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2 **Woody encroachment and soil carbon stocks in subalpine areas in the Central** 3 **Spanish Pyrenees**

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14

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

27 (i) woody encroachment has occurred from 1956 to 2015 due to the expansion of
28 coniferous forests and shrublands (at the expense of grasslands), (ii) land cover and soil
29 depth had significant effects on soil properties (except for pH), being larger in the
30 uppermost 0-10 cm depth, (iii) SOC and N contents and stocks were higher in the
31 grasslands sites, and (iv) the woody encroachment process initially produced a decrease
32 in the SOC stocks (shrublands), but no differences were observed considering the
33 complete soil profile between grasslands and young and old forests. Further studies,
34 describing SOC stabilization and quantifying above-ground carbon (shrub and tree
35 biomass) are required.

36

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

39

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
43 vulnerability to Climate Change (Giorgi, 2006; Nogués Bravo et al., 2008; López-Moreno
44 et al., 2017) and to the abandonment of agricultural and pastoral activities since the mid-
45 twentieth century (MacDonald et al., 2000; Strijker, 2005; Hatna and Bakker, 2011;
46 Lasanta et al., 2017). One of the consequences is the transformation of many fine-grained
47 cultural landscapes into homogeneous coarse-grained landscapes (Van Eetvelde and
48 Antrop, 2003; Lasanta et al., 2005) with socioeconomic and environmental effects on the
49 local population and nearby areas (Mottet et al., 2006; Gellrich et al., 2007; Bernués et
50 al., 2014).

51 In Mediterranean mountains, grassland ecosystems are extremely important as
52 producers of many goods and services for society (provisioning, regulation, support,
53 culture) and as contributors to the support of a rich biodiversity (Millennium Ecosystem
54 Assessment, 2005; Raudsepp-Hearne et al., 2010; Bernués et al., 2014). Grasslands
55 provide forage for large wild herbivores and for livestock, so they play a prominent role
56 in the mountain economy and even in many country economy's (Villagrasa et al., 2015).
57 It should not be forgotten that livestock farming contributes directly to food security and
58 provides livelihood to almost one billion people in the world (FAO, 2009). Grasslands
59 also play an important regulatory role; they act as firewalls in areas where the rural
60 abandonment favors the expansion of woody communities, which increase the risk of
61 fires (i.e., Lloret et al., 2002; Beilin et al., 2014). In addition, grasslands regulate the
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
64 al., 2011; López-Moreno et al., 2011; Nadal-Romero et al., 2013). The support services
65 provided by grasslands are based on their high plant diversity, with species adapted to
66 extreme conditions or at its distribution limit, which increases the number of endemism
67 and provides to the grasslands a high ecological value (Canals and Sebastiá, 2000).
68 Consequently, the loss of grasslands reduces plant and faunal biodiversity (Ratajczak et
69 al., 2012; Canals et al., 2014). In addition, grasslands, that were created, used and
70 maintained by men for centuries or millennia, give rise to a cultural landscape of high
71 aesthetic value, offering cultural services such as recreational (tourism), educational and
72 spiritual services (Lamarque et al., 2014) and contributing to the well-being of rural areas
73 (Hejcman et al., 2013; Huber et al., 2013). On the other hand, grasslands are an important
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).

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

82 In the Central Spanish Pyrenees, the semi-natural grasslands reach in altitude to
83 approximately 2,400 m a.s.l., coinciding with the upper limit of the subalpine belt and the
84 maximum level for the development of shrubland, while *Pinus uncinata* would reach up
85 to 2,200 m a.s.l. (García-Ruiz et al., 1990; Badía and Fillat, 2008). These grasslands were
86 created by human fires from the mid-Holocene, but more actively from the Bronze Age
87 to the Middle Ages, when much of the subalpine belt was deforested to graze transhumant
88 livestock (Bal et al., 2011; Cunil, 2012; Pérez-Sanz et al., 2003). However, since the
89 middle of the 20th century, subalpine grasslands have undergone a major transformation
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
92 were less adapted to the environment (García-Ruiz and Lasanta, 1990). The abandonment
93 of grassland activities has favored the expansion of woody species into grasslands
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
96 2,000 m a.s.l. have been woody-encroached, in the Central Spanish Pyrenees, while 35%
97 the shrubs have been populated with trees. These changes are exacerbated by climatic
98 warming, which increase the temperatures and reduces the snow cover and snowfall
99 period (Batllori and Gutiérrez, 2008; López-Moreno et al., 2017) causing a cascading
100 effect on ecosystem processes, accelerating the invasion of grasslands by high

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

103 So far, the process of woody encroachment in subalpine grasslands has been studied
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
107 (traditional management, conservation policies as suppressing fires or grazing practices,
108 the creation of plantations) (Bartolomé et al., 2008), (iii) the negative effects on
109 biodiversity (Komac et al., 2011b) and on the diversity of grasslands due to the invasive
110 effect of *Brachypodium pinnatum* (Canals et al., 2014) or *Erica scoparia* (L.) (Bartolomé
111 et al., 2005a), (iv) the changes in the biomass and greenery of grasslands as a consequence
112 of the woody encroachment and Climate Change (Gartzia et al., 2016a), (vi) the loss of
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
117 well (Canals et al., 2014; Armas-Herrera et al., 2016; San Emeterio et al., 2016).

118 However, the effects of woody encroachment on soil properties and soil organic
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
121 organic carbon is an indicator of soil quality associated with aggregate stability, with
122 direct implications for water infiltration and soil erosion, as well as for biodiversity, and
123 plant and fauna development. It is known, that one of the main ecosystem effects of
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

126 carbon-binding capacity (Ritchie, 2014; Rutledge et al., 2014; Aldezábal et al., 2015),
127 and may be even more important than forests in the "generation of carbon credits"
128 (Albrecht and Kandji, 2003). Recently, the influence of land use changes in soil carbon
129 reserves has been studied due to the differences in carbon sequestration depending on
130 land uses and land covers (Post and Kwon, 2006; Guo and Gifford, 2002; García-Pausas
131 et al., 2011).

132 The main objective of this study is to analyze soil properties and soil organic carbon
133 stocks in different land uses in the subalpine belt in a small sector of the Central Spanish
134 Pyrenees (Borau Valley). The specific objectives were: (i) to determine land use changes
135 in the study area and quantify the grassland lost during the last 60 years, and (ii) to assess
136 the effect of woody encroachment on soil properties and SOC dynamics. This leads to the
137 following research hypotheses: (i) subalpine grasslands contain high soil organic carbon
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
142 land use changes, is necessary to take effective measures to ensure the flow of the multiple
143 goods and services that these ecosystems provide to society.

144

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 km², 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

151 the last century. During the 20th century, the Borau Valley lost 82% of its population,
152 going from 394 inhabitants in 1900 to 74 inhabitants in 2001. During the same period,
153 90% of the agricultural area was abandoned, decreasing from 1,794 ha to 168.8 ha
154 between 1974 and 2000, and 75% of the livestock was lost (Vicente-Serrano, 2001).

155 The Borau Valley presents a great lithological homogeneity, with a predominance
156 of intensely folded calcareous marls and sandstones, typical for flysch formations.
157 Geomorphological processes have historically been very active, due to both the
158 lithological plasticity and the strong anthropization of the area: massive deforestation and
159 massive ploughings to establish crops and to feed the livestock population in summer in
160 high altitudes (more than 21,000 sheep in the 19th century, according to Vicente-Serrano,
161 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.

168 Chauvelier (1987) distinguished two well-differentiated ecosystems in the Central
169 Pyrenees: between 840 and 1,800 m, a forest ecosystem appears on the footslope hills and
170 montane belts. From 1,700-1,800 m a supraforest ecosystem is developed which
171 corresponds to the subalpine vegetation. Both vegetation systems were greatly affected
172 by human action, which resulted in the lowering of the upper forest level, and the
173 deforestation of the slopes at low altitudes for cultivation. These interventions led the
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),

176 while most of the territory was occupied by crops in the montane belt and by grasslands
177 in the subalpine belt. Therefore, natural forests occupy only small areas. In the montane
178 belt now woody encroachment processes prevail (typical species are *Echinopartum*
179 *horridum*, *Genista scorpius*, *Buxus sempervirens*, *Juniperus communis* and *Crataegus*
180 *monogyna*). Above 1,700-1,800 m (subalpine belt) small gloves of *Pinus uncinata* appear
181 and great extensions of subalpine grasslands predominate, occupying an area that was
182 climatically forestry, but that the human action turned into grasslands by means of the
183 clearings and the fires (García-Ruiz et al., 2015). The grasslands located at lower altitudes
184 (1700-1850 m) correspond to the *Mesobromion erecti* community (*Eryngio-*
185 *Plantaginetum mediae* association), which is a very productive grassland, much
186 appreciated by livestock. At higher altitudes there is a transition to the *Nardium strictae*
187 community (*Alchemillo-Nardetum strictae* association) and above this area a transition to
188 *Festucio eskiae* (*Carici-Festucetum Eskiae* association), with a progressive loss of
189 pasture quality (Remón Aldabe, 1997).

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.

194

195 2.2. Land use change analyses

196 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 then a second map from
200 1956 was created. Two maps were generated, including the following LULC classes: (i)

201 dry cultivated fields, (ii) meadows, (iii) subalpine grasslands, (iv) shrublands, (v)
202 subalpine grasslands-shrublands, (vi) coniferous, (vii) deciduous species, and (viii) alpine
203 grasslands and rock outcrops. The LULC maps were overlain to assess changes that
204 occurred over the period of study. All these analyses were done using ArcGIS 10.3.
205 Measurement of the areas where changes or no changes had occurred enabled
206 computation of a LULC transition matrix and the Kappa index, and which were calculated
207 using Idrisi (Selva, Clark University, 17, January 2012).

208

209 2.3. *Experimental design and sampling*

210 Field survey techniques, aerial photograph interpretation and LULC maps were
211 used to select four different land covers at 1,800 m (see Figure 1): (i) grasslands (mainly
212 cover by *Nardion Strictae*) representing current grazing areas (control areas) to describe
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 *Echinopartum Horridum* and *J. Communis*, and
217 (iv) old forest, representing the coniferous forest that already existed in 1956. For each of
218 the four land covers, three plots were selected, all with similar topographic conditions
219 (altitude, slope and exposition).

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

226

227 *2.4. Laboratory analysis and soil characterization*

228 Soil samples were air dried and passed through a 2 mm mesh sieve in the laboratory.
229 Soil pH and electrical conductivity (EC) were measured in a deionized water – soil
230 suspension (1:2.5). Soil texture was determined using a particle size analyzer (Mastersizer
231 2000). Soil organic matter (SOM) was determined using the Walkley-Black method.
232 Total carbon (C_{total}) and total nitrogen (N) were determined by dry combustion (Vario
233 Max). Carbonate concentration (CaCO₃) was determined using the Bernard calcimeter
234 method, although low values were found so this variable was discarded. Consequently
235 soil organic carbon (SOC) was calculated using the van Bemmelen factor of 0.58, using
236 as universal conversion factor. Bulk density values were estimated from undisturbed
237 cores and pedotransfer equations (proposed by Guo and Gifford (2002) and Post and
238 Kwon (2000)). SOC and N contents were expressed in g km⁻¹ soil, while SOC and N
239 stocks were expressed in Mg ha⁻¹ (calculated by incorporating their respective depth and
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
242 in detail in Nadal-Romero et al. (2016).

243

244 *2.5. Statistical analyses*

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

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

252

253 **3. Results**

254 *3.1. Land use changes*

255 Figures 2 and 3 show the spatial distribution of the LULC in 1956 and 2015. By
256 1956, the main LULCs correspond to subalpine grasslands (32.1%) and coniferous
257 (27.2%). Substantial changes have occurred by 2015 (Table 1):

258 (1) Coniferous was in 2015 the most extensive land cover (33%), and grasslands
259 were the second largest LULC (25.2%), but they have been marked spatial
260 shrinkage (1110 ha).

261 (2) The expansion of coniferous forest at the expense of grasslands (441 ha) was
262 one of the main changes that occurred in this period, together with the change from
263 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).

267 (4) Woody encroachment due to the expansion of shrubland is also observed due to
268 changes from subalpine grasslands to shrublands (96 ha) and to grasslands-
269 shrublands (587 ha).

270

271 *3.2. Soil properties and soil carbon stocks*

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

274 appear for all properties except for pH, and P content presented only significant
275 differences related to land cover (Table 2).

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

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

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

289 The highest Corg concentrations were obtained in the grassland sites (at 0-10 cm)
290 and the lowest values in the shrub areas (Figure 4). At 0-10 cm significant differences
291 were observed between grasslands and the different land covers. No differences were
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).

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

300 Total SOC stocks ranged between 91.7 Mg ha⁻¹ (shrubs) to 147.9 Mg ha⁻¹
301 (grasslands) (Figure 5a). The percentage of SOC in the first 10 cm averaged 36.8, 38.7,
302 35.7 and 41.1 % for grassland, shrubs, young forest and old forest respectively.
303 Significant differences related to land cover at 0-10 cm were found between grasslands
304 and the different land covers. At 10-20 cm no significant differences were observed.
305 Below 20 cm no differences were found between grasslands and young forests, and
306 significant differences were observed between grasslands and young forests and shrubs
307 and old forests. Considering the complete soil profiles, significant differences were
308 observed between grasslands and shrubs, but no differences were found between young
309 forest and old forest.

310 Total N stocks ranged between 9.8 Mg ha⁻¹ (old forests) to 19.4 Mg ha⁻¹ (grasslands)
311 (Figure 5b). At 0-10 cm no significant differences were observed. At 10-20 cm significant
312 differences were observed between old forests and the other land covers. Below 20 cm
313 significant differences were observed between grasslands and the other land cover types.
314 No significant differences were observed related to depth at grasslands sites. Considering
315 the complete soil profile N stocks were significant different at grasslands compare with
316 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

322 shrublands (at the expense of grasslands). A reduction of grasslands has occurred (about
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
326 crisis of the transhumance, since the beginning of the 19th century, reduced the livestock
327 pressure and contributed to shrub and forest expansion reducing the area occupied by
328 summer grasslands (Urbión Sierra, Spain). However, global warming has also contributed
329 to the advance of shrubs and pines in the upper limit of the forest, as do many thermophilic
330 species (Gottfried et al., 2012).

331 Shrub expansion is a worldwide phenomenon (Brandt et al., 2013; Matson and Bart,
332 2013; Ratajczak et al., 2012; Xie and Sha, 2012). Our results agree with several studies
333 carried out recently in different European mountain areas. Literature reviewed indicated
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
338 encroachment has been reported previously. In the eastern Pyrenees, Batllori and
339 Gutiérrez (2008) reported a densification of forest close to the tree line. In the Central
340 Pyrenees, Gartzia et al. (2014) described the expansion of shrubland and forest at the
341 expense of summer grasslands and Sanjuan et al. (2016) reported the expansion of dense
342 pine forest and a decline in the area of subalpine grasslands.

343

344 *4.2. Subalpine grasslands: consequences of woody encroachment on soil properties*

345 Our initial results support our first hypothesis that subalpine grasslands contain
346 significantly higher soil organic carbon contents and stocks (see Figures 4 and 5). Similar

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

357 Our results also confirm our second hypothesis: woody encroachment in the
358 subalpine belt may lead to significant changes in soil properties. Significant changes in
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
363 recorded in the upper soil layers, and no differences were observed in the deeper soil
364 layers, suggesting that these changes are mainly due to changes in the litter quality (deeper
365 soils are less affected by litter quality changes). Similar results were observed in Hooker
366 and Compton (2003).

367 The SOC and N concentrations were higher in the mineral soil of grasslands if
368 compared with other uses. Similar findings were also observed in comparable studies by
369 Guidi et al. (2014). Our results revealed that both SOC and N stocks were also affected
370 by LULC changes. Total SOC stocks (as the sum of all soil layers) ranged between 86.8
371 Mg C ha⁻¹ and 147.9 Mg C ha⁻¹, following approximated order: grasslands > young forest

372 > old forest > shrublands. Total N stocks ranged between 9.8 Mg N ha⁻¹ and 19.4 Mg N
373 ha⁻¹, following approximated order: grasslands > young forest > shrublands > old forest.
374 N content and stock is related to the herbaceous vegetation that usually proliferates on
375 grasslands, with legumes plants with a high N-fixing capacity, which increase soil N
376 content (Hooper and Vitousek, 1998).

377 Changes in the magnitude and direction of soil properties and SOC stocks after
378 woody encroachment present a high uncertainty and opposite results can be found in the
379 literature (Li et al., 2016; Hunkinzer et al., 2017). Gosheva et al. (2017) concluded that
380 forest expansion on former grasslands is not associated with an increase of SOC
381 sequestration and their results reported a decrease of the SOC values. Other studies
382 reported an increase in SOC stocks related to shrublands expansion (Li et al., 2016).
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
388 between grasslands and young and old forest. So, despite the potential capacity of shrub
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
394 stocks after 90 years was observed. Changes in root dynamics can be partly responsible
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

397 been tested by different authors, indicating that grass roots disappear at very high woody
398 densities (see Figure 1) (Jackson et al., 2002; Coetsee et al., 2013). Hudak et al. (2003)
399 suggested the loss of grass roots as a possible mechanism of declining soil Corg
400 concentration at sites where a close canopy prohibited the growth of grass. These changes
401 in root sizes can also change aggregate size fractions and aggregate stabilization affecting
402 to SOC stocks (Guidi et al., 2014).

403 Several investigations studied the spatial variability of soil properties using
404 different spatial and statistical methods (i.e. Loescher et al., 2014; Bogunovic et al.,
405 2017). Soils are highly variable, making designing sampling strategies a challenging task.
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
408 area (at least as closely as possible). Our 36 soil sampling-plots (randomly collected in
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.

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

420

421 **5. Conclusions**

422 This study has provided novel information on the effects of woody encroachment
423 of subalpine grassland on soil properties and SOC stocks in a mountain Mediterranean
424 area. Our results support our hypothesis that subalpine grasslands contain high soil
425 organic carbon and that woody encroachment in the subalpine belt may lead to significant
426 changes in soil properties. However, contrary to our hypothesis woody encroachment did
427 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 and
433 conifer forests substantially affects the soil system.
- 434 3. During the woody encroachment process in the subalpine belt of the Central
435 Spanish Pyrenees the SOC stock in the mineral phases initially decreases from
436 $147.9 \text{ Mg C ha}^{-1}$ to $91.7 \text{ Mg C ha}^{-1}$.
- 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

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

447

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455

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1956 (has)	<u>Dry cultivated fields</u>	<u>Meadows</u>	<u>Subalpine grasslands</u>	<u>Shrublands</u>	<u>Subalpine grasslands- Shrublands</u>	<u>Coniferous</u>	<u>Deciduous species</u>	<u>Coniferous - Deciduous species</u>	<u>Alpine grasslands and rock outcrops</u>	Total	%
2015 (has)											
<u>Dry cultivated fields</u>	<i>35.8</i>	0	0	0	0	0	0	0	0	35.8	0.2
<u>Meadows</u>	27.5	<i>312.3</i>	0	1	0	0	0	0	0	340.5	2.1
<u>Subalpine grasslands</u>	0	12.6	<i>4013.2</i>	0.1	0.1	0.3	0	0	0.8	4027.1	25.3
<u>Shrublands</u>	20.6	0	95.6	<i>752.8</i>	0	0	0	0	4.7	873.7	5.5
<u>Subalpine grasslands- Shrublands</u>	0	26.6	586.7	0.1	<i>447.3</i>	0.1	0	0	33.4	1094.1	6.9
<u>Coniferous</u>	0	40.7	441.0	281.2	165.8	<i>4335.8</i>	0	0	16.9	5281.4	33.1
<u>Deciduous species</u>	0	0	0	0	0	0	<i>271.2</i>	0	0	271.2	1.7
<u>Coniferous - Deciduous species</u>	0	0	0	0	0	0	0	<i>214.6</i>	0	214.6	1.3
<u>Alpine grasslands and rock outcrops</u>	0	10.9	1.0	18.9	0.2	9.1	0	0	<i>3769.7</i>	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

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

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		C_{org}	N	C_{total}	SOC stock	TN stock	C_{org}:N ratio	SOM	pH	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
	p	<i>0.000</i>	<i>0.000</i>	<i>0.000</i>	<i>0.000</i>	<i>0.000</i>	<i>0.000</i>	<i>0.000</i>	0.247	<i>0.000</i>	<i>0.000</i>	<i>0.000</i>	<i>0.000</i>	<i>0.007</i>	<i>0.000</i>
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	<i>0.000</i>	<i>0.000</i>	<i>0.000</i>	<i>0.000</i>	0.000	0.000	<i>0.000</i>	0.831	<i>0.000</i>	<i>0.000</i>	<i>0.000</i>	<i>0.000</i>	0.397	<i>0.000</i>
Land cover x 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	<i>0.008</i>	0.505	1	0.774	0.267	<i>0.003</i>	<i>0.000</i>	0.883	0.782

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800 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
801 where the differences occurred between groups.

802 C_{org}: organic carbon content; N: nitrogen content; C_{total}: 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;

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

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	Grasslands				Shrubs				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 _{org} (%)	50.4±15.5 aA	30±10.7 B	23.4±7.9 aB	18.4±5 aB	25.9±2 bA	18.9±5.9 B	13.3±1.4 bcBC	8±0.9 bc	33±5.5 bA	23.4±4 B	17.5±6 acB	18.3±5.4 aB	31.6±1.3 bA	20.5±2.1 B	12.9±0.3 bcc	10.1±0.6 bd
N (%)	5.6±1.4 aA	4±1.7 aA	3.3±1.1 a	2.8±0.7 aA	2.9±0.4 bA	2.5±0.8 aA	1.8±0.2 bB	1.5±0.6 bB	3.6±0.9 bA	2.4±0.2 aB	2±0. 3 ^{bb}	2±0.2 abb	2.4±0.3 bA	1.8±0 bB	1.6±0.2 bB	1.4±0.1 bBC
C _{total} (g kg ⁻¹)	6.3±1.7 aA	4.1±1.7 aAB	3.2±1.2 aB	2.5±0.8 aB	3.3±0.3 bA	2.6±0.9 aA	1.7±0.1 bB	1.3±0.5 bB	4.5±1 abA	2.8±0.4 aB	2.1±0.2 bB	2.1±0.2 abB	3.7±0.6 bA	2.3±0.1 bB	1.7±0.2 bBC	1.3±0 bc
SOC (Mg ha ⁻¹)	54.4±11.9 aA	37.4±0.9 AB	30.8±8.9 ^{aB}	25.4±6 aB	33.8±2.1 bA	25.9±6.8 B	19.2±1.7 bBC	12.2±1.2 bBC	40.7±5.3 bA	31.1±4.3 AB	24.3±7 abB	25.2±6.3 aB	39.5±1.2 bA	28±2.3 B	18.7±0.4 bc	15.1±0.8 bd
TN (Mg ha ⁻¹)	6.1±0.1	5.0±1.8 a	4.4±1.2 a	3.9±0.8 a	3.8±0.5 A	3.3±1 aAB	2.6±0.3 bAB	2.3±0.8 bB	4.4±0.9 A	3.2±0.4 aB	2.7±0.3 bB	2.8±0.3 bB	2.9±0.4 A	2.5±0.1 bB	2.3±0.3 bB	2.1±0.2 bB
CN ratio	8.8±0.6 aA	7.6±0.6 aB	7±0.5 B	6.5±0.3 aB	9±0.5 aA	7.5±0.1 aB	7.4±0.6 B	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 aA	5.2±1.8 BC	4±1.4 aBC	3.2±0.9 aB	4.5±0.3 bA	3.3±1 B	2.3±0.2 bBC	1.4±0.2 bc	5.7±1 bA	4±0.7 AB	3±1 abB	3.2±0.9 acB	5.5±0.2 bA	3.5±0.4 B	2.2±0.1 bc	1.8±0.1 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 (μS cm ⁻¹)	121.9±50.6 aA	61.1±13.7 B	45.3±3 aB	43.3±2 aB	66.8±20.8 aA	40.3±9.7 BC	31.8±6.6 abBC	18±2.1 bB	88.4±50.2 aA	37.2±17.9 B	29.1±9.2 bB	25±6.1 cB	47.2±7.8 bA	37.5±4.4 AB	25±7.4 bBC	18.2±0.7 bBC
Clay (%)	3.4±2 aA	6.4±2.1 a	12.4±4.7 aB	15.5±1.5 ^B	10±1.6 bA	12.5±2.3 bAB	13.9±0.8 aB	15.2±2.7 B	9.7±2.2 bA	15.1±2.3 bB	16.5±2.7 aB	17.5±2.7 B	10.5±1.4 bA	15.1±0.9 bA	22±3 bB	21.7±6.4 B
Sand (%)	73.1±5.4 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±4 bA	48.5±4.5 bB	44.4±3 acB	41.9±3.6 aB	58.9±2 bA	44.2±2 bB	34.7±3.5 bBC	31.9±9.3 acC
Silt (%)	23.5±3.4 aA	30.1±1.2 aB	39.5±0.5 aC	44.3±2.3 aD	30.3±2.4 b	30±2 a	32.5±5.2 b	34.6±2 b	30.1±2.1 bA	36.4±2.3 cB	39.1±1 aBC	40.6±0.8 aC	30.6±0.8 bA	40.7±2.2 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±8.7 bA	84.2±9.3 B	85±10.8 B	90.8±3.3 C	84.1±31.2 ab	92.9±12.7	94.6±6.4	110.4±5.5 b	56.4±18.9 b	67.8±19	60.6±24.6	78.1±43.9
BD (g cm ⁻³)	1.1±0.1 aA	1.3±0.1 AB	1.3±0.1 aB	1.4±0.1 aB	1.3±0 bA	1.4±0.1 bB	1.5±0 cBC	1.5±0 cC	1.2±0.1 aA	1.3±0 bA	1.4±0.1 cB	1.4±0.1 cB	1.3±0 bA	1.4±0 B	1.5±0 bc	1.5±0 bd

812 Table 3. Mean and standard deviations of the studied soil properties. Soil characteristics resulting from former land covers, revegetation processes (secondary
813 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_{org}: organic carbon; C_{inorg}: inorganic carbon; S N: nitrogen content; OC: soil organic carbon stock; TN: nitrogen stocks; OM: organic matter; EC: electrical
818 conductivity; CaCO₃: carbonate content; P: organic phosphorus; BD: bulk density; FC: field capacity.

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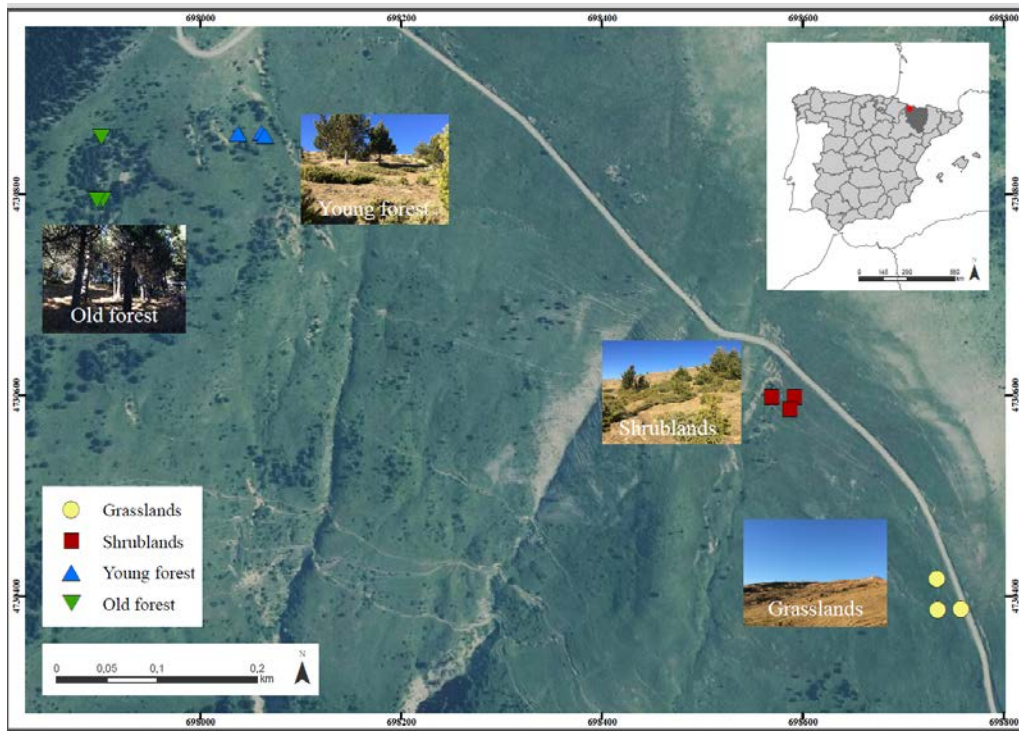
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828 Figure 1. Location of sampling points and the study area (Central Spanish Pyrenees)

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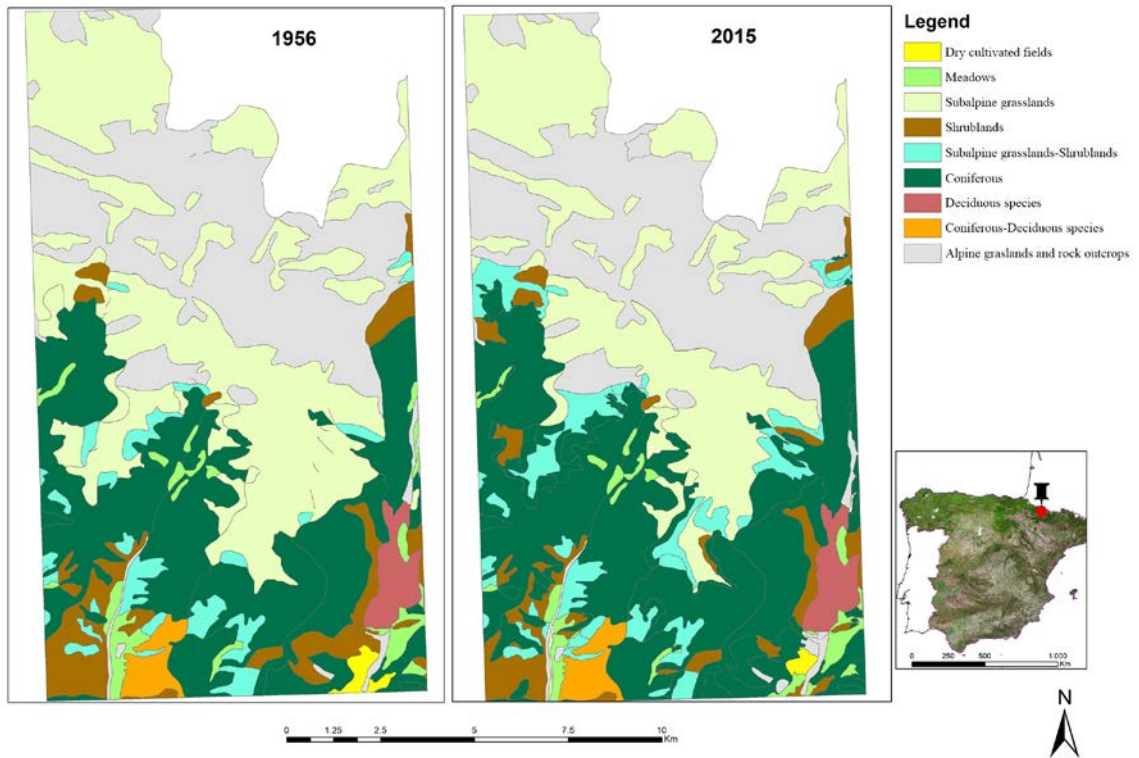
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845 Figure 2. Maps with LULC categories in the Borau Valley in 1956 and 2015 and location

846 of the study area in Spain

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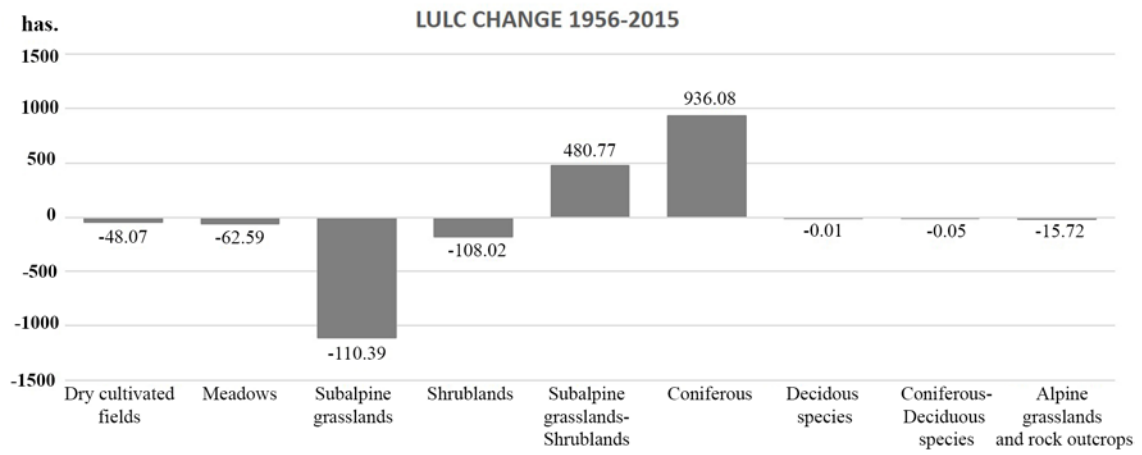
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860 Figure 3. LULC changes between 1956 and 2015

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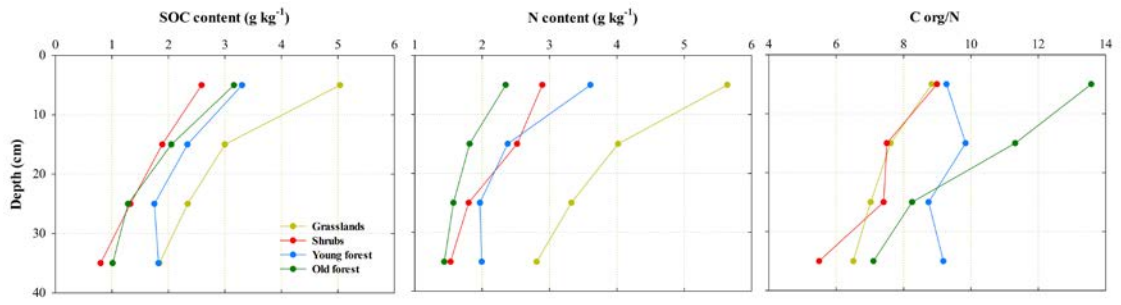
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880 Figure 4. Soil organic carbon (SOC) and nitrogen (N) contents and Corg/N ratios in the
881 different land covers and depths.

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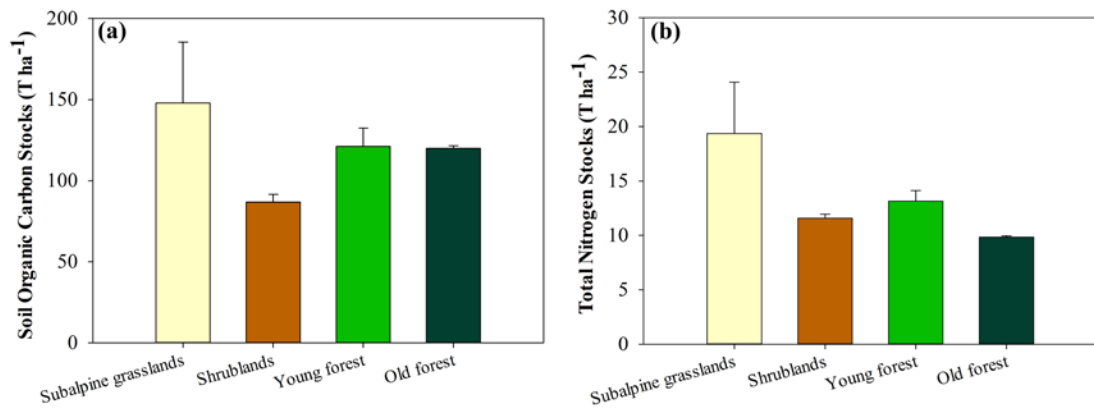
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902 Figure 5. Soil organic carbon (SOC) and nitrogen (N) stocks in the different land covers.

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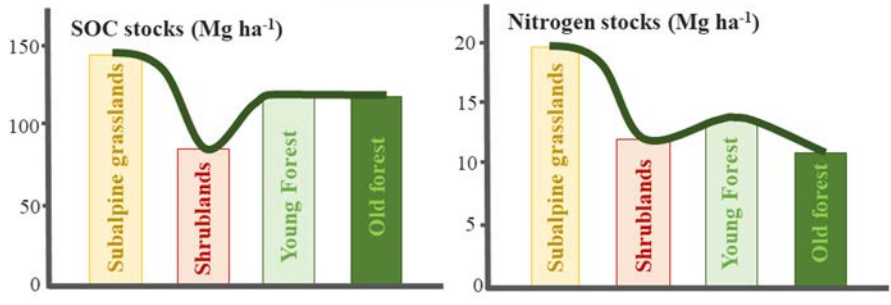
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