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2	Woody encroachment and soil carbon stocks in subalpine areas in the Central
3	Spanish Pyrenees
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15	Abstract
16	Woody encroachment has been an ongoing process in the subalpine belt of Mediterranean
17	mountains, after land abandonment, the disappearance of the transhumant system and the
18	decrease of the livestock number. The main objectives of this study were: (i) to identify
19	land use/land cover (LULC) changes from 1956 to 2015, and (ii) to investigate the effects
20	of LULC changes in physical and chemical soil properties and soil organic carbon (SOC)
21	and nitrogen (N) stocks. It is hypothesized that woody encroachment in the subalpine belt
22	may lead to significant changes in soil properties, and will generate an increase in the
23	SOC stocks. A land use gradient was identified in the subalpine belt of the Central
24	Spanish Pyrenees: (i) subalpine grasslands, (ii) shrublands, (iii) young forests, and (iv)
25	old forests. Mineral soil samples were collected every 10 cm, down to 40 cm, at three
26	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 27 coniferous forests and shrublands (at the expense of grasslands), (ii) land cover and soil 28 depth had significant effects on soil properties (except for pH), being larger in the 29 uppermost 0-10 cm depth, (iii) SOC and N contents and stocks were higher in the 30 grasslands sites, and (iv) the woody encroachment process initially produced a decrease 31 in the SOC stocks (shrublands), but no differences were observed considering the 32 complete soil profile between grasslands and young and old forests. Further studies, 33 describing SOC stabilization and quantifying above-ground carbon (shrub and tree 34 biomass) are required. 35

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Keywords: grasslands, secondary succession, soil carbon and nitrogen stocks, soil
properties, subalpine belt

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# 40 **1.** Introduction

41 The management of agropastoral ecosystems is one of the major challenges facing 42 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 43 et al., 2017) and to the abandonment of agricultural and pastoral activities since the mid-44 45 twentieth 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 46 cultural landscapes into homogeneous coarse-grained landscapes (Van Eetvelde and 47 48 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 49 50 al., 2014).

In Mediterranean mountains, grassland ecosystems are extremely important as 51 52 producers of many goods and services for society (provisioning, regulation, support, culture) and as contributors to the support of a rich biodiversity (Millennium Ecosystem 53 54 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 55 56 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 57 provides livelihood to almost one billion people in the world (FAO, 2009). Grasslands 58 also play an important regulatory role; they act as firewalls in areas where the rural 59 60 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 61 62 hydrological cycle because it has been demonstrated that spontaneous revegetation or 63 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 64 65 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 66 and provides to the grasslands a high ecological value (Canals and Sebastiá, 2000). 67 Consequently, the loss of grasslands reduces plant and faunal biodiversity (Ratajczak et 68 69 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 70 aesthetic value, offering cultural services such as recreational (tourism), educational and 71 72 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 73 74 source of ecological information and local knowledge on adaptation of management 75 systems to adapt to Global Change (Fernández 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 82 approximately 2,400 m a.s.l., coinciding with the upper limit of the subalpine belt and the 83 maximum level for the development of shrubland, while Pinus uncinata would reach up 84 85 to 2,200 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 86 to the Middle Ages, when much of the subalpine belt was deforested to graze transhumant 87 88 livestock (Bal et al., 2011; Cunil, 2012; Pérez-Sanz et al., 2003). However, since the middle of the 20th century, subalpine grasslands have undergone a major transformation 89 90 due to the decline of livestock activities: the disappearance of transhumance, the sharp 91 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 92 of grassland activities has favored the expansion of woody species into grasslands 93 94 (Bartolomé et al., 2005a; Anthelme et al., 2007). Gartzia et al. (2014) pointed out that 95 19% of dense pastures below 2,100 m a.s.l. and 24% of low pastures between 1,980 and 2,000 m a.s.l. have been woody-encroached, in the Central Spanish Pyrenees, while 35% 96 97 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 98 99 period (Batllori and Gutiérrez, 2008; López-Moreno et al., 2017) causing a cascading effect on ecosystem processes, accelerating the invasion of grasslands by high 100

101 competitive woody species threatening ecosystem functions and services (Komac et al.,102 2013).

So far, the process of woody encroachment in subalpine grasslands has been studied 103 104 in the Pyrenees (Komac et al., 2011a, 2011b, 2013; Madruga et al., 2011), and some 105 consequences have been analyzed: (i) the increase of the biomass of woody vegetation 106 with the consequent fire risk (Bartolomé et al., 2005b), (ii) grassland management (traditional management, conservation polices as suppressing fires or grazing practices, 107 108 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 109 110 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 111 of the woody encroachment and Climate Change (Gartzia et al., 2016a), (vi) the loss of 112 113 connectivity of grasslands (Gartzia et al., 2016b), and (viii) the influence of land use 114 changes on subalpine areas on river torrentiality (Gómez-Villar et al., 2014; Sanjuán et 115 al., 2016). The effects of prescribed fires of successional shrubs on soil function, on the 116 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). 117

However, the effects of woody encroachment on soil properties and soil organic 118 119 carbon (SOC) stocks have been less studied, despite the importance of this question 120 within a Global Change context (Farley et al., 2013; Lo et al., 2015). In addition, soil organic carbon is an indicator of soil quality associated with aggregate stability, with 121 122 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 123 124 grasslands is their ability to carbon sequestration and to regulate greenhouse gas 125 emissions (Farley et al., 2013). Grasslands form perennial and dense covers with a high

carbon-binding capacity (Ritchie, 2014; Rutledge et al., 2014; Aldezábal 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 Kwon, 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 132 133 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 134 135 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 136 following research hypotheses: (i) subalpine grasslands contain high soil organic carbon 137 138 stocks, (ii) woody encroachment in the subalpine belt may lead to significant changes in 139 soil properties, and (iii) woody encroachment and forest expansion in subalpine belts will 140 generate an increase in the SOC stocks. Understanding the mechanisms behind 141 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 142 goods and services that these ecosystems provide to society. 143

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## 145 2. Materials and Methods

146 *2.1. Study area* 

147 The study was developed in the subalpine grasslands of the Borau Valley (Central 148 Spanish Pyrenees) (Figure 1). The Borau Valley covers an area of 41.72 km<sup>2</sup>, with an 149 altitudinal gradient ranging from 840 m a.s.l. to 2,566 m a.s.l. at the top. The Borau Valley 150 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 1,794 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 (more than 21,000 sheep in the 19th century, according to Vicente-Serrano, 2001).

162 The climate can be classified as a sub-Mediterranean mountain climate with 163 continental influence, which is reflected in a decrease of the precipitation during summer 164 season. At the nearby station of Esposa (979 m a.s.l.) the annual rainfall is 1086 mm and 165 the average temperature is 9.9°C. The seasonal distribution of precipitation is relatively 166 homogeneous, although maximum values were recorded during autumn and winter, and 167 minimum values during summer, causing a slight water deficit in this season.

Chauvelier (1987) distinguished two well-differentiated ecosystems in the Central 168 169 Pyrenees: between 840 and 1,800 m, a forest ecosystem appears on the footslope hills and montane belts. From 1,700-1,800 m a supraforest ecosystem is developed which 170 corresponds to the subalpine vegetation. Both vegetation systems were greatly affected 171 172 by human action, which resulted in the lowering of the upper forest level, and the deforestation of the slopes at low altitudes for cultivation. These interventions led the 173 174 forested spots (mainly pine and oak) to be concentrated in the less favorable areas where 175 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 176 177 in the subalpine belt. Therefore, natural forests occupy only small areas. In the montane belt now woody encroachment processes prevail (typical species are Echinospartum 178 179 horridum, Genista scorpius, Buxus sempervirens, Juniperus communis and Crataegus monogyna). Above 1,700-1,800 m (subalpine belt) small gloves of *Pinus uncinata* appear 180 and great extensions of subalpine grasslands predominate, occupying an area that was 181 climatically forestry, but that the human action turned into grasslands by means of the 182 183 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-184 Plantaginetum mediae association), which is a very productive grassland, much 185 appreciated by livestock. At higher altitudes there is a transition to the Nardium strictae 186 187 community (Alchemillo-Nardetum strictae association) and above this area a transition to Festucio eskiae (Carici-Festucetum Eskiae association), with a progressive loss of 188 pasture quality (Remón Aldabe, 1997). 189

190 The soils are classified as Phaeozems (FAO, 2014), characteristic of relatively 191 humid grasslands and forest areas in a moderately continental climate. The soils present 192 a dark top horizon, rich in humus, with or without secondary carbonates, but with a high 193 saturation of bases in the first meter.

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195 *2.2. Land use change analyses* 

Aerial photographs from 1956 and orthophotographs from 2015, at a scale of 197 1:33.000 and 1:25.000 respectively, were used to analyze the evolution of land uses and 198 land covers (LULC). A first map was produced based on the orthophotograph from 2015 199 (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) 201 subalpine grasslands-shrublands, (vi) coniferous, (vii) deciduous species, and (viii) alpine 202 203 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. 204 205 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 206 using Idrisi (Selva, Clarck University, 17, January 2012). 207

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### 2.3. Experimental design and sampling

Field survey techniques, aerial photograph interpretation and LULC maps were 210 used to select four different land covers at 1,800 m (see Figure 1): (i) grasslands (mainly 211 cover by Nardion Strictae) representing current grazing areas (control areas) to describe 212 213 the status before woody encroachment and forest expansion, (ii) shrubs, representing 214 areas that have suffered woody encroachment and occupied by J. communis and Silybum 215 marianum, (iii) young forest, representing areas with forest expansion in the last 50 years 216 (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 217 the four land covers, three plots were selected, all with similar topographic conditions 218 219 (altitude, slope and exposition).

An extensive field survey and soil sampling was carried out in December 2016. At 220 each plot, soil samples were sampled in the field at 10 cm increments: 0-10 cm, 10-20 221 222 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 223 224 one soil single composite sample per depth and plot. In total 48 composite samples were 225 collected and analyzed in the laboratory.

### 227 2.4. Laboratory analysis and soil characterization

Soil samples were air dried and passed through a 2 mm mesh sieve in the laboratory. 228 Soil pH and electrical conductivity (EC) were measured in a deionized water - soil 229 suspension (1:2.5). Soil texture was determined using a particle size analyzer (Mastersizer 230 2000). Soil organic matter (SOM) was determined using the Walkley-Black method. 231 Total carbon (Ctotal) and total nitrogen (N) were determined by dry combustion (Vario 232 233 Max). Carbonate concentration (CaCO3) was determined using the Bernard calcimeter method, although low values were found so this variable was discarded. Consequently 234 235 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 236 cores and pedotransfer equations (proposed by Guo and Gifford (2002) and Post and 237 Kwon (2000)). SOC and N contents were expressed in g km<sup>-1</sup> soil, while SOC and N 238 stocks were expressed in Mg ha<sup>-1</sup> (calculated by incorporating their respective depth and 239 240 thickness and bulk density). Finally, available phosphorous (P) was determined by the 241 Bray method. Sampling and analysis methods concerning these properties are explained in detail in Nadal-Romero et al. (2016). 242

243

## 244 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 analysis were carried out using SPSS Statistics 20.

252

- 253 **3. Results**
- 254 *3.1. Land use changes*

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

264 (3) Coniferous forest was expanded, with the main transitions being from subalpine
265 grasslands (441 ha), shrublands (281 ha) and subalpine grasslands-shrublands (166
266 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 grasslandsshrublands (587 ha).

270

271 *3.2. Soil properties and soil carbon stocks* 

Our results from the statistical tests showed that some soil properties weresignificantly different depending on both land cover and depth. Significant differences

appear for all properties except for pH, and P content presented only significantdifferences 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).

289 The highest Corg concentrations were obtained in the grassland sites (at 0-10 cm) and the lowest values in the shrub areas (Figure 4). At 0-10 cm significant differences 290 were observed between grasslands and the different land covers. No differences were 291 292 observed at 10-20 cm between land covers, and below 20 cm significant differences were 293 observed between grasslands and young forests and shrubs and old forests. Significant 294 differences were also observed between different depths in the shrub and old forest sites, 295 and only between 0-10 cm and the other depths in grasslands and young forests (Table 296 2).

Significant differences related to N concentrations and CN ratios were also found
(Figure 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<sup>-1</sup> (shrubs) to 147.9 Mg ha<sup>-1</sup> 300 (grasslands) (Figure 5a). The percentage of SOC in the first 10 cm averaged 36.8, 38.7, 301 35.7 and 41.1 % for grassland, shrubs, young forest and old forest respectively. 302 Significant differences related to land cover at 0-10 cm were found between grasslands 303 304 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 305 306 significant differences were observed between grasslands and young forests and shrubs 307 and old forests. Considering the complete soil profiles, significant differences were observed between grasslands and shrubs, but no differences were found between young 308 309 forest and old forest.

Total N stocks ranged between 9.8 Mg ha<sup>-1</sup> (old forests) to 19.4 Mg ha<sup>-1</sup> (grasslands) (Figure 5b). At 0-10 cm no significant differences were observed. At 10-20 cm significant differences were 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 grasslands sites. Considering the complete soil profile N stocks were significant different at grasslands compare with other land covers (Table 2).

317

# 318 4. Discussion

## 319 *4.1. LULC changes: woody encroachment of subalpine grasslands*

320 Substantial land use changes have occurred from 1956 to 2015 in the study area.321 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 322 323 7% of the total grassland area was changed). The main reasons for these major changes 324 were the decline in livestock and livestock pressure. Similar results were observed in the 325 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 19<sup>th</sup> century, reduced the livestock 326 pressure and contributed to shrub and forest expansion reducing the area occupied by 327 summer grasslands (Urbión Sierra, Spain). However, global warming has also contributed 328 329 to the advance of shrubs and pines in the upper limit of the forest, as do many thermophilic species (Gottfried et al., 2012). 330

331 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 332 carried out recently in different European mountain areas. Literature reviewed indicated 333 334 that forest regrowth is an ongoing process in the Alps (Gehrig-Fasel et al., 2007; 335 Fondevilla et al., 2016; Caviezel et al., 2017) and in the Apennines (Palombo et al., 2013). 336 In the Iberian Range, Sanjuán et al. (2017) reported a marked trend to dense forest and 337 the spatial contraction of shrublands and grasslands. In the Pyrenees, woody encroachment has been reported previously. In the eastern Pyrenees, Batllori and 338 Gutiérrez (2008) reported a densification of forest close to the tree line. In the Central 339 340 Pyrenees, Gartzia et al. (2014) described the expansion of shrubland and forest at the expense of summer grasslands and Sanjuan et al. (2016) reported the expansion of dense 341 342 pine forest and a decline in the area of subalpine grasslands.

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344 *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 Figures 4 and 5). Similar

values were observed in subalpine grasslands in Mediterranean mountains at similar 347 altitudes. Conen et al. (2008) in the Swiss Alps, reported similar Corg concentrations in 348 grasslands: mean values were 58.2 g kg<sup>-1</sup> (at 0-5 cm) and 44.9 g kg<sup>-1</sup> (at 5-10 cm) for 349 mineral soil at 1,795 m. Catoni et al. (2016) in the Ligurian Alps (1,780 m) reported Corg 350 values around 50 g kg<sup>-1</sup> in grasslands areas. Jiménez and Villar (2017) found also similar 351 values in grassland soils of Monte Perdido Massif (Ordesa National Park, Central 352 Pyrenees). About SOC stocks, Sjögersten et al. (2011) and Hunziker et al. (2017) reported 353 354 mean SOC values about 100 Mg C ha<sup>-1</sup> in grassland areas, which is comparable with the results of the present study, all of them suggesting a high capacity of grasslands to store 355 SOC. 356

Our results also confirm our second hypothesis: woody encroachment in the 357 subalpine belt may lead to significant changes in soil properties. Significant changes in 358 359 most of the soil parameters studied were observed in relation to land cover type and soil 360 depth (except for pH). The soil texture ranged from sandy loam to medium loam with 361 significant differences between LULC and depth. CN ratios increased from grasslands to 362 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 363 layers, suggesting that these changes are mainly due to changes in the litter quality (deeper 364 365 soils are less affected by litter quality changes). Similar results were observed in Hooker and Compton (2003). 366

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<sup>-1</sup> and 147.9 Mg C ha<sup>-1</sup>, following approximated order: grasslands > young forest > old forest > shrublands. Total N stocks ranged between 9.8 Mg N ha<sup>-1</sup> and 19.4 Mg N
ha<sup>-1</sup>, 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 legumes 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 377 woody encroachment present a high uncertainty and opposite results can be found in the 378 379 literature (Li et al., 2016; Hunkinzer et al., 2017). Gosheva et al. (2017) concluded that forest expansion on former grasslands is not associated with an increase of SOC 380 381 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). 382 383 Gutiérrez-Girón et al. (2015) reported a significant increases in SOC and total N stocks 384 in the Sierra de Guadarrama (Central Spain). Montané et al. (2007) also showed that 385 woody encroachment did not decrease SOC stocks in grasslands.

386 The low SOC accumulation associated with shrublands may be related to its short 387 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 388 389 encroachment to accumulate SOC, we think that long encroachment periods are needed. 390 Thuille and Schulze (2006), Hiltbrunner et al. (2013) and Van Hall et al. (2017) also 391 observed first a decline followed by an increase of the mineral SOC stock. Huzinker et 392 al. (2017) carried out a chronosequence study in the Alps and concluded that the SOC 393 stocks 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 394 395 of these changes. The loss of grass roots associated with the first stage of woody 396 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 Figure 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 403 404 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. 405 406 Assuming the high variability and uncertainty of soil properties, the most important issue 407 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 408 409 three plots per land use) were selected considering local differences due to slope, 410 exposition and vegetation variability. However, our results present high variability in the 411 analyzed soil properties. This variability was higher in the top layers, decreasing in depth. 412 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).

420

# 421 **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.

428 The following conclusions can be made:

429 1. Significant land use changes have occurred from 1956 to 2015 in the study area:
430 woody encroachment has been observed due to the expansion of coniferous forests
431 and shrublands (at the expense of grasslands)

432 2. The results illustrate that LULC changes from subalpine grassland to shrubs and433 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<sup>-1</sup> to 91.7 Mg C ha<sup>-1</sup>.

437 4. No significant differences with regard to complete SOC stocks were observed
438 between grasslands and young forest, suggesting that long encroachment periods
439 are needed.

440

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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1956 (has)	Dry cultivated fields	Meadows	Subalpine grasslands	Shrublands	<u>Subalpine grasslands</u> - Shrublands	Coniferous	Deciduous species	<u>Coniferous</u> - 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	0.2
Meadows	27.5	312.3	0	1	0	0	0	0	0	340.5	2.1
Subalpine grasslands	0	12.6	4013.2	0.1	0.1	0.3	0	0	0.8	4027.1	25.3
Shrublands	20.6	0	95.6	752.8	0	0	0	0	4.7	873.7	5.5
<u>Subalpine grasslands</u> - Shrublands <u>Coniferous</u>	0	26.6	586.7 441.0	0.1	<i>447.3</i> 165.8	0.1 4335.8	0	0	33.4	1094.1 5281.4	6.9 33.1
Deciduous species	0	0	0	0	0	0	271.2	0	0	271.2	1.7
<u>Coniferous</u> - Deciduous species	0	0	0	0	0	0	0	214.6	0	214.6	1.3
Alpine grasslands and rock outcrops	0	10.9	1.0	18.9	0.2	9.1	0	0	3769.7	3809.8	23.9
Total	83.9	403.1	5137.5	1053.7	613.4	4345.3	271.2	214.6	3825.5	15948.2	100
%	0.5	2.5	32.2	6.6	3.8	27.2	1.7	1.3	24.0		100

Table 1. Transition matrix between LULC classes in 1956 (vertical) and 2015 (horizontal). The diagonal numbers in italics indicate the has of each LULC
 category that did not change

		Corg	Ν	Ctotal	SOC stock	TN stock	C <sub>org</sub> N ratio	SOM	рН	EC	Clay	Sand	Silt	Р	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
	р	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 x	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
soil depth	р	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

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

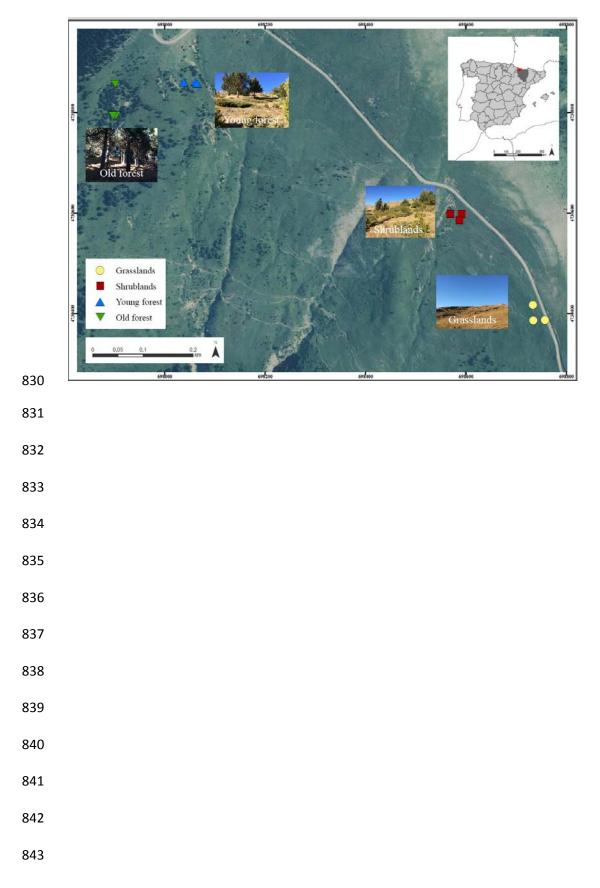
802 C<sub>org</sub>: organic carbon content; N: nitrogen content; Ctotal: carbon content; SOC stock: soil organic carbon stock; TN: nitrogen stocks; SOM: soil organic matter;
 803 EC: electrical conductivity; P: assimilable phosphorus; BD: bulk density;

*p* in italics are significantly different at p < 0.05

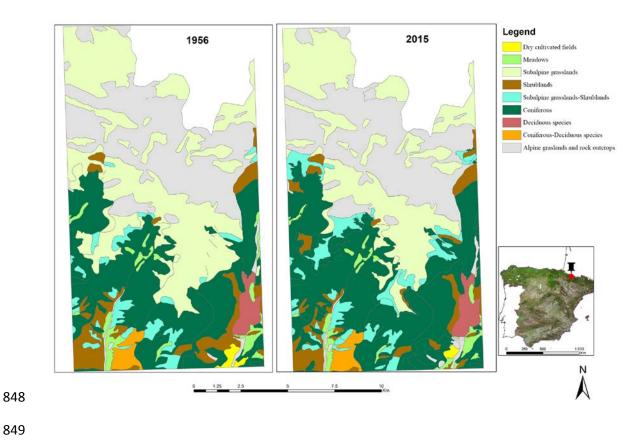
		Grassl	ands			Shr	ubs			Young	forest		Old forest				
	0-10 cm	10-20 cm	20-30 cm	> 30 cm	0-10 cm	10-20 cm	20-30 cm	> 30 cm	0-10 cm	10-20 cm	20-30 cm	> 30 cm	0-10 cm	10-20 cm	20-30 cm	> 30 cm	
C <sub>org</sub>	50.4±15.5	30±10.7	23.4±7.9	18.4±5	25.9±2	18.9±5.9	13.3±1.4	8±0.9	33±5.5	23.4±4	17.5±6	18.3±5.4	31.6±1.3	20.5±2.1	12.9±0.3	10.1±0.6	
(%)	aA	в	<sup>aB</sup>	<sub>aB</sub>	<sub>ba</sub>	в	bcBC	bC	bA	в	<sub>acB</sub>	aB	bA	в	bcc	bD	
N	5.6±1.4	4±1.7	3.3±1.1	2.8±0.7	2.9±0.4	2.5±0.8	1.8±0.2	1.5±0.6	3.6±0.9	2.4±0.2	2±0.	2±0.2	2.4±0.3	1.8±0	1.6±0.2	1.4±0.1	
(%)	aA	aA	a	<sub>aA</sub>	bA	aA	bB	bB	bA	aB	3 <sup>bB</sup>	abB	bA	bB	bв	bBC	
Ctotal	6.3±1.7	4.1±1.7	3.2±1.2	2.5±0.8	3.3±0.3	2.6±0.9	1.7±0.1	1.3±0.5	4.5±1	2.8±0.4	2.1±0.2	2.1±0.2	3.7±0.6	2.3±0.1	1.7±0.2	1.3±0	
(g kg <sup>-1</sup> )	aA	aAB	aB	aB	bA	aA	bB	bB	abA	aB	bB	abB	bA	bB	bBC	bC	
SOC	54.4±11.9	37.4±0.9	30.8±8.9 <sup>aB</sup>	25.4±6	33.8±2.1	25.9±6.8	19.2±1.7	12.2±1.2	40.7±5.3	31.1±4.3	24.3±7	25.2±6.3	39.5±1.2	28±2.3	18.7±0.4	15.1±0.8	
(Mg ha <sup>-1</sup> )	aA	<sub>АВ</sub>		aB	bA	в	bBC	bBC	bA	<sub>АВ</sub>	abB	aB	bA	в	ьс	bD	
TN	6.1±0.1	5.0±1.8	4.4±1.2	3.9±0.8	3.8±0.5	3.3±1	2.6±0.3	2.3±0.8	4.4±0.9	3.2±0.4	2.7±0.3	2.8±0.3	2.9±0.4	2.5±0.1	2.3±0.3	2.1±0.2	
(Mg ha <sup>-1</sup> )		a	a	a	A	aAB	bAB	bB	A	aB	bB	bB	A	bB	bB	bB	
CN ratio	8.8±0.6 aA	7.6±0.6 aB	7±0.5 в	6.5±0.3 aB	9±0.5 aA	7.5±0.1 aB	7.4±0.6 в	5.5±1.4 acC	9.3±0.8 a	9.8±1.8 a	8.7±1.7	9.2±2.8 ab	13.6±1.4 bA	11.3±1.1 bB	8.3±1 c	7.1±0.9 aC	
SOM	8.7±2.7	5.2±1.8	4±1.4	3.2±0.9	4.5±0.3	3.3±1	2.3±0.2	1.4±0.2	5.7±1	4±0.7	3±1	3.2±0.9	5.5±0.2	3.5±0.4	2.2±0.1	1.8±0.1	
(%)	aA	BC	aBC	aB	bA	в	bBC	ьс	bA	Ав	abB	acB	bA	в	bC	bD	
pH	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	121.9±50.6	61.1±13.7	45.3±3	43.3±2	66.8±20.8	40.3±9.7	31.8±6.6	18±2.1	88.4±50.2	37.2±17.9	29.1±9.2	25±6.1	47.2±7.8	37.5±4.4	25±7.4	18.2±0.7	
(µS cm <sup>-1</sup> )	aA	в	aB	aB	aA	вс	abBC	ьв	aA	в	bB	cB	bA	<sub>АВ</sub>	bBC	bBC	
Clay	3.4±2	6.4±2.1	12.4±4.7	15.5±1.5 <sup>B</sup>	10±1.6	12.5±2.3	13.9±0.8	15.2±2.7	9.7±2.2	15.1±2.3	16.5±2.7	17.5±2.7	10.5±1.4	15.1±0.9	22±3	21.7±6.4	
(%)	aA	a	aB		bA	bAB	aB	в	bA	bB	aB	в	bA	bA	bВ	в	
Sand	73.1±5.4	63.5±3.1	48.1±4.2	40.2±3.1	59.7±4	57.5±4.2	53.6±5.8	50.2±4.8	60.2±4	48.5±4.5	44.4±3	41.9±3.6	58.9±2	44.2±2	34.7±3.5	31.9±9.3	
(%)	aA	aB	aC	aD	ь	a	a	ab	bA	bB	acB	aB	bA	bB	bBC	acC	
Silt	23.5±3.4	30.1±1.2	39.5±0.5	44.3±2.3	30.3±2.4	30±2	32.5±5.2	34.6±2	30.1±2.1	36.4±2.3	39.1±1	40.6±0.8	30.6±0.8	40.7±2.2	43.3±1	46.4±3	
(%)	aA	aB	aC	aD	b	a	b	b	bA	св	aBC	aC	bA	bB	<sub>aB</sub>	aC	
P ( mg kg <sup>-1</sup> )	106.2±22.4	86.1±22.4	93.1±28.3	95.8±32.2	64.7±8.7 bA	84.2±9.3 в	85±10.8 в	90.8±3.3 c	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	1.1±0.1	1.3±0.1	1.3±0.1	1.4±0.1	1.3±0	1.4±0.1	1.5±0	1.5±0	1.2±0.1	1.3±0	1.4±0.1	1.4±0.1	1.3±0	1.4±0	1.5±0	1.5±0	
( g cm <sup>-3</sup> )	aA	AB	aB	aB	bA	bB	cBC	cC	aA	bA	cB	cB	bA	в	ьс	bD	

- Table 3. 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 conditions.
- 814 Note:
- 815 Means with the different lower case letter superscripts within a row are significantly different at 0.05 level of significance (p < 0.05)
- 816 Means with the different upper case letter superscripts within a column are significantly different at 0.05 level of significance (p < 0.05)
- 817 C<sub>org</sub>: organic carbon; C<sub>inorg</sub>: inorganic carbon; S N: nitrogen content; OC: soil organic carbon stock; TN: nitrogen stocks; OM: organic matter; EC: electrical
   818 conductivity; CaCO<sub>3</sub>: carbonate content; P: organic phosphorus; BD: bulk density; FC: field capacity.

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



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



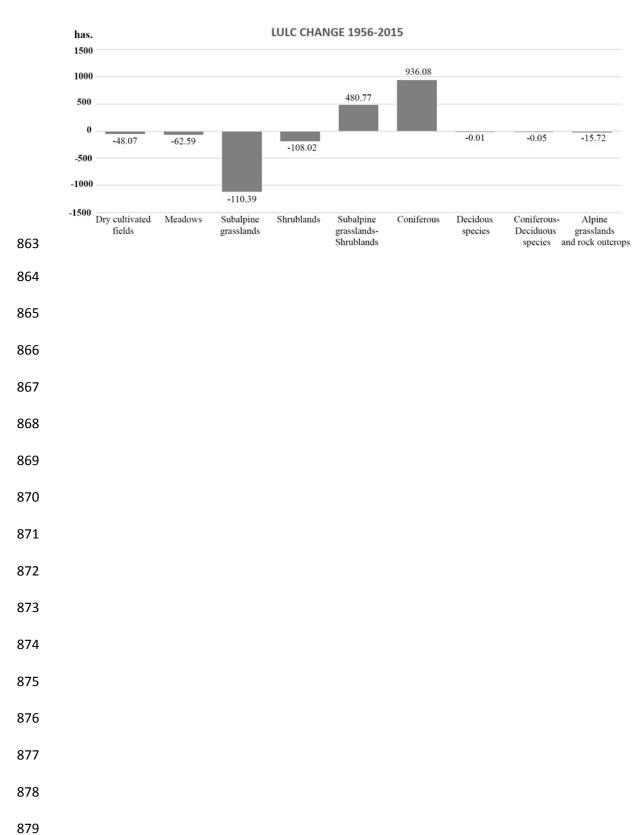
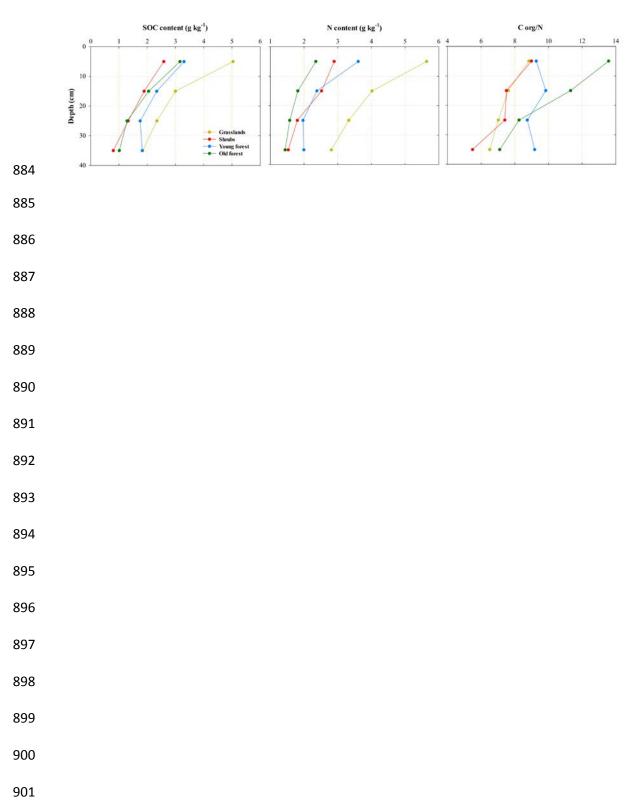
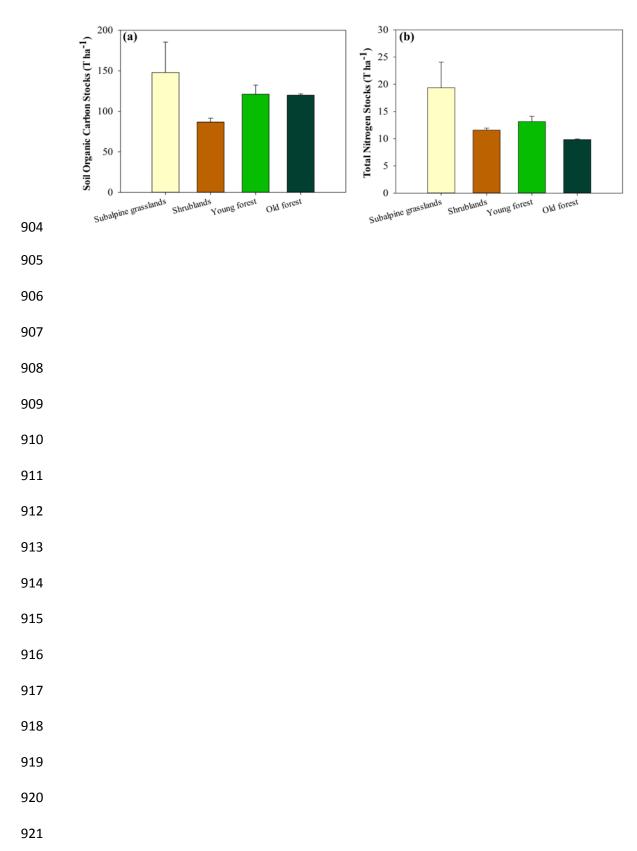


Figure 3. LULC changes between 1956 and 2015

- 880 Figure 4. Soil organic carbon (SOC) and nitrogen (N) contents and Corg/N ratios in the
- different land covers and depths.
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902 Figure 5. Soil organic carbon (SOC) and nitrogen (N) stocks in the different land covers.

