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Woody encroachment and soil carbon stocks in subalpine areas in the Central Spanish Pyrenees



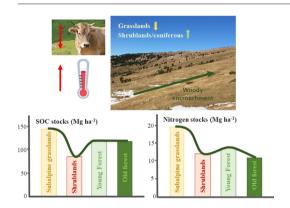
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HIGHLIGHTS

- Woody encroachment has been an ongoing process in the subalpine belt of Mediterranean.
- We analyzed LULC changes and the effects of these changes in soil properties.
- Encroachment has occurred due to the expansion of coniferous forests and shrublands.
- SOC and N contents and stocks were higher in the grasslands sites.
- The woody encroachment process initially produced a decrease in the SOC

GRAPHICAL ABSTRACT



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ABSTRACT

Woody encroachment has been an ongoing process in the subalpine belt of Mediterranean mountains, after land abandonment, the disappearance of the transhumant system and the decrease of the livestock number. The main objectives of this study were: (i) to identify land use/land cover (LULC) changes from 1956 to 2015, and (ii) to investigate the effects of LULC changes in physical and chemical soil properties and soil organic carbon (SOC) and nitrogen (N) stocks. It is hypothesized that woody encroachment in the subalpine belt may lead to significant changes in soil properties, and will generate an increase in the SOC stocks. A land use gradient was identified in the subalpine belt of the Central Spanish Pyrenees: (i) subalpine grasslands, (ii) shrublands, (iii) young forests, and (iv) old forests. Mineral soil samples were collected every 10 cm, down to 40 cm, at three points per each LULC and a total of 48 samples were analyzed. The results showed that (i) woody encroachment has occurred from 1956 to 2015 due to the expansion of coniferous forests and shrublands (at the expense of grasslands), (ii) land cover and soil depth had significant effects on soil properties (except for pH), being larger in the uppermost 0–10 cm depth, (iii) SOC and N contents and stocks were higher in the grassland sites, and (iv) the woody encroachment process initially produced a decrease in the SOC stocks (shrublands), but no differences were observed considering the complete soil profile between grasslands and young and old forests. Further studies, describing SOC stabilization and quantifying above-ground carbon (shrub and tree biomass) are required.

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1. Introduction

The management of agropastoral ecosystems is one of the major challenges facing our society today, especially in Mediterranean mountain areas, due to their high vulnerability to Climate Change (Giorgi, 2006; Nogués Bravo et al., 2008; López-Moreno et al., 2017) and to the abandonment of agricultural and pastoral activities since the midtwentieth century (MacDonald et al., 2000; Strijker, 2005; Hatna and Bakker, 2011; Lasanta et al., 2017). One of the consequences is the transformation of many fine-grained cultural landscapes into homogeneous coarse-grained landscapes (Van Eetvelde and Antrop, 2003; Lasanta et al., 2005) with socioeconomic and environmental effects on the local population and nearby areas (Mottet et al., 2006; Gellrich et al., 2007; Bernués et al., 2014).

In Mediterranean mountains, grassland ecosystems are extremely important as producers of many goods and services for society (provisioning, regulation, support, culture) and as contributors to the support of a rich biodiversity (Millennium Ecosystem Assessment, 2005; Raudsepp-Hearne et al., 2010; Bernués et al., 2014). Grasslands provide forage for large wild herbivores and for livestock, so they play a prominent role in the mountain economy and even in many country economy's (Villagrasa et al., 2015). It should not be forgotten that livestock farming contributes directly to food security and provides livelihood to almost one billion people in the world (FAO, 2009). Grasslands also play an important regulatory role; they act as firewalls in areas where the rural abandonment favors the expansion of woody communities, which increase the risk of fires (i.e., Lloret et al., 2002; Beilin et al., 2014). In addition, grasslands regulate the hydrological cycle because it has been demonstrated that spontaneous revegetation or afforestation processes of grassland areas reduce the discharge of rivers (García-Ruiz et al., 2011; López-Moreno et al., 2011; Nadal-Romero et al., 2013). The

support services provided by grasslands are based on their high plant diversity, with species adapted to extreme conditions or at its distribution limit, which increases the number of endemism and provides to the grasslands a high ecological value (Canals and Sebastià, 2000). Consequently, the loss of grasslands reduces plant and faunal biodiversity (Ratajczak et al., 2012; Canals et al., 2014). In addition, grasslands, that were created, used and maintained by men for centuries or millennia, give rise to a cultural landscape of high aesthetic value, offering cultural services such as recreational (tourism), educational and spiritual services (Lamarque et al., 2014) and contributing to the well-being of rural areas (Hejcman et al., 2013; Huber et al., 2013). On the other hand, grasslands are an important source of ecological information and local knowledge on adaptation of management systems to adapt to Global Change (Fernández-Giménez and Fillat, 2012; Harsch et al., 2009).

An important portion of Mediterranean mountain grasslands are semi-natural, being the result of human intervention for millennia, eliminating woody species with fires or shrub clearing to generate extensive summer pastures that were used by extensive livestock (Didier, 2001; Roepke and Krause, 2013; Sanjuán et al., 2017). The persistence of these grasslands largely depends on the continuity of grazing and the clearing of woody plants (When et al., 2011; Gartzia et al., 2014).

In the Central Spanish Pyrenees, the semi-natural grasslands reach in altitude to approximately 2400 m a.s.l., coinciding with the upper limit of the subalpine belt and the maximum level for the development of shrubland, while *Pinus uncinata* would reach up to 2200 m a.s.l. (García-Ruiz et al., 1990; Badía and Fillat, 2008). These grasslands were created by human fires from the mid-Holocene, but more actively from the Bronze Age to the Middle Ages, when much of the subalpine belt was deforested to graze transhumant livestock (Bal et al., 2011; Cunill et al., 2012; Pérez-Sanz et al., 2013). However, since the middle

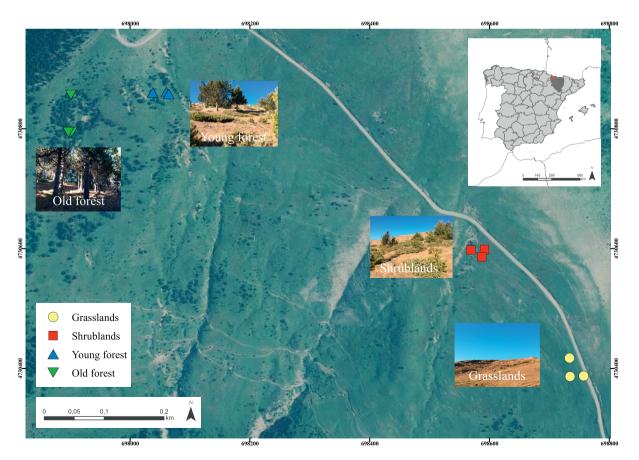


Fig. 1. Location of sampling points and the study area (Central Spanish Pyrenees).

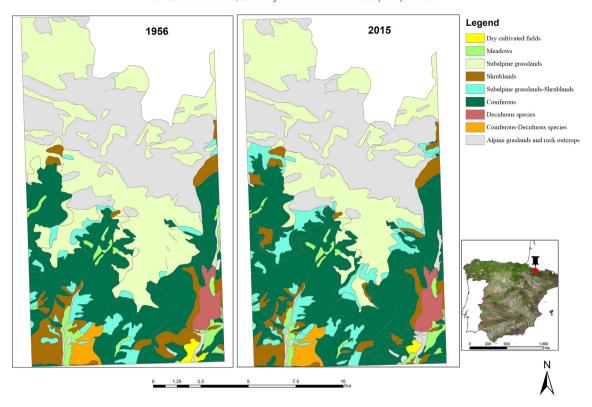


Fig. 2. Maps with LULC categories in the Borau Valley in 1956 and 2015 and location of the study area in Spain.

of the 20th century, subalpine grasslands have undergone a major transformation due to the decline of livestock activities: the disappearance of transhumance, the sharp decline of livestock numbers and the replacement of native breeds by other breeds that were less adapted to the environment (García-Ruiz and Lasanta, 1990). The abandonment of grassland activities has favored the expansion of woody species into grasslands (Bartolomé et al., 2005a; Anthelme et al., 2007). Gartzia et al. (2014) pointed out that 19% of dense pastures below 2100 m a.s. l. and 24% of low pastures between 1980 and 2000 m a.s.l. have been woody-encroached, in the Central Spanish Pyrenees, while 35% the shrubs have been populated with trees. These changes are exacerbated by climatic warming, which increase the temperatures and reduces the snow cover and snowfall period (Batllori and Gutiérrez, 2008; López-Moreno et al., 2017) causing a cascading effect on ecosystem processes, accelerating the invasion of grasslands by high competitive woody species threatening ecosystem functions and services (Komac et al., 2013).

So far, the process of woody encroachment in subalpine grasslands has been studied in the Pyrenees (Komac et al., 2011a, 2011b, 2013; Madruga et al., 2011), and some consequences have been analyzed:

(i) the increase of the biomass of woody vegetation with the consequent fire risk (Bartolomé et al., 2005b), (ii) grassland management (traditional management, conservation polices as suppressing fires or grazing practices, the creation of plantations) (Bartolomé et al., 2008), (iii) the negative effects on biodiversity (Komac et al., 2011b) and on the diversity of grasslands due to the invasive effect of Brachypodium pinnatum (Canals et al., 2014) or Erica scoparia (L.) (Bartolomé et al., 2005a), (iv) the changes in the biomass and greenery of grasslands as a consequence of the woody encroachment and Climate Change (Gartzia et al., 2016a), (vi) the loss of connectivity of grasslands (Gartzia et al., 2016b), and (viii) the influence of land use changes on subalpine areas on river torrentiality (Gómez-Villar et al., 2014; Sanjuán et al., 2016). The effects of prescribed fires of successional shrubs on soil function, on the amount and stability of organic matter, and on the nutrient cycle have been studied as well (Canals et al., 2014; Armas-Herrera et al., 2016; San Emeterio et al., 2016).

However, the effects of woody encroachment on soil properties and soil organic carbon (SOC) stocks have been less studied, despite the importance of this question within a Global Change context (Farley et al., 2013; Lo et al., 2015). In addition, soil organic carbon is an indicator of

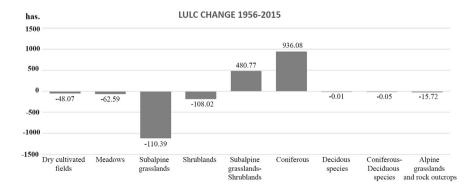


Fig. 3. LULC changes between 1956 and 2015.

soil quality associated with aggregate stability, with direct implications for water infiltration and soil erosion, as well as for biodiversity, and plant and fauna development. It is known, that one of the main ecosystem effects of grasslands is their ability to carbon sequestration and to regulate greenhouse gas emissions (Farley et al., 2013). Grasslands form perennial and dense covers with a high carbon-binding capacity (Ritchie, 2014; Rutledge et al., 2014; Aldezabal et al., 2015), and may be even more important than forests in the "generation of carbon credits" (Albrecht and Kandji, 2003). Recently, the influence of land use changes in soil carbon reserves has been studied due to the differences in carbon sequestration depending on land uses and land covers (Post and Know, 2006; Guo and Gifford, 2002; García-Pausas et al., 2011).

The main objective of this study is to analyze soil properties and soil organic carbon stocks in different land uses in the subalpine belt in a small sector of the Central Spanish Pyrenees (Borau Valley). The specific objectives were: (i) to determine land use changes in the study area and quantify the grassland lost during the last 60 years, and (ii) to assess the effect of woody encroachment on soil properties and SOC dynamics. This leads to the following research hypotheses: (i) subalpine grasslands contain high soil organic carbon stocks, (ii) woody encroachment in the subalpine belt may lead to significant changes in soil properties, and (iii) woody encroachment and forest expansion in subalpine belts will generate an increase in the SOC stocks. Understanding the mechanisms behind agropastoral ecosystem responses to climate, in interaction with land management and land use changes, is necessary to take effective measures to ensure the flow of the multiple goods and services that these ecosystems provide to society.

2. Materials and methods

2.1. Study area

The study was developed in the subalpine grasslands of the Borau Valley (Central Spanish Pyrenees) (Fig. 1). The Borau Valley covers an area of 41.72 km², with an altitudinal gradient ranging from 840 m a.s. l. to 2566 m a.s.l. at the top. The Borau Valley exemplifies the process of territorial uncoordination that has occurred in the Pyrenees in the last century. During the 20th century, the Borau Valley lost 82% of its population, going from 394 inhabitants in 1900 to 74 inhabitants in 2001. During the same period, 90% of the agricultural area was abandoned, decreasing from 1794 ha to 168.8 ha between 1974 and 2000, and 75% of the livestock was lost (Vicente-Serrano, 2001).

The Borau Valley presents a great lithological homogeneity, with a predominance of intensely folded calcareous marls and sandstones, typical for flysch formations. Geomorphological processes have historically been very active, due to both the lithological plasticity and the strong anthropization of the area: massive deforestation and massive ploughings to establish crops and to feed the livestock population in summer in high altitudes (>21,000 sheep in the 19th century, according to Vicente-Serrano, 2001).

The climate can be classified as a sub-Mediterranean mountain climate with continental influence, which is reflected in a decrease of the precipitation during summer season. At the nearby station of Esposa (979 m a.s.l.) the annual rainfall is 1086 mm and the average temperature is 9.9 °C. The seasonal distribution of precipitation is relatively homogeneous, although maximum values were recorded during autumn and winter, and minimum values during summer, causing a slight water deficit in this season.

Chauvelier (1987) distinguished two well-differentiated ecosystems in the Central Pyrenees: between 840 and 1800 m a.s.l., a forest ecosystem appears on the footslope hills and montane belts. From 1700 to 1800 m a.s.l. a supraforest ecosystem is developed which corresponds to the subalpine vegetation. Both vegetation systems were greatly affected by human action, which resulted in the lowering of the upper forest level, and the deforestation of the slopes at low altitudes for

1956 (has)	Dry cultivated fields	Meadows	Subalpine grasslands	Shrublands	Subalpine grasslands-shrublands	Coniferous	Deciduous species	Coniferous-deciduous species	Alpine grasslands and rock outcrops	Total	
2015 (has)											
Dry cultivated fields	35.8	0	0	0	0	0	0	0	0	35.8	
Meadows	27.5	312.3	0	1	0	0	0	0	0	340.5	
Subalpine grasslands	0	12.6	4013.2	0.1	0.1	0.3	0	0	0.8	4027.1	
Shrublands	20.6	0	92.6	752.8	0	0	0	0	4.7	873.7	
Subalpine grasslands-shrublands	0	26.6	586.7	0.1	447.3	0.1	0	0	33.4	1094.1	
Coniferous	0	40.7	441.0	281.2	165.8	4335.8	0	0	16.9	5281.4	
Deciduous species	0	0	0	0	0	0	271.2	0	0	271.2	
Coniferous-deciduous species	0	0	0	0	0	0	0	214.6	0	214.6	
Alpine grasslands and rock outcrops	0	10.9	1.0	18.9	0.2	9.1	0	0	3769.7	3809.8	
Total	83.9	403.1	5137.5	1053.7	613.4	4345.3	271.2	214.6	3825.5	15,948.2	
%	5.0	2.5	32.2	9 9	8 6	27.2	17	13	24.0		

Highlighted in bold are the total has for each LULC in 1956 and 2015.

cultivation. These interventions led the forested spots (mainly pine and oak) to be concentrated in the less favorable areas where agriculture was not applicable (shady and steep slopes and sectors far from the village), while most of the territory was occupied by crops in the montane belt and by grasslands in the subalpine belt. Therefore, natural forests occupy only small areas. In the montane belt now woody encroachment processes prevail (typical species are Echinospartum horridum, Genista scorpius, Buxus sempervirens, Juniperus communis and Crataegus monogyna). Above 1700–1800 m (subalpine belt) small gloves of Pinus uncinata appear and great extensions of subalpine grasslands predominate, occupying an area that was climatically forestry, but that the human action turned into grasslands by means of the clearings and the fires (García-Ruiz et al., 2015). The grasslands located at lower altitudes (1700–1850 m) correspond to the Mesobromion erecti community (Eryngio-Plantaginetum mediae association), which is a very productive grassland, much appreciated by livestock. At higher altitudes there is a transition to the Nardion strictae community (Alchemillo-Nardetum strictae association) and above this area a transition to Festuca eskia (Carici-Festucetum eskiae association), with a progressive loss of pasture quality (Remón Aldave, 1997).

The soils are classified as Phaeozems (FAO, 2014), characteristic of relatively humid grasslands and forest areas in a moderately continental climate. The soils present a dark top horizon, rich in humus, with or without secondary carbonates, but with a high saturation of bases in the first meter.

2.2. Land use change analyses

Aerial photographs from 1956 and orthophotographs from 2015, at a scale of 1:33,000 and 1:25,000 respectively, were used to analyze the evolution of land uses and land covers (LULC). A first map was produced based on the orthophotograph from 2015 (downloaded from the National Geographical Institute), and them a second map from 1956 was created. Two maps were generated, including the following LULC classes: (i) dry cultivated fields, (ii) meadows, (iii) subalpine grasslands, (iv) shrublands, (v) subalpine grasslands-shrublands, (vi) coniferous, (vii) deciduous species, and (viii) alpine grasslands and rock outcrops. The LULC maps were overlain to assess changes that occurred over the period of study. All these analyses were done using ArcGIS 10.3. Measurement of the areas where changes or no changes had occurred enabled computation of a LULC transition matrix and the Kappa index, and which were calculated using Idrisi (Selva, Clarck University, 17, January 2012).

2.3. Experimental design and sampling

Field survey techniques, aerial photograph interpretation and LULC maps were used to select four different land covers at 1800 m (see Fig. 1): (i) grasslands (mainly cover by Nardion strictae) representing current grazing areas (control areas) to describe the status before woody encroachment and forest expansion, (ii) shrubs, representing areas that have suffered woody encroachment and occupied by J. communis and Silybum marianum, (iii) young forest, representing areas with forest expansion in the last 50 years (mainly conifers) and the presence of Echinospartum horridum and J. communis, and (iv) old forest, representing the coniferous forest that already existed in 1956. For each of the four land covers, three plots were selected, all with similar topographic conditions (altitude, slope and exposition).

An extensive field survey and soil sampling was carried out in December 2016. At each plot, soil samples were sampled in the field at 10 cm increments: 0–10 cm, 10–20 cm, 20–30 cm and >30 cm (maximum around 50 cm). We collected 3 soil samples per plot systematically and depth. Subsamples (total subsamples 144) were combined into one soil single composite sample per depth and plot. In total 48 composite samples were collected and analyzed in the laboratory.

2.4. Laboratory analysis and soil characterization

Soil samples were air dried and passed through a 2 mm mesh sieve in the laboratory. Soil pH and electrical conductivity (EC) were measured in a deionized water-soil suspension (1:2.5). Soil texture was determined using a particle size analyzer (Mastersizer 2000), Soil organic matter (SOM) was determined using the Walkley-Black method. Total carbon (Ctotal) and total nitrogen (N) were determined by dry combustion (Vario Max). Carbonate concentration (CaCO3) was determined using the Bernard calcimeter method, although low values were found so this variable was discarded. Consequently soil organic carbon (SOC) was calculated using the van Bemmelen factor of 0.58, using as universal conversion factor. Bulk density values were estimated from undisturbed cores and pedotransfer equations (proposed by Guo and Gifford (2002) and Post and Kwon (2000)). SOC and N contents were expressed in g km⁻¹ soil, while SOC and N stocks were expressed in Mg ha⁻¹ (calculated by incorporating their respective depth and thickness and bulk density). Finally, available phosphorous (P) was determined by the Bray method. Sampling and analysis methods concerning these properties are explained in detail in Nadal-Romero et al. (2016).

2.5. Statistical analyses

Besides, the descriptive analysis, all data were tested for normal distribution using the Chi-square test. Analysis of variance, a two-way ANOVA, was used to compare the differences among land covers and depths (homogeneity of variance was tested using Levene's test). A posteriori, LSD post-hoc tests were used to confirm where the differences occurred between groups.

In all the cases, we considered differences to be statistically significant at p < 0.005. All statistical analyses were carried out using SPSS Statistics 20.

3. Results

3.1. Land use changes

Figs. 2 and 3 show the spatial distribution of the LULC in 1956 and 2015. By 1956, the main LULCs correspond to subalpine grasslands

Table 2 F values and significance (p) of ANOVA analysis for all properties in all soil samples. LSD post-hoc tests are shown in Table 3 and were used to confirm where the differences occurred between groups.

		$C_{\rm org}$	N	Ctotal	SOC stock	TN stock	C _{org} N ratio	SOM	pН	EC	Clay	Sand	Silt	P	BD
Land cover	F	11.386	21.191	12.766	12.251	21.791	14.755	11.386	1.452	9.428	16.612	24.348	27.175	4.924	12.251
	р	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.247	0.000	0.000	0.000	0.000	0.007	0.000
Soil depth	F	27.222	13.851	25.208	31.153	9.536	15.085	27.185	0.291	21.787	24.636	54.116	66.825	1.021	31.153
	p	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.831	0.000	0.000	0.000	0.000	0.397	0.000
Land cover × soil depth	F	0.940	0.457	0.675	0.606	0.509	3.181	0.941	0.067	0.616	1.319	3.685	6.300	0.470	0.606
	p	0.505	0.892	0.725	0.782	0.857	0.008	0.505	1	0.774	0.267	0.003	0.000	0.883	0.782

Core: organic carbon content; N: nitrogen content; Ctotal: carbon content; SOC stock: soil organic carbon stock; TN: nitrogen stocks; SOM: soil organic matter; EC: electrical conductivity; P: assimilable phosphorus; BD: bulk density.

p in italics is significantly different at p < 0.05.

(32.1%) and coniferous (27.2%). Substantial changes have occurred by 2015 (Table 1):

- (1) Coniferous was in 2015 the most extensive land cover (33%), and grasslands were the second largest LULC (25.2%), but they have been marked spatial shrinkage (1110 ha).
- (2) The expansion of coniferous forest at the expense of grasslands (441 ha) was one of the main changes that occurred in this period, together with the change from subalpine grasslands to grasslands-shrublands (587 ha).
- (3) Coniferous forest was expanded, with the main transitions being from subalpine grasslands (441 ha), shrublands (281 ha) and subalpine grasslands-shrublands (166 ha).
- (4) Woody encroachment due to the expansion of shrubland is also observed due to changes from subalpine grasslands to shrublands (96 ha) and to grasslands-shrublands (587 ha).

3.2. Soil properties and soil carbon stocks

Our results from the statistical tests showed that some soil properties were significantly different depending on both land cover and depth. Significant differences appear for all properties except for pH, and P content presented only significant differences related to land cover (Table 2).

Land cover and depth significantly affected the texture composition of the soil samples. The lowest clay contents were observed in grasslands. Significant differences in the texture composition were observed between grasslands and the other land covers, but mainly limited to the first 20 cm. Significant differences were also observed between different depths in the grasslands, young forests and old forests, and only for the clay content at the shrublands (Tables 2 and 3).

The highest EC values were observed at grasslands and these values decreased in depth in all cases (Table 3). Significant differences related to land cover and depth were observed. For pH, low values were observed in all the samples, and no significant differences were found (Table 2).

Significant differences related to the available P concentration were found only in the first 10 cm between land covers; although no differences were observed between grasslands and young forests (Table 2).

The highest Corg concentrations were obtained in the grassland sites (at 0–10 cm) and the lowest values in the shrub areas (Fig. 4). At 0–10 cm significant differences were observed between grasslands and the different land covers. No differences were observed at 10–20 cm between land covers, and below 20 cm significant differences were observed between grasslands and young forests and shrubs and old forests. Significant differences were also observed between different depths in the shrub and old forest sites, and only between 0 and 10 cm and the other depths in grasslands and young forests (Table 2).

Significant differences related to N concentrations and CN ratios were also found (Fig. 4 and Table 2). N contents decreased with depth and values were higher in the grasslands. The highest CN ratios were found in the old forests (Table 3).

Total SOC stocks ranged between 91.7 Mg ha⁻¹ (shrubs) to 147.9 Mg ha⁻¹ (grasslands) (Fig. 5a). The percentage of SOC in the first 10 cm averaged 36.8, 38.7, 35.7 and 41.1% for grassland, shrubs, young forest and old forest respectively. Significant differences related to land cover at 0–10 cm were found between grasslands and the different land covers. At 10–20 cm no significant differences were observed. Below 20 cm no differences were found between grasslands and young forests, and significant differences were observed between grasslands and young forests and shrubs and old forests. Considering the complete soil profiles, significant differences were observed between grasslands and shrubs, but no differences were found between young forest and old forest.

Total N stocks ranged between 9.8 Mg ha^{-1} (old forests) to 19.4 Mg ha^{-1} (grasslands) (Fig. 5b). At 0–10 cm no significant differences were observed. At 10–20 cm significant differences were

Mean and standard deviations of the studied soil properties. Soil characteristics resulting from former land covers, revegetation processes (secondary succession and afforestation) (PS = Pinus sylvestris and PN = Pinus nigra) and natural forest

	Grasslands				Shrubs				Young forest				Old forest			
	0-10 cm	10-20 cm	10–20 cm 20–30 cm >30 cm	>30 cm	0-10 cm	10-20 cm	20-30 cm	>30 cm	0-10 cm	10-20 cm	20-30 cm	>30 cm	0-10 cm	10-20 cm	20-30 cm	>30 cm
Corg (%)	$50.4\pm15.5^{\mathrm{aA}}$	$30 \pm 10.7^{\mathrm{B}}$	$23.4 \pm 7.9^{aB} 18.4 \pm 5^{aB}$		$25.9 \pm 2^{\text{bA}}$	$18.9 \pm 5.9^{\mathrm{B}}$	$13.3\pm1.4^{\rm bcBC}$	8 ± 0.9^{bC}	$33 \pm 5.5^{\mathrm{bA}}$	23.4 ± 4^{B}	١.	m	31.6 ± 1.3^{bA}	20.5 ± 2.1^{B}	$12.9 \pm 0.3^{\rm bcC}$	$10.1\pm0.6^{\rm bD}$
N (%)	$5.6\pm1.4^{\mathrm{aA}}$	$4 \pm 1.7^{\mathrm{aA}}$	$3.3 \pm 1.1^{\mathrm{a}}$	$2.8 \pm 0.7^{\mathrm{aA}}$	$2.9\pm0.4^{\mathrm{bA}}$	$2.5 \pm 0.8^{\mathrm{aA}}$	$1.8 \pm 0.2^{\mathrm{bB}}$	$1.5\pm0.6^{\mathrm{bB}}$	$3.6 \pm 0.9^{\rm bA}$	$2.4 \pm 0.2^{\mathrm{aB}}$	$2 \pm 0.3^{\mathrm{bB}}$	2 ± 0.2^{abB}	$2.4 \pm 0.3^{\mathrm{bA}}$	$1.8 \pm 0^{\mathrm{bB}}$	$1.6\pm0.2^{\mathrm{bB}}$	$1.4\pm0.1^{\mathrm{bBC}}$
Ctotal (g kg ⁻¹)	Ctotal (g kg $^{-1}$) 6.3 \pm 1.7 ^{aA}	$4.1 \pm 1.7^{\text{aAB}}$	$3.2\pm1.2^{\mathrm{aB}}$	$2.5 \pm 0.8^{\mathrm{aB}}$	$3.3 \pm 0.3^{\rm bA}$	$2.6\pm0.9^{\mathrm{aA}}$	$1.7 \pm 0.1^{\mathrm{bB}}$	$1.3 \pm 0.5^{\rm bB}$	4.5 ± 1^{abA}	$2.8\pm0.4^{\rm aB}$				$2.3 \pm 0.1^{\text{bB}}$	$1.7 \pm 0.2^{\mathrm{bBC}}$	1.3 ± 0^{bC}
SOC (Mg ha ⁻¹)	54.4 ± 11.9^{aA}	37.4 ± 0.9^{AB}	$30.8 \pm 8.9^{\mathrm{aB}}$	25.4 ± 6^{aB}	$33.8 \pm 2.1^{\text{bA}}$	25.9 ± 6.8^{B}	$19.2 \pm 1.7^{\text{bBC}}$	$12.2 \pm 1.2^{\text{bBC}}$	$40.7 \pm 5.3^{\text{bA}}$	31.1 ± 4.3^{AB}	24.3 ± 7^{abB}	25.2 ± 6.3^{aB}	$39.5 \pm 1.2^{\text{bA}}$	28 ± 2.3^{B}	18.7 ± 0.4^{bC}	$15.1 \pm 0.8^{\rm bD}$
$TN (Mg ha^{-1})$	6.1 ± 0.1	$5.0\pm1.8^{\rm a}$	$4.4\pm1.2^{\rm a}$	$3.9\pm0.8^{\rm a}$	3.8 ± 0.5^{A}	$3.3 \pm 1^{\mathrm{aAB}}$	$2.6 \pm 0.3^{\mathrm{bAB}}$	$2.3 \pm 0.8^{\mathrm{bB}}$	4.4 ± 0.9^{A}	$3.2\pm0.4^{\rm aB}$	$2.7 \pm 0.3^{\mathrm{bB}}$	$2.8 \pm 0.3^{\mathrm{bB}}$	2.9 ± 0.4^{A}	$2.5\pm0.1^{\mathrm{bB}}$	$2.3 \pm 0.3^{\mathrm{bB}}$	$2.1 \pm 0.2^{\mathrm{bB}}$
CN ratio	$8.8 \pm 0.6^{\mathrm{aA}}$	$7.6\pm0.6^{\rm aB}$	$7 \pm 0.5^{\mathrm{B}}$	$6.5\pm0.3^{\rm aB}$	$9 \pm 0.5^{\mathrm{aA}}$	$7.5\pm0.1^{\rm aB}$	$7.4 \pm 0.6^{\mathrm{B}}$	$5.5 \pm 1.4^{\mathrm{acC}}$	$9.3\pm0.8^{\rm a}$	$9.8\pm1.8^{\rm a}$	8.7 ± 1.7	$9.2 \pm 2.8^{\mathrm{ab}}$	$13.6\pm1.4^{\rm bA}$	$11.3 \pm 1.1^{\text{bB}}$	8.3 ± 1^{C}	$7.1\pm0.9^{\mathrm{aC}}$
SOM (%)	8.7 ± 2.7^{aA}	$5.2 \pm 1.8^{\mathrm{BC}}$	$4\pm1.4^{\mathrm{aBC}}$	3.2 ± 0.9^{aB}	$4.5 \pm 0.3^{\rm bA}$	3.3 ± 1^{B}	$2.3 \pm 0.2^{\mathrm{bBC}}$	$1.4 \pm 0.2^{\rm bC}$	$5.7 \pm 1^{\text{bA}}$	$4\pm0.7^{\mathrm{AB}}$	3 ± 1^{abB}	$3.2 \pm 0.9^{\mathrm{acB}}$	$5.5 \pm 0.2^{\text{bA}}$	3.5 ± 0.4^{B}	2.2 ± 0.1^{bC}	$1.8 \pm 0.1^{\mathrm{bD}}$
Hd	5.5 ± 0.5	5.5 ± 0.7	5.5 ± 0.7	5.5 ± 0.8	5.4 ± 0.1	5.4 ± 0.3	5.2 ± 0.2	5.3 ± 0	5.3 ± 0.1	5.2 ± 0.1	5.2 ± 0.2	5.2 ± 0.1	5.6 ± 0.1	5.4 ± 0.1	5.3 ± 0.1	5.4 ± 0.1
EC (μ S cm ⁻¹)	121.9 ± 50.6^{aA}	61.1 ± 13.7^{B}	$45.3 \pm 3^{\mathrm{aB}}$	43.3 ± 2^{aB}	$66.8 \pm 20.8^{\mathrm{aA}}$	$40.3 \pm 9.7^{\mathrm{BC}}$	31.8 ± 6.6^{abBC}	$18 \pm 2.1^{\text{bB}}$	$88.4\pm50.2^{\mathrm{aA}}$	37.2 ± 17.9^{B}	$29.1 \pm 9.2^{\rm bB}$	$25 \pm 6.1^{\mathrm{cB}}$	$47.2 \pm 7.8^{\text{bA}}$	37.5 ± 4.4^{AB}	$25 \pm 7.4^{\text{bBC}}$	$18.2 \pm 0.7^{\mathrm{bBC}}$
Clay (%)	3.4 ± 2^{aA}	$6.4\pm2.1^{\rm a}$	12.4 ± 4.7^{aB}	15.5 ± 1.5^{B}	$10\pm1.6^{\mathrm{bA}}$	$12.5 \pm 2.3^{\text{bAB}}$	13.9 ± 0.8^{aB}	15.2 ± 2.7^{B}	$9.7 \pm 2.2^{\text{bA}}$	$15.1 \pm 2.3^{\text{bB}}$	16.5 ± 2.7^{aB}	17.5 ± 2.7^{B}	$10.5 \pm 1.4^{\text{bA}}$	$15.1 \pm 0.9^{\rm bA}$	$22 \pm 3^{\text{bB}}$	21.7 ± 6.4^{B}
Sand (%)	$73.1 \pm 5.4^{\mathrm{aA}}$	63.5 ± 3.1^{aB}	48.1 ± 4.2^{aC}	40.2 ± 3.1^{aD}	59.7 ± 4^{b}	57.5 ± 4.2^{a}	53.6 ± 5.8^{a}	50.2 ± 4.8^{ab}	$60.2 \pm 4^{\text{bA}}$	$48.5 \pm 4.5^{\text{bB}}$	$44.4 \pm 3^{\text{acB}}$	41.9 ± 3.6^{aB}	$58.9 \pm 2^{\text{bA}}$	$44.2 \pm 2^{\text{bB}}$	$34.7 \pm 3.5^{\text{bBC}}$	$31.9 \pm 9.3^{\rm acc}$
Silt (%)	$23.5\pm3.4^{\mathrm{aA}}$	$30.1 \pm 1.2^{\mathrm{aB}}$	$39.5 \pm 0.5^{\rm aC}$	44.3 ± 2.3^{aD}	$30.3 \pm 2.4^{\rm b}$	$30 \pm 2^{\rm a}$	32.5 ± 5.2^{b}	$34.6 \pm 2^{\rm b}$	$30.1 \pm 2.1^{\rm bA}$	$36.4 \pm 2.3^{\mathrm{cB}}$	39.1 ± 1^{aBC}	$40.6\pm0.8^{\mathrm{aC}}$	$30.6 \pm 0.8^{\rm bA}$	$40.7 \pm 2.2^{\text{bB}}$	43.3 ± 1^{aB}	46.4 ± 3^{aC}
P $(mg kg^{-1})$	106.2 ± 22.4	86.1 ± 22.4	93.1 ± 28.3	95.8 ± 32.2	$64.7 \pm 8.7^{\text{bA}}$	84.2 ± 9.3^{B}	$85 \pm 10.8^{\mathrm{B}}$	$90.8 \pm 3.3^{\circ}$	84.1 ± 31.2^{ab}	92.9 ± 12.7	94.6 ± 6.4	110.4 ± 5.5	56.4 ± 18.9^{b}	67.8 ± 19	60.6 ± 24.6	78.1 ± 43.9
$BD (g cm^{-3})$	$1.1\pm0.1^{\mathrm{aA}}$	$1.3\pm0.1^{\rm AB}$	$1.3\pm0.1^{\rm aB}$	$1.4\pm0.1^{\rm aB}$	$1.3 \pm 0^{\mathrm{bA}}$	$1.4\pm0.1^{\rm bB}$	$1.5\pm0^{\rm cBC}$	$1.5\pm0^{\rm cC}$	$1.2\pm0.1^{\mathrm{aA}}$	$1.3\pm0^{\mathrm{bA}}$	$1.4\pm0.1^{\rm cB}$	$1.4\pm0.1^{\rm cB}$	$1.3 \pm 0^{\mathrm{bA}}$	$1.4\pm0^{\rm B}$	$1.5\pm0^{\mathrm{bC}}$	$1.5\pm0^{\mathrm{bD}}$

Note: Means with the different lower case letter superscripts within a row are significantly different at 0.05 level of significance (p < 0.05) Means with the different upper case letter superscripts within a column are significantly different at 0.05 level of significance (p < 0.05)

soil organic carbon stock; TN: nitrogen stocks; OM: organic matter; EC: electrical conductivity; CaCO3; carbonate content; P: organic phosphorus; BD: bulk density; FC: field inorganic carbon; S N: nitrogen content; OC: Corg: organic carbon; Cinorg: observed between old forests and the other land covers. Below 20 cm significant differences were observed between grasslands and the other land cover types. No significant differences were observed related to depth at grassland sites. Considering the complete soil profile N stocks were significant different at grasslands compare with other land covers (Table 2).

4. Discussion

4.1. LULC changes: woody encroachment of subalpine grasslands

Substantial land use changes have occurred from 1956 to 2015 in the study area. Woody encroachment has been observed due to the expansion of coniferous and shrublands (at the expense of grasslands). A reduction of grasslands has occurred (about 7% of the total grassland area was changed). The main reasons for these major changes were the decline in livestock and livestock pressure. Similar results were observed in the Iberian Range by García-Ruiz et al. (2016) and Sanjuán et al. (2017), concluding that the crisis of the transhumance, since the beginning of the 19th century, reduced the livestock pressure and contributed to shrub and forest expansion reducing the area occupied by summer grasslands (Urbión Sierra, Spain). However, global warming has also contributed to the advance of shrubs and pines in the upper limit of the forest, as do many thermophilic species (Gottfried et al., 2012).

Shrub expansion is a worldwide phenomenon (Brandt et al., 2013; Matson and Bart, 2013; Ratajczak et al., 2012; Xie and Sha, 2012). Our results agree with several studies carried out recently in different European mountain areas. Literature reviewed indicated that forest regrowth is an ongoing process in the Alps (Gehrig-Fasel et al., 2007; Fondevilla et al., 2016; Caviezel et al., 2017) and in the Apennines (Palombo et al., 2013). In the Iberian Range, Sanjuán et al. (2017) reported a marked trend to dense forest and the spatial contraction of shrublands and grasslands. In the Pyrenees, woody encroachment has been reported previously. In the eastern Pyrenees, Batllori and Gutiérrez (2008) reported a densification of forest close to the tree line. In the Central Pyrenees, Gartzia et al. (2014) described the expansion of shrubland and forest at the expense of summer grasslands and Sanjuán et al. (2016) reported the expansion of dense pine forest and a decline in the area of subalpine grasslands.

4.2. Subalpine grasslands: consequences of woody encroachment on soil properties

Our initial results support our first hypothesis that subalpine grasslands contain significantly higher soil organic carbon contents and stocks (see Figs. 4 and 5). Similar values were observed in subalpine grasslands in Mediterranean mountains at similar altitudes. Conen et al. (2008) in the Swiss Alps, reported similar Corg concentrations in grasslands: mean values were 58.2 g kg $^{-1}$ (at 0–5 cm) and 44.9 g kg $^{-1}$ (at 5–10 cm) for mineral soil at 1795 m. Catoni et al. (2016) in the Ligurian

Alps (1780 m) reported Corg values around 50 g kg⁻¹ in grasslands areas. Jiménez and Villar (2017) found also similar values in grassland soils of Monte Perdido Massif (Ordesa National Park, Central Pyrenees). About SOC stocks, Sjögersten et al. (2011) and Hunziker et al. (2017) reported mean SOC values about 100 Mg C ha⁻¹ in grassland areas, which is comparable with the results of the present study, all of them suggesting a high capacity of grasslands to store SOC.

Our results also confirm our second hypothesis: woody encroachment in the subalpine belt may lead to significant changes in soil properties. Significant changes in most of the soil parameters studied were observed in relation to land cover type and soil depth (except for pH). The soil texture ranged from sandy loam to medium loam with significant differences between LULC and depth. CN ratios increased from grasslands to young and old forest sites, but did not differ between both forest sites. Main changes were recorded in the upper soil layers, and no differences were observed in the deeper soil layers, suggesting that these changes are mainly due to changes in the litter quality (deeper soils are less affected by litter quality changes). Similar results were observed in Hooker and Compton (2003).

The SOC and N concentrations were higher in the mineral soil of grasslands if compared with other uses. Similar findings were also observed in comparable studies by Guidi et al. (2014). Our results revealed that both SOC and N stocks were also affected by LULC changes. Total SOC stocks (as the sum of all soil layers) ranged between 86.8 Mg C ha⁻¹ and 147.9 Mg C ha⁻¹, following approximated order: grasslands > young forest > old forest > shrublands. Total N stocks ranged between 9.8 Mg N ha⁻¹ and 19.4 Mg N ha⁻¹, following approximated order: grasslands > young forest > shrublands > old forest. N content and stock is related to the herbaceous vegetation that usually proliferates on grasslands, with legume plants with a high N-fixing capacity, which increase soil N content (Hooper and Vitousek, 1998).

Changes in the magnitude and direction of soil properties and SOC stocks after woody encroachment present a high uncertainty and opposite results can be found in the literature (Li et al., 2016; Hunziker et al., 2017). Gosheva et al. (2017) concluded that forest expansion on former grasslands is not associated with an increase of SOC sequestration and their results reported a decrease of the SOC values. Other studies reported an increase in SOC stocks related to shrublands expansion (Li et al., 2016). Gutiérrez-Girón et al. (2015) reported a significant increases in SOC and total N stocks in the Sierra de Guadarrama (Central Spain). Montané et al. (2007) also showed that woody encroachment did not decrease SOC stocks in grasslands.

The low SOC accumulation associated with shrublands may be related to its short development period. Considering the complete soil profile, no differences were observed between grasslands and young and old forest. So, despite the potential capacity of shrub encroachment to accumulate SOC, we think that long encroachment periods are needed. Thuille and Schulze (2006), Hiltbrunner et al. (2013) and Van Hall et al. (2017) also observed first a decline followed by an increase of the mineral SOC stock. Hunziker et al. (2017) carried out a chronosequence study in the Alps and concluded that the SOC stocks

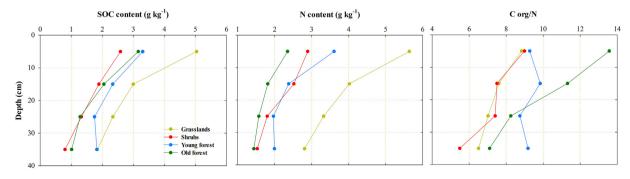


Fig. 4. Soil organic carbon (SOC) and nitrogen (N) contents and Corg/N ratios in the different land covers and depths.

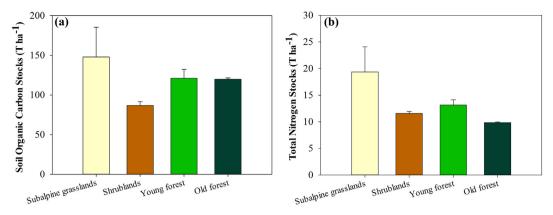


Fig. 5. Soil organic carbon (SOC) and nitrogen (N) stocks in the different land covers.

decrease for 40 years old shrub stands, but a significant increase in total SOC stocks after 90 years was observed. Changes in root dynamics can be partly responsible of these changes. The loss of grass roots associated with the first stage of woody encroachment can lead to a decrease in below-ground carbon storage. This hypothesis has been tested by different authors, indicating that grass roots disappear at very high woody densities (see Fig. 1) (Jackson et al., 2002; Coetsee et al., 2013). Hudak et al. (2003) suggested the loss of grass roots as a possible mechanism of declining soil Corg concentration at sites where a close canopy prohibited the growth of grass. These changes in root sizes can also change aggregate size fractions and aggregate stabilization affecting to SOC stocks (Guidi et al., 2014).

Several investigations studied the spatial variability of soil properties using different spatial and statistical methods (i.e. Loescher et al., 2014; Bogunovic et al., 2017). Soils are highly variable, making designing sampling strategies a challenging task. Assuming the high variability and uncertainty of soil properties, the most important issue is to be sure that the results from our samples represent the different characteristics of the area (at least as closely as possible). Our 36 soil sampling-plots (randomly collected in three plots per land use) were selected considering local differences due to slope, exposition and vegetation variability. However, our results present high variability in the analyzed soil properties. This variability was higher in the top layers, decreasing in depth. Related to LULC, variability was higher in grassland soil samples.

Our results require additional research to elucidate new questions that arose from this study: (i) grass root analysis, aggregate size and density fractionation experiments should be carried out to complement the information about soil carbon sequestration; and (ii) we should estimate SOC stocks in soil organic layers and above-ground (plant biomass), as some authors suggest that the decrease in SOC stocks can be compensated by the development of an organic layer on top of the mineral soil, that acts as carbon pool, and for the above-ground carbon stocks (i.e. Sjögersten et al., 2011; Hudak et al., 2012).

5. Conclusions

This study has provided novel information on the effects of woody encroachment of subalpine grassland on soil properties and SOC stocks in a mountain Mediterranean area. Our results support our hypothesis that subalpine grasslands contain high soil organic carbon and that woody encroachment in the subalpine belt may lead to significant changes in soil properties. However, contrary to our hypothesis woody encroachment did not generate an increase in the SOC accumulation.

The following conclusions can be made:

 Significant land use changes have occurred from 1956 to 2015 in the study area: woody encroachment has been observed due to the

- expansion of coniferous forests and shrublands (at the expense of grasslands)
- 2. The results illustrate that LULC changes from subalpine grassland to shrubs and conifer forests substantially affects the soil system.
- 3. During the woody encroachment process in the subalpine belt of the Central Spanish Pyrenees the SOC stock in the mineral phases initially decreases from 147.9 Mg C ha $^{-1}$ to 91.7 Mg C ha $^{-1}$.
- 4. No significant differences with regard to complete SOC stocks were observed between grasslands and young forest, suggesting that long encroachment periods are needed.

Woody encroachment is a current process that will continue in the future, probably enhanced by the continuously decreasing livestock and by global warming. The information obtained in this study indicated that subalpine grasslands may be an important source of carbon storage in mountain areas. Consequently forest management in Mediterranean mountains should consider woody encroachment as a real process and a present-day problem in future management practices.

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