at each plot. After removing plant residues, the sub-samples were passed through a 2 mm sieve and then thoroughly mixed in the composite sample. Finally, the soil samples were kept at 3º C prior to the analyses.

Many chemical, physical, and biochemical parameters of soils sampled at each plot were determined. The following chemical properties of soil were analysed: (1) texture (sand, silt, and clay contents), following the method of Guitian Ojea and Carballas (1976); (2) pH in a 1:5 (w/v) aqueous solution by a portable pH-meter; (3) content of carbonates, according to Porta (1998); (4) total carbon content, according to Zhang and Biswas (2017); (5) water-soluble carbon, following Danielsson (1982); (6) organic carbon, by the potassium dichromate oxidation method (Nelson and Sommers 1996); (7) total nitrogen, by Kjeldahl method (Bremner 1982); and (8) total phosphorous, by ICP spectrometry after nitric-perchloric acid digestion. The water extract was obtained by shaking for two hours a mixture of soil and distilled water (1:10 soil:water ratio), centrifuging and filtering. Based on carbon and nitrogen measurements, the C:N ratio was calculated according to Lucas-Borja et al. (2012).

Among the physical properties, bulk density was determined, using a soil ring with a volume of 98.1 cm^3, a diameter of 5 cm, and a height of 5 cm. The sampled soil was oven-dried (at 104 °C for 24 h), and weighed. Soil water repellence (SWR) and unsaturated hydraulic conductivity were measured in ten randomly chosen points at each plot. The water drop penetration time (Woudt 1959) method was adopted for SWR measurement, since this method is one of the most common in the literature (Letey et al. 2000; Buczko and Bens 2006; Tarchitzky et al. 2007), while unsaturated hydraulic conductivity was measured using the Mini Disk Infiltrometer

(Decagon Devices, Inc.). More details about these measurements are reported in the studies by Zema et al. (2021a; 2021b).

Soil samples were also biochemically characterized adopting the following parameters**:** (1) basal soil respiration, measured in a multiple sensor respirometer (Micro-Oxymax, Columbus, OH, USA) and expressed as µg of CO_2 g^{-1} of soil $hour^{-1}$; (2) dehydrogenase activity, measured by modifying the method reported by Von Mersi and Schinner (1991) and expressed as the reduction of p-iodonitrotetrazolium chloride (INT) to p-iodonitrotetrazolium formazan, as µg of INTF g^{-1} of dry soil $hour^{-1}$; (3) urease activity, measured according to the method of Tabatabai (1994), which uses urea as the substrate and borate buffer (pH = 10) (Kandeler and Gerber 1988), and expressed as µmol $N\text{-}NH^{4+}$ g^{-1} of dry soil $hour^{-1}$; (4) acid-phosphatase and (5) β-glucosidase activities, determined according to Tabatabai and Bremner (1969) and Eivazi and Tabatabai (1977) and expressed as µmol p-NP g^{-1} of dry soil $hour^{-1}$; (6) polyphenol oxydase, measured according to Anothai and Chairin (2020).

Moreover, the bacterial and fungal biomass was estimated by determining the fatty acids. First, phospholipids were measured using the procedure described by Frostegård et al. (1993) and Bardgett et al. (1996) after extraction from 2 g of soil using a chloroform-methanol extraction based on Bligh and Dyer (1959) and fractionation. Phospholipids were then transformed by alkaline methanolysis into fatty acid methyl esters (FAMEs), which were quantified by a gas chromatograph (Trace GC Ultra Thermo Scientific) fitted with a 30-m capillary column (Thermo TR-FAME 30m x 0.25 mm ID x 0.25 µm film), using helium as carrier gas. The temperature, initially set up at 150 ºC for 0.5 min, was increased to 180 ºC at a rate of 2 ºC min^{-1} and then to 240 ºC at 4 ºC min^{-1}. The bacterial biomass was quantified using the contents of fatty acids i15:0, a15:0, 15:0, i16:0, i17:0, cy17:0, and cy19:0 (Frostegård et al. 1993; Bardgett et al. 1996), while the fungal biomass using the content of fatty acid 18:2ω6 (Federle 1986; Zelles et al. 1995; Bååth 2003). The $Gram^{+}$ (i15:0, a15:0, i16:0, and i17:0) and the $Gram^{-}$ (cy17:0 and cy19:0) specific fatty acids were also measured, to have an indication of the ratio between the $Gram^{+}$ and $Gram^{-}$ bacterial biomass. The ratio of monounsaturated to saturated PLFAs was expressed as mono/sat, and all results were given in nmol g^{-1}.

2.3. Characterization of EMF

The indicators of *ecosystem functions* were first classified into five categories (in brackets the related indicators): (1) nutrient cycling (based on total carbon, water-soluble carbon, nitrogen, phosphorous, C/N of soil); (2) belowground carbon stocks (basal respiration and organic carbon of soil); (3) waste decomposition (dehydrogenase, β-glucosidase, urease, acid-phosphatase, polyphenol oxydase, bacterial biomass, fungal biomass, G^+ biomass, and G^- biomass of soil); (4) wood production (tree basal area, mean age, density, and height); (5) and water cycle (SWR and soil hydraulic conductivity) (Table 2).

Each indicator of ecosystem functions (EF) was normalized to values (EF') between 0 and 1, according to equation (1):

$$EF' = [EF - \min(EF)]/[\max(EF) - \min(EF)] \quad (1)$$

with EF' and EF indicating the transformed and original values of each ecosystem function, and min and max indicating the minimum and maximum values. This normalization allows a comparison of non-homogenous variables associated to the different ecosystem functions considered in this study, as expressed by different measuring units. This type of normalization indicates where an individual stand is positioned within the whole variability range (0 to 1) for a given ecosystem function. Then, the ecosystem multifunctionality (EMF) was calculated as the average value among the ecosystem functions of each category (Jing et al. 2020).

The same method was used to compute the normalized average values of *ecosystem structure* (rock, vegetation, bare soil, and dead wood coverage, and the number of plant species) and *ecosystem properties* (soil texture, bulk density, pH, electrical conductivity, and carbonates).

The method adopted in this study to estimate EMF is widely utilized and accepted in the current literature on ecosystem multifunctionality (Maestre et al. 2012; Meyer et al. 2018; Luo et al. 2023). Moreover, this method was applied in the study site for evaluating the tree age influence (Lucas-Borja and Delgado-Baquerizo 2019) as well as the impacts of wildfire and post-fire mulching (Carmona-Yáñez et al. 2023) on ecosystem multifunctionality in semi-arid pine forests of the same environment.

Table 2 - Groups of categories of ecosystem multifunctionality, and related properties and indicators to characterize ecosystem multifunctionality of the four forest stands in the study area (“Los Palancares y Tierra Muerta” forest, Castilla La Mancha, Central-Eastern Spain).

Categories of ecosystem multifunctionality	**Properties**	**Indicators**
Ecosystem structure	Soil cover	Rock, vegetation, bare soil, and dead wood coverage
	Plant diversity	Number of plant species (richness)
Ecosystem properties	Physico-chemical soil properties	Soil texture
		Bulk density
		pH
		EC
		Carbonates
Ecosystem functions	Nutrient cycling	Total carbon
		Total nitrogen
		C/N
		Phosphorous
		Water soluble carbon
	Belowground carbon stocks	Basal respiration
		Organic carbon
	Waste decomposition	Dehydrogenase activity
		β-glucosidase activity
		Urease activity
		Phosphatase activity
		Polyphenol oxydase activity

		Bacterial biomass
		Fungal biomass
		Gram$^+$ biomass
		Gram$^-$ biomass
	Wood production	Tree basal area
		Mean tree age
		Tree density
		Tree height
	Water cycle	Soil water repellency
		Soil hydraulic conductivity

2.4. Statistical analysis

A one-way ANOVA was used to evaluate the effect of forest management and stand composition on EMF. A *post-hoc* test using Tukey's Honest Significant Differences (HSD) was applied to identify significant differences between pairs of forest stands. Then, a correlation analysis among categories of ecosystem multifunctionality was carried out using Spearman's coefficients. Moreover, a Principal Coordinates analysis (PCoA) based on Euclidean distance was performed, in order to evaluate whether and to what extent forest composition and management alter the multivariate space of ecosystem multifunctionality. In more detail, all indicators of ecosystem functions were normalized with z-score before computing the Euclidean distance. The first two axes of PCoA were used to visualize the differences in multiple ecosystem functions between forest stands. A significance level of $P < 0.05$ was considered throughout the statistical analyses. All statistical analyses were conducted using the R (version 4.0.3) (Team 2013).

3. RESULTS

3.1. Effects of forest management and stand composition on ecosystem multifunctionality

Regarding the ecosystem structure and properties, both forest management and stand composition altered plant diversity (always significantly lower compared to SBPC, with the lowest value measured in SBPA). In contrast, almost all soil covers were not influenced by these factors, with the exception of vegetation cover. The vegetation cover was significantly lower in SBHO than the other stands. It is also worth mentioning that, for the reference stand, both rock and bare soil were practically absent (Table 3).

Soil properties significantly changed between pairs of stands, as response to forest management and stand composition. In more detail, we found that: (i) in some cases, soil structure was significantly different between pairs of stands (i.e., content of sand between mixed and pure stands, silt between the managed and unmanaged pure stands, and clay between the unmanaged pure stand and one of the mixed stand, SBHO, on one side, and the unmanaged and pure stand); (ii) pH was significantly different among all stands, SBPC and SBSJ showing the lowest and highest values, respectively; (iii) the highest bulk density was found in SBSJ, while the lowest in SBPC, the other two stands showed intermediate but significantly different values; (iv) carbonates were significantly higher in SBPA and lower in SBSJ compared to SBPC, while SBHO showed similar values as the control stand (Table 3).

Concerning the individual ecosystem functions of the four forest stands, the indicators of the soil enzymatic and microbial activities that were related to waste decomposition were higher in the mixed stands compared to SBPA and SBPC, the latter showing the lowest values. Soil organic carbon and basal respiration were the highest in SBPC, which, however, showed the lowest indicators associated to the water cycle (soil hydraulic conductivity and water repellency). No unambiguous trends among the studied stand types (managed vs. unmanaged and pure vs. mixed) were observed for the nutrient cycling, although SBPC showed the highest total carbon and C/N of soil. This stand was also characterized by the highest wood production, mainly associated to the indicators of tree size and age (Table 4).

Based on these values, the ecosystem functions were calculated and compared among the stands (Figure 2).

Table 3 - Mean values ± standard error (n = 3) of indicators of ecosystem structure (soil covers and plant diversity) and physico-chemical properties of soil composing ecosystem multifunctionality (EMF) for different forest stands in the study area ("Los Palancares y Tierra Muerta" forest, Castilla La Mancha, Central-Eastern Spain).

Indicator		**Forest stands**			
		SBPA	**SBSJ**	**SBHO**	**SBPC**
		Ecosystem structure			
Plant diversity		5.33±0.88a	6.67±0.33ab	7.33±0.33b	12.00±0.58c
Soil covers	Rock (%)	3.33±3.33a	13.33±8.82a	13.33±3.33a	0±0a
	Vegetation (%)	53.33±6.67b	50.00±5.77b	26.67±6.67a	70.00±5.77b
	Bare soil (%)	13.33±8.82a	23.33±14.53a	13.33±6.67a	0±0a
	Dead wood (%)	30±11.55a	13.33±13.33a	46.67±14.57a	30±5.77a
		Ecosystem properties			
Soil properties	Sand (%)	76.0±1.15a	84.0±1.15b	71.0±1.15c	76.0±1.15a
	Silt (%)	8.0±1.15a	6.0±1.15ab	9.0±1.15b	12.0±1.15b
	Clay (%)	16.0±1.15a	10.0±1.15b	20.0±1.15c	12.0±1.15b
	pH	6.82±0.09a	7.18±0.09b	6.53±0.09c	5.79±0.09d
	Carbonates (%)	1.97±0.03a	0.17±0.03b	0.93±0.03c	0.86±0.03c
	Bulk density (g/cm^3)	0.83±0.02a	1.61±0.07b	1.31±0.11c	0.53±0.01d

Notes: SBPA = pure and managed stand of Spanish black pine; SBSJ = mixed stand of Spanish black pine and Spanish juniper; SBHO = mixed stand of Spanish black pine and holm oak; SBPC = pure and unmanaged stand of Spanish black pine. Different letters indicate significant differences among forest stands after post-hoc Tukey's Honest Significant Differences tests.

Table 4 **-** Mean values ± standard error (n = 3) of indicators of waste decomposition, belowground carbon stocks, water cycle, nutrient cycling and wood production composing ecosystem multifunctionality (EMF) for different forest stands in the study area ("Los Palancares y Tierra Muerta" forest, Castilla La Mancha, Central-Eastern Spain).

Ecosystem functions	**Indicator**	**Forest stands**			
		SBPA	**SBSJ**	**SBHO**	**SBPC**
Waste decomposition	Dehydrogenase (μmol INTF g^{-1} soil h^{-1})	3.10±0.26ab	2.44±0.26b	3.32±0.26a	3.45±0.26a
	β-glucosidase (μmol PNF g^{-1} soil h^{-1})	2.35±0.01a	6.97±0.42b	8.69±0.23c	2.40±0.04a
	Acid-phosphatase (μmol PNF g^{-1} soil h^{-1})	0.61±0.08a	0.78±0.08ab	0.94±0.08b	0.86±0.08b
	Polyphenol oxidase (μmol mol g^{-1} SOC h^{-1})	42.60±0.57a	51.60±0.58b	37.94±0.12c	9.43±0.29d
	Urease (μmol N-NH_4^+ g^{-1} soil h^{-1})	2.85±0.52a	6.17±0.52b	6.70±0.52b	4.27±0.52a
	Gram^{+} biomass (nmol g^{-1})	2.74±0.06a	3.04±0.09b	1.28±0.03c	1.13±0.04c
	Gram^{-} biomass (nmol g^{-1})	0.44±0.01ab	0.49±0.04a	0.47±0.03ab	0.38±0.02b

	Fungi biomass (nmol g^{-1})	2.34±0.12a	2.18±0.11a	2.30±0.16a	1.40±0.06b
	Bacteria biomass (nmol g^{-1})	3.17±0.06a	3.53±0.12b	1.75±0.05c	1.51±0.06c
Belowground carbon stocks	Basal respiration (mgC-CO$_2$ kg^{-1} day^{-1})	48.59±2.34a	55.30±2.34a	79.53±2.34b	82.93±2.34b
	Organic carbon (%)	10.23±0.8a	10.10±0.59a	14.63±0.58b	15.30±1.12b
Water cycle	Soil hydraulic conductivity (mm/h^{-1})	0.00008±0.00002a	0.00049±0.00022ab	0.00015±0.00001ab	0.000002±0.00000a
	Soil water repellency (WDPT, s)	49.33±2.96a	119.28±2.05b	2.42±0.36c	7.23±0.23c
Nutrient cycling	Total carbon (%)	10.23±0.80a	10.10±0.59a	14.63±0.58b	15.30±1.12b
	Total nitrogen (%)	0.44±0.04a	0.58±0.02b	0.80±0.06c	0.52±0.03a
	Phosphorous (ppm)	118.19±1.73a	10.03±1.73b	55.99±1.73c	51.48±1.73c
	C:N	28.61±1.33a	17.71±0.37b	19.90±1.60b	31.23±0.70a
	Water soluble carbon (mg/kg)	457.35±14.57ab	617.33±81.70b	824.88±73.71b	813.91±46.85b
Wood	Tree basal area	31.87±2.14a	23.10±1.65b	24.73±1.83b	51.27±2.23c

production	(m^2/ha)				
	Tree height (m)	21.67±0.67a	22.33±0.67a	23.33±1.67a	40.33±0.88b
	Mean tree age (years)	99.67±4.67a	97.33±3.48a	98.00±2.65a	143.00±5.57b
	Tree density (number trees per ha)	891.67±55.71a	1006.23±2.85b	1061.33±33.84c	1205.00±8.33d

Notes: *SBPA = pure and managed stand of Spanish black pine; SBSJ = mixed stand of Spanish black pine and Spanish juniper; SBHO = mixed stand of Spanish black pine and holm oak; SBPC = pure and unmanaged stand of Spanish black pine. Different letters indicate significant differences among forest stands after post-hoc Tukey's Honest Significant Differences tests.*

Among these ecosystem functions, compared to the reference stand (SBPC plots), the average EMF was significantly lower in SBPA and SBSJ, and similar in SBHO. These differences must be associated to the individual functions synthesized by EMF. In more detail, while the same changes as for the average EMF was detected in belowground carbon stocks, wood production was significantly lower in SBPA, SBSJ and SBHO compared to the SBPC plots. Nutrient cycling significantly decreased in SBPA and SBSJ, SBHO plots showing similar values as the SBPC plots. In contrast, water cycle of the managed stand (SBPA) and mixed forests (SBSJ and SBHO) was similar as the pure and unmanaged stand (SBPC). Finally, waste decomposition was significantly higher in SBHO and SBSJ plots, while the SBPA stand did not show significant differences, when these stands were compared to SBPC (Figure 2).

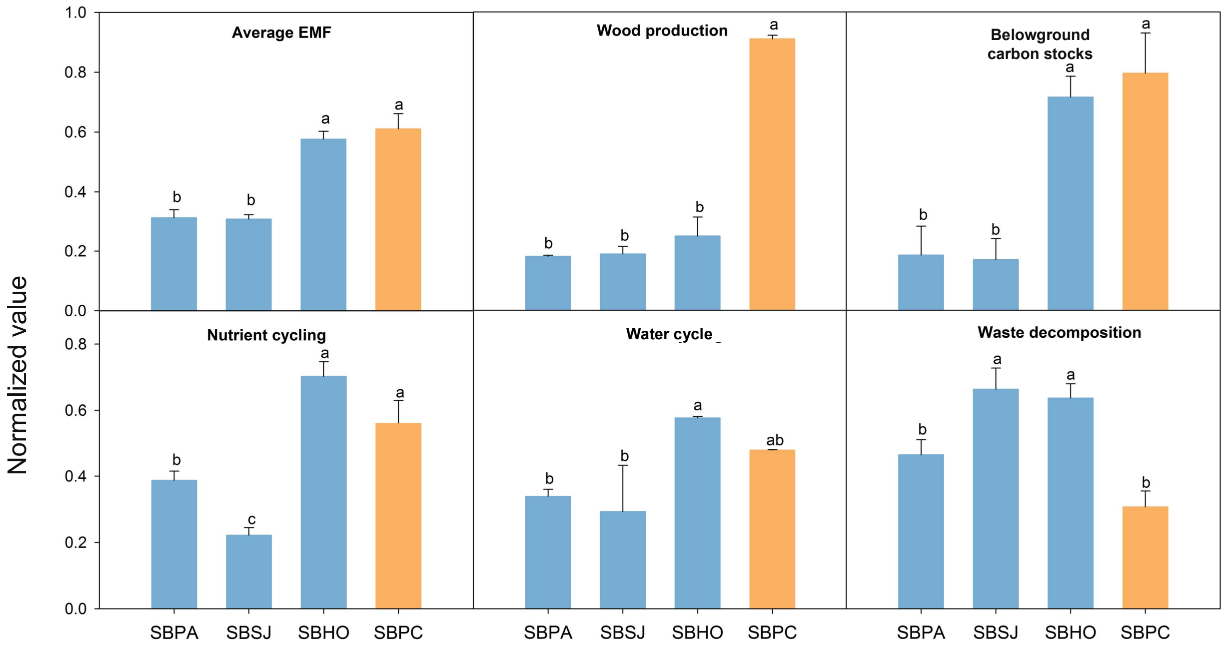

Figure 2 – Mean ± standard error of functions composing ecosystem multifunctionality (EMF) for different forest stands in the study area ("Los Palancares y Tierra Muerta" forest, Castilla La Mancha, Central-Eastern Spain). *Legend: SBPA = pure and managed stand of Spanish black pine; SBSJ = mixed stand of Spanish black pine and Spanish juniper; SBHO = mixed stand of Spanish black pine and holm oak; SBPC = pure and unmanaged stand of Spanish black pine. Different letters indicate significant differences among forest stands after post-hoc Tukey's Honest Significant Differences tests.*

3.2. Associations between ecosystem multifunctionality, and ecosystem properties and structure

The correlation analysis showed significant Spearman’s coefficients between several pairs of indicators of ecosystem properties, structure, functions, and EMF (Figure 3). In more detail, the highest positive correlations were found between waste decomposition on one side, and rock cover and bulk density on the other side as well as average EMF and silt content ($r = 0.9$, $p < 0.05$ in all cases). The pairs waste decomposition on one side, vs. soil C:N and vegetation on the other side, as well as nutrient cycling vs. sand content ($r = -0.8$, $p < 0.05$ in all cases) showed the highest negative (as absolute values) and significant Spearman’s coefficients (Figure 3).

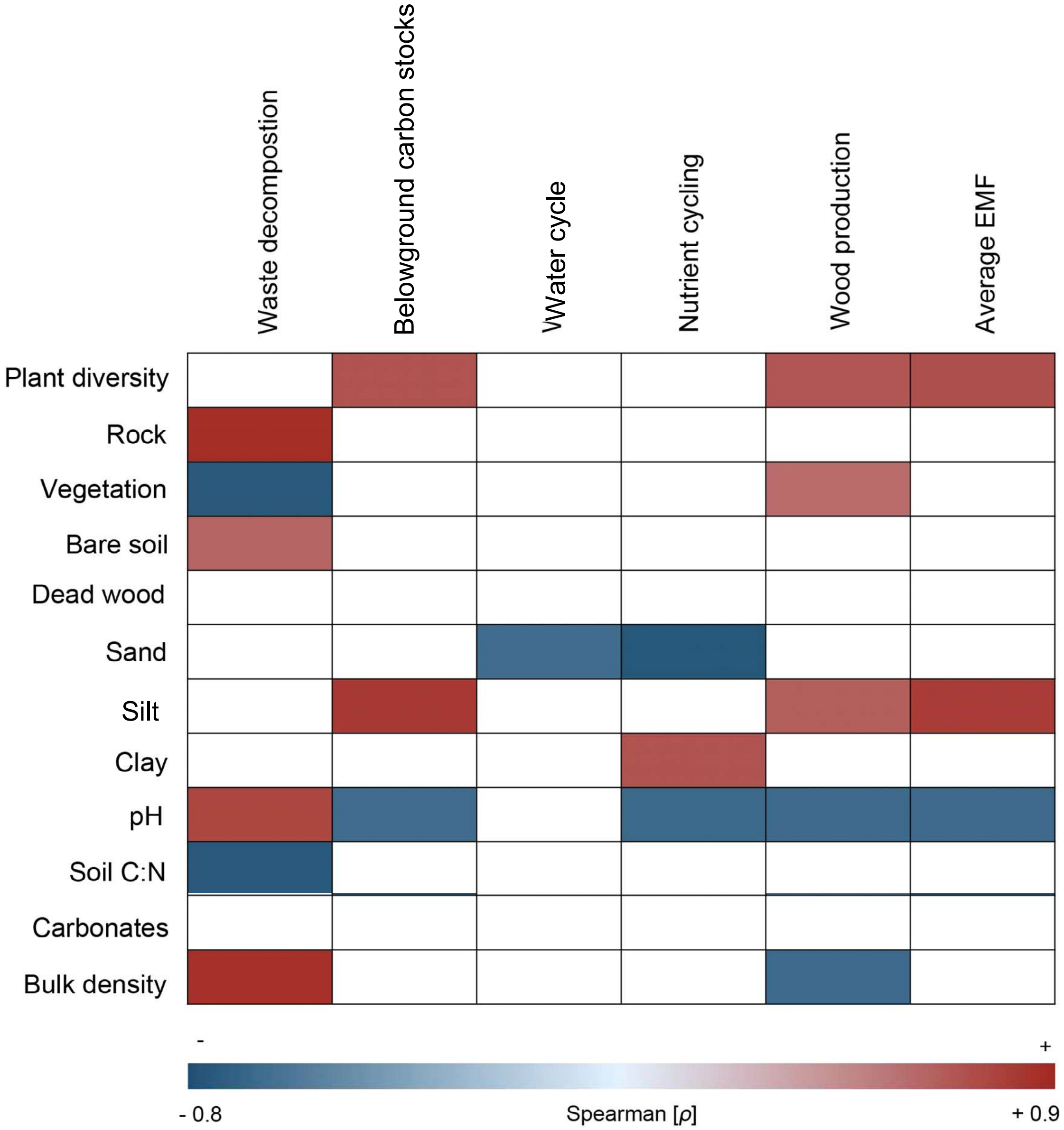

Figure 3 - Correlation matrix reporting Spearman's coefficients between pairs of indicators of ecosystem multifunctionality (in the rows), and ecosystem functions and EMF (ecosystem multifunctionality) (in the columns) for different forest stands in the study area ("Los Palancares y Tierra Muerta" forest, Castilla La Mancha, Central-Eastern Spain). *The colour palette relates to the values of Spearman's coefficients. Significance levels: * P < 0.05; ***P < 0.001.*

3.3. Multidimensional analysis of ecosystem multifunctionality

The PCoA showed that, in the space of multiple ecosystem functions, the first two axes of variation (functional dimensions) explained more than 90% of the multi-dimensional functional space variation (Figure 4A). The functional dimensions #1 and #2 explained 80.5% and 13.2%, respectively, of this variation. The first functional dimension (#1) was noticeably influenced by some indicators of waste decomposition (bacteria and Gram$^+$ biomass, phosphatase and polyphenol oxydase activities), water cycle (soil water repellency), belowground carbon stocks (soil basal respiration and organic carbon), nutrient cycling (total and water-soluble carbon) and wood production (tree density). Tree density influenced also the second axis together with tree basal area (Figure 4B). Of all indicators, only bacteria and Gram$^+$ biomass, and polyphenol oxydase activity had positive loadings on the functional dimension #1 (Figure 4B). Moreover, EMF was significantly and positively correlated to the functional dimension #1 ($r^2 = 0.75$, $p < 0.001$), while no correlation was found with the functional dimension #2 (Figure 4C). Finally, EMF was negatively and significantly correlated with the functional dimension #1 ($r^2 = 0.50$, $p < 0.05$), while the correlation (again negative and significant) to the functional dimension #2 was lower ($r^2 = 0.38$, $p < 0.05$) (Figure 4D).

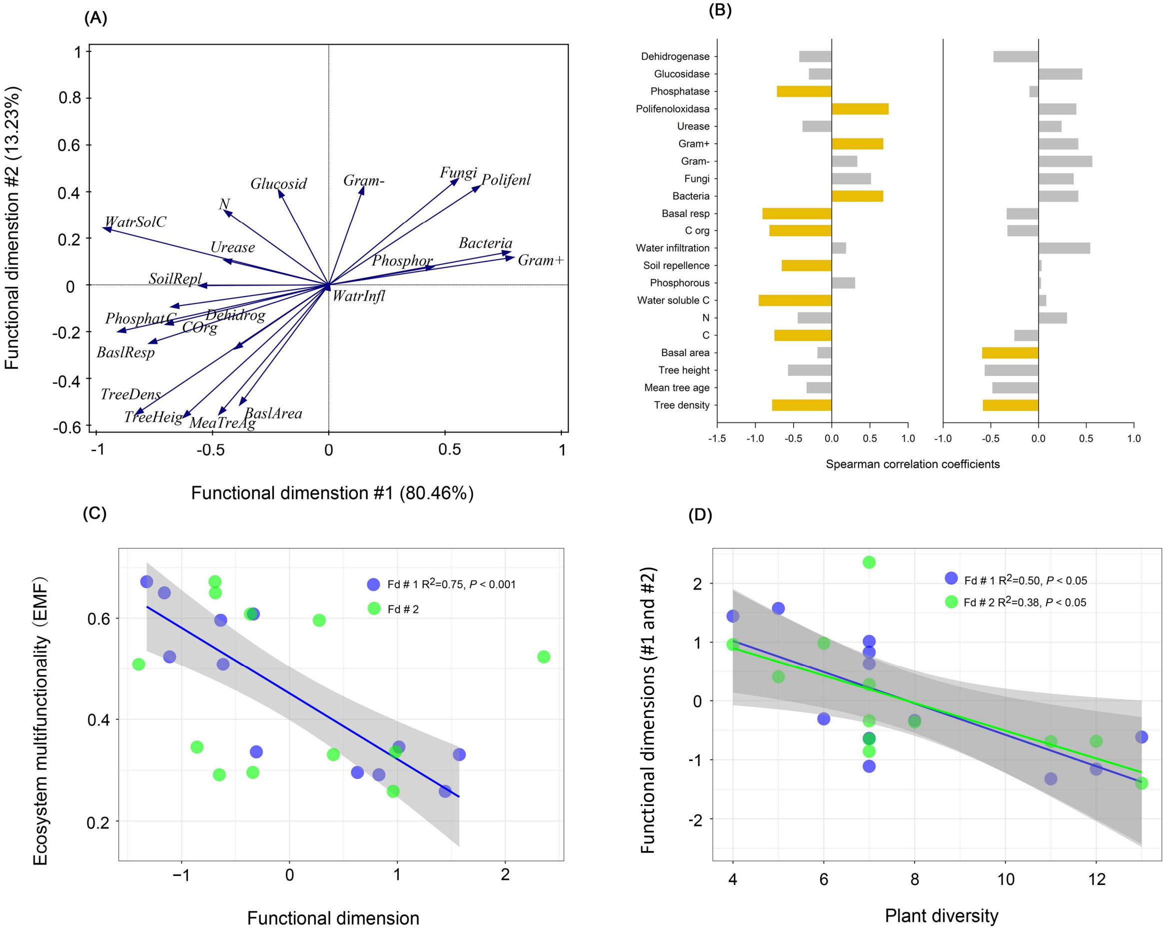

Figure 4 - Unconstrained principal coordinate analyses (PCoA) with Bray–Curtis distance analysis of multiple ecosystem functions (A); bar plots of the Spearman correlation coefficients between multiple ecosystem functions and functional dimensions (B); relationships between functional dimensions (Fd #1 and #2, each grouping the significant related variables of Figure 4B) and ecosystem multifunctionality (EMF, C), and plant diversity (D). *Orange bars evidence the correlations that are considered as significant ($p < 0.05$).*

4. DISCUSSIONS

4.1. Effects of stand composition on forest multifunctionality

About the ecosystem structure, stand composition has been generally acknowledged as a crucial factor influencing plant diversity, and functional composition (Lucas-Borja and Delgado-Baquerizo 2019). The significant influence of stand composition on plant diversity found in this study is in line with previous studies stating that forest stand structure is a successful functional strategy (Lucas-Borja and Delgado-Baquerizo 2019; Wang et al. 2022), since, according to the present study, this function is left undisturbed. Moreover, the low impact of stand composition on ground cover properties is somehow expected, since the whole forest have been left undisturbed for more than 100 years, resulting in similar microclimatic conditions, parental rock material and forest species.

The significant changes found in ecosystem properties due to stand composition were presumably due to the fact that the associations of two forest species were able to alter soil structure (with slight but somewhat significant changes in sand or clay contents), and mainly some important physico-chemical properties, such as pH, C:N ratio and bulk density. Other studies have analyzed the effects of stand composition on soil properties. For instance, (Hou et al. 2019) showed that the changes in soil physical properties are dependent on tree stand types, since many abiotic and biotic factors can describe the effects of vegetation composition on soil processes and properties. The reasons for changes in the analyzed soil properties between stands with different composition may be several, such as the different root density and litter chemistry of pure stands in comparison to mixed forest stands. In general, the litter quality of tree species influences soil properties, providing suitable conditions for an increase in organic matter (Kooch et al. 2017). Moreover, the decomposition rate of litter may be variable under different stand composition and forest management, thus producing different amounts of acidic substances that accumulate in the topsoil at the different forest stands (Wang et al. 2012), and therefore the significant changes in pH among all stands detected in this study. The studies of Kooch (2012) and Kooch et al. (2017) support the theory that the litter pH of some species has an acidic condition with high C:N ratios and C, which affects the pH and C:N of the soil. The tree species can influence the soil properties through changes in pH and carbon exudates (Prescott and Grayston 2013; Thoms and Gleixner 2013; Heděnec et al. 2023). Moreover, the differentiated organic matter supply to soil due to the different stand composition may be a reason for the different bulk density, resulting in differences in waterholding capacity, soil nutrient storage, and gas penetration across the studied forest stands (Yue et al. 2017; Hou et al. 2019).

About the studied ecosystem functions, forest stand structure was a significant driver of waste decomposition and wood production. In more detail, the variability in waste decomposition due to stand structure, was mainly due to the higher activities of some enzymes (namely β-glucosidase, polyphenol oxydase and urease) in mixed stands compared to the pure forests. This influence may be attributed to differences in litter quality, root exudates, herbivory and nutrient uptake due to the different species (Grayston and Prescott 2005). The increases in the Gram^{+} and Gram^{-} biomass in SBSJ stands may have a secondary role on waste decomposition, and these increases should presumably due to the higher soil water content, which promotes the relative abundance of those bacteria (Lange et al. 2014; Chen et al. 2019). This result shows that forest composition plays beneficial effects on ecosystem functioning by influencing some soil microbial communities (associated with enzyme contents) and their activity. However, different tree stands do not equally affect all soil enzymes (Wang et al. 2012). High-quality litter layers are generally beneficial for most soil microbial communities (Bandyopadhyaya et al. 2002), and biodiversity is known to be relatively strongly related to available energy resources and essential nutrients (Bedano et al. 2005). Fungal and bacterial community composition (in terms of abundance and diversity) are closely related to tree composition, and microbial communities associated with mixed species are more active (Khlifa et al. 2017). These results indicate that soil fungal and bacterial communities play an important role in maintaining multiple ecosystem functions in different forest stands (Xu et al. 2021). It is also worth mentioning the clear association between waste decomposition on one side and some important physico-chemical properties of soil (pH, C:N and bulk density) on the other side, as shown by the correlation analysis. This association is in line with other studies, which have demonstrated that, in different forest stands, microbial activity is noticeably affected by variations in available nutrients, particularly carbon, nitrogen, and soil chemistry among the studied forest stands (Cheng et al. 2013; Allan et al. 2015; Xu et al. 2021).

The lack of significance differences in wood production found in the pure stand compared to the other mixed forest was ascribed to the same tree height and age among the stands, despite the different basal area and tree density. The low impact of growth of basal area (Hein and Dhôte 2006), stem biomass (Thurm et al. 2016), stem volume (Pretzsch et al. 2015) or total above-ground biomass (Pretzsch et al. 2010) on wood production may be surprising at a first sight. However, literature is generally quite contrasting on this aspect, as stated by Zhang et al. (2012) and Zeller et al. (2017), showing that pure and mixed forest structures can increase or decrease wood production. Presumably, in our experimental stands, the growth conditions (resource supply and environmental factors) of individual trees were highly dependent on the surrounding forest structure (Pretzsch 2014).

The effects of stand composition on the remaining ecosystem functions (nutrient cycling, belowground carbon stocks and water cycle) were dependent on the specific tree species. Compared to the reference forest, the significant reduction in nutrient cycling function in the SBSJ stand may result from the significantly lower soil organic, water soluble and total carbon contents of this stand. These variations may be due to the differences in the quantity and quality of root and litter inputs to forest soil between the tree species, and this played a noticeably influence on nutrient availability, enzymatic and microbial activities and biogeochemical cycles. The influence of stand composition on these processes through differences in the quantity and quality of litter due to variations in tree species was also acknowledged by Bell et al. (2015). This effect may be directly associated with the differences in tree and other plant species composition between overstory and understory in stands (Turbé et al. 2010; Liu et al. 2019). In addition, different root patterns due to the variable stand composition of the mixed stands (i.e., in the SBHO stand) may be another reason for the changes in nutrient cycling, determining differences in the distribution of carbon in soil profiles (Spielvogel et al. 2014). Depending on root depth and distribution, the production of organic carbon by fine root turnover and exudates are the major entry routes in mineral soils (Rothe et al. 2002; Rumpel and Kögel-Knabner 2011).

Belowground carbon stocks significantly decreased in the SBSJ stand compared to the pure forest. In that mixed stand, both basal respiration and organic matter content was significantly lower in the topsoil,. This variability could be related to the fact that the organic matter content in forest soils depends and derives primarily from above-ground litter and forest vegetation biomass (Shao et al. 2017), which, in turn, can be attributed to a complex set of factors affecting soil respiration - such as litter quality, root density, and respiratory activity - and to the environment, regarding soil moisture and temperature (Vesterdal et al. 2012; Zhang et al. 2023). Higher soil respiration in forest stands is due to favorable conditions for microbial activity, including an adequate supply of substrate, especially in the aboveground soil layer. Moreover, the high coefficient of correlation between belowground carbon stocks and plant diversity indirectly confirms the influence of stand composition on this ecosystem function. It is also worth mentioning that, when calculating multifunctionality, the aboveground carbon was excluded as indicator of belowground carbon stock from the analyses, due to the lack of relevant data. However, this is not a limitation of this study, since an indirect indication of this ecosystem feature was incorporated in wood production. Furthermore, the same indicators and functions were measured and compared among all selected plots and stands, and it is important to avoid considering correlated indicators when assessing EMF.

In spite of the non-significant differences in water cycle between pure and mixed stands, the presence of holm oak (SBHO) may have significantly increased this function compared to the stand with juniper (SBSJ). Excluding the impacts of water infiltration, whose differences among different forest stands were not significant, this effect may be due to soil water repellency, which is variable among stands with different composition, and especially juniper is able to increase soil hydrophobicity under semi-arid conditions (Zema et al. 2021b).

4.2. Effects of management on forest multifunctionality

Forestry operations (e.g., wood harvesting, soil compaction) noticeably reduced the plant diversity in the managed stand compared to the reference forest, although without any alterations in the soil surface conditions. In line with our study, Heydari et al. (2021) found higher species richness and plant diversity (in terms of evenness) in old and unmanaged forests. The beneficial aspects (e.g., economic production, reduced fire susceptibility, increased accessibility) and the negative impacts (e.g., increased soil compaction, higher runoff and erosion, and short-term loss of habitat) of tree harvesting have been debated for years (Gomez et al. 2002). In this regard, forest management may modify below-canopy microclimatic conditions by changing the structural features of a stand (Ehbrecht et al. 2019; Blumröder et al. 2021). Beaudet et al. (2004) also showed that frequent cutting and removal of dead wood increase solar radiation and change microclimatic conditions in forest areas. Therefore, competition for resources may increase exclusion between species, leading to a reduction in species diversity (Wilson and Tilman 1993).

In contrast to what found for soil covers, significant variations in all the studied physico-chemical properties of soil between managed and unmanaged stands were found. As widely demonstrated, machinery used during forest operations may generate changes in soil, such as soil compaction, thus reducing size and continuity of soil macropores through which roots preferentially grow (Lucas-Borja et al. 2020). Therefore, these changes in soil properties after using machinery in forest harvesting operations may lead to modifications in plant species that better adapt to compacted soil, and in soil properties, discriminating managed and unmanaged forest stands.

Compared to the reference stand, forest management significantly influenced wood production, which significantly decreased in the managed stand in comparison to the unmanaged forest. It is important highlighting that this study did not measure the wood extracted during harvesting operations, which, in sum to remaining trees, might generate the same or even higher wood production in managed stands compared to unmanaged stands. Therefore, for forest management, we ascribe the changes in this ecosystem function to the differences in basal area, stem biomass and volume and to total above-ground biomass.

The significant decrease in nutrient cycling due to forest management compared to the unmanaged stand should be explained by the lower total and organic carbon as well as phosphorous contents in the managed forest. Here, this lower carbon content also impacted on the decreased belowground carbon stocks. In terms of biomass, it is worthy noting that increased tree density and basal area may generate a parallel increase in carbon pools and nutrient content in soil, as showed by the indicators of nutrient cycling function. As demonstrated by Ushio et al. (2010) and Lucas-Borja et al. (2010), forest management not only can alter physico-chemical properties of forest soils and quantity and quality of substrate, but also more directly impacts soil microbial community. In our study this effect of forest management on waste decomposition was not noticed, presumably due to similar patterns in many enzymatic activities between the managed and unmanaged stands, although significant differences were noticed in the composition of bacteria and fungi.

Finally, the absence of impacts of forest management on water cycle detected in our study is expected, since the water infiltration and soil water repellency were similar between managed and unmanaged stands. The differences in soil hydraulic conductivity are usually attributed to a combination of soil properties and covers (Doerr et al. 2000; Cawson et al. 2012), and the soil covers were comparable between managed and unmanaged stands, in line to other studies in the same forest area (Zema et al. 2021b).

4.3. Effects of stand composition and management on overall forest multifunctionality

The average EMF was significantly affected by forest management (leading to a decrease in the managed stand compared to reference forest), while stand composition was an important EMF driver only for one mixed stand. Therefore, the results of this study suggest that multifunctional properties of Mediterranean forests primarily vary with forestry operations (which highlights the importance of sustainable management plans to optimise the EMF of semi-arid forests, as those of Cuenca Mountains and, more in general, Spanish Black Pine forest stands), and secondarily with forest mixing. About the latter result, similar findings were reported by Lucas-Borja et al. (2016), who stated that the characteristic and functional forest complexity leads to larger litterfall, soil organic matter accumulation, and nutrient content on the forest floor in different tree stands, consequently favoring the nutrient and carbon cycling functions. In line with our result, numerous studies have focused on the effect of terrestrial plant diversity on multifunctionality (Bradford et al. 2014; López-Rojo et al. 2019; Lucas-Borja and Delgado-Baquerizo 2019; Liu et al. 2022).

It is also important noting that the noticeable negative correlation between EMF and the first functional dimension #1 (the latter being associated to some indicators of waste decomposition, belowground carbon stocks, nutrient cycling and wood production by negative loadings) of PCoA reveals that EMF increases with bacteria biomass, phosphatase, basal respiration, organic, total and water-soluble carbon, and tree density. This means that forest management should be targeted to forestry operation that avoid decreases in microbial biomass, enzymatic activities, organic matter and nutrient contents of soil, and adopt an optimal tree density, limiting indiscriminate cutting and forest logging. Finally, the significant negative correlation between EMF and plant diversity highlights the essential role of tree species richness and evenness on those ecosystem functions.

5. Conclusions

The comparison of ecosystem structure, properties and functions as well as EMF between pairs of Mediterranean forest stands, characterized by different tree composition or subjected to management, showed that, regarding ecosystem structure, both forest management and stand composition altered plant diversity but not soil covers (except the vegetation cover), and, about ecosystem properties, soil characteristics significantly changed between pairs of stands (especially texture, pH and bulk density), as responses to forest management and stand composition.

In reply to the second research question, the study showed that, among the ecosystem functions, forest stand composition was a significant driver of waste decomposition and wood production, while its effect on the remaining ecosystem functions (nutrient cycling, belowground carbon stocks and water cycle) depends on the specific tree species. The forest management significantly influenced wood production, belowground carbon stocks, nutrient cycling, but not water cycle and waste decomposition.

About the first research question, this study demonstrated that, in Spanish Black Pine stands under semi-arid conditions, EMF can be significantly reduced by management compared to the unmanaged forest, and can be similar or different between pure and mixed stands according to the specific species. More in general, the study suggests that the multiple functions played by Mediterranean forest ecosystems primarily vary with management operations, and secondarily with stand composition. However, a forest management with mixed species may have a non-negative or even a positive effect on overall EMF, and this indicates that management operations should be targeted to increase plant diversity (for instance, reforestation with mixed species, preferably using Fagaceae rather than Cupressaceae with Pinaceae, as shown in this study). The essential role of forestry operations on EMF emphasises the caution of a suitable management for preserving the important functions of the Mediterranean forest ecosystems, which are crucial hotspots for global biodiversity conservation and climate change mitigation.

Overall, this study contributes to better understand how a Spanish Black Pine forest, one of the most widespread forest species in the Iberian Peninsula, functions under different management operations and stand compositions. This knowledge gives landscape planners working in semi-arid conditions more insight about those practices and silvicultural layouts that may support the multiple functions and services provided by delicate and endangered ecosystems, such as the Mediterranean forests. Moreover, this study, revealing the effects of forest tree composition and management on ecosystem functioning, provides a scientific basis for predicting community dynamics and ecosystem functionality in semi-arid forests. However, much research is needed to explore the ecosystem functioning under other forest management techniques and different stand compositions.

DECLARATIONS

Conflict of interest statement

The authors have no relevant financial, non-financial or competing interests to disclose.

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Data availability statement

Data will be made available upon specific request tot he authors.

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