



# Fir expansion not controlled by moderate densities of large herbivores: a Mediterranean mountain grassland conservation issue

Sandrine Chauchard<sup>1</sup> · Christopher Carcaillet<sup>2,3</sup> · Frédéric Guibal<sup>4</sup>

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## Abstract

• **Key message** Whilst livestock has maintained grasslands over centuries in south European mountains, current ungulate densities are insufficient to control tree and forest expansion effectively. The present results based on fir population dynamics and radial/height growth raise questions about the sustainable management of new forests that are former mountain grasslands. This empirical case study contributes to debate about methods for long-term management of grasslands in Mediterranean mountains.

• **Context** Forest expansion following the cessation of grazing is a threat to biodiversity in mountain grasslands that are components of Mediterranean cultural landscapes.

• **Aims** We hypothesised that ungulates could mitigate tree recruitment, thus conserving mountain grasslands. We tested the efficiency of grazing and browsing by domestic and wild ungulates (sheep, roe and red deer) at inhibiting silver fir (*Abies alba* Mill.) encroachment in a conservation area, one of the main browsed trees.

• **Methods** The fir trees' age structure was analysed by dendrochronology, and the individual growth patterns and scars were recorded in their tree-ring series.

• **Results** Fir density has increased since the 1960s, simultaneously with the increase in ungulate populations. The scar occurrences only appeared during the 1970s. Most scars were observed on seedlings and small saplings. The impacts of ungulates are observed on the radial and height growth of trees, mostly on trees < 100 cm, but it did not however result in controlling the recruitment of fir.

• **Conclusion** The hypothesis that a moderate density of ungulates might inhibit tree encroachment is therefore rejected, even in the case of a highly palatable species such as silver fir. Ungulates, solely, would not be an appropriate policy for grassland conservation in Mediterranean mountains.

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**Contribution of the co-authors** SC, CC, and FG designed the study and sampled the material; SC made the lab measurements, collected and analysed the data; SC wrote the first draft of the manuscript, CC and FG contributed to the writing.

✉ Sandrine Chauchard  
sandrine.chauchard@univ-lorraine.fr

<sup>1</sup> Université de Lorraine, AgroParisTech, INRA, UMR Silva, 54000 Nancy, France

<sup>2</sup> Ecole Pratique des Hautes Etudes (EPHE), PSL University Paris, Paris, France

<sup>3</sup> Laboratoire d'Ecologie des Hydrosystèmes Naturels et Anthropisés (UMR5023 CNRS, ENTPE), Université Claude Bernard Lyon, 69622 Villeurbanne, France

<sup>4</sup> IMBE, CNRS, Aix Marseille Univ, Univ. Avignon, IRD, Europôle Méditerranéen de l'Arbois, BP 80, 13545 Aix en Provence Cedex 4, France

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## 1 Introduction

The woody invasion of grasslands during the twentieth century is the most common and widespread ecological landscape transformation in European mountains (Archer et al. 2000). This is particularly linked to the cessation of livestock grazing following abandonment of unproductive lands. This transformation and the abandonment of traditional agriculture have triggered natural reforestation in many European mountain areas (e.g. Walther 1986; Romero-Calcerrada and Perry 2004; Cocca et al. 2012). Spontaneous forest expansion is viewed in different ways depending on the geographical, cultural and economic contexts (Sitzia et al. 2010), either as an

opportunity to increase wilderness (e.g. Vera 2000) or as a threat to the conservation of cultural landscapes (e.g. Höchtl et al. 2005). As far as land management is concerned, contrasting conservation policies could be considered: passive management to rewild abandoned areas or active prevention of forest expansion in order to maintain cultural landscapes (Navarro and Pereira 2012).

In Mediterranean areas, forest expansion following the cessation of livestock grazing could be a threat to biodiversity (Preiss et al. 1997; Falcucci et al. 2007; Kunstler et al. 2017), notably in grasslands that are components of mountainous cultural landscapes. As highly diverse areas that provide habitats for species of conservation value, these mountain grasslands are currently protected under the European Union Habitats Directive, which underpins the “Natura 2000” conservation network. These sites need appropriate management to maintain their favourable conservation status, and the challenge is to identify suitable processes for conserving them (Redecker et al. 2002), for instance, grazing and mowing regimes which may hamper woody species’ dynamics and thus preserve the existing habitat (Halada et al. 2011).

For centuries, livestock has been essential in creating and diversifying Mediterranean mountain grasslands (Blondel and Aronson 1999). This is why using herbivores for grassland conservation has long been promoted (WallisDeVries et al. 1998; Redecker et al. 2002; Bakker et al. 2006). Herbivores, especially ungulates, can play a crucial role by controlling both the structure and species composition of Mediterranean (Fortuny et al. 2014, 2017; Osem et al. 2017) or temperate plant communities (Boulanger et al. 2015) and may exert a profound influence on ecosystem processes (Hobbs 1998; Estes et al. 2011). Grazing, browsing and trampling can have detrimental effects on all stages of tree development, from germination and growth to seed production (Gill 1992; Rooney and Waller 2003; Vanderberghe et al. 2007). In both European and national conservation areas, low grazing pressure is thus regularly prescribed to control woody encroachments and to favour grasslands (Bakker et al. 2006). In many conservation plans, the domestic ungulates theoretically substitute for wild large herbivores (auroch, bison, deer, etc.) that have disappeared or become rare, probably due to game hunting since prehistoric times (Vera 2000). However, because this theory is hotly disputed (Bradshaw et al. 2003; Birks 2005; Mitchell 2005), the process needs to be assessed. Wild ungulates may also limit tree encroachment in areas where their populations have risen over recent decades (Maillard et al. 1999; Avededo et al. 2005; Falcucci et al. 2007). Wild and domestic ungulates could thus be combined to mitigate woody encroachment and support the conservation of Mediterranean mountain grasslands.

We hypothesised that domestic and wild ungulates could mitigate tree regeneration and growth, thus limiting encroachment of trees and conserving grasslands in low-altitude

mountains. To test the efficiency of grazing management by wild and domestic ungulates, we analysed the recruitment dynamics and growth of silver fir (*Abies alba* Mill.) in a Mediterranean mountain context where threatened grasslands are subject to a conservation programme. Fir was chosen as a test species because it is preferentially browsed by ungulates (Güthorl 1994; Motta 1996; Kupferschmid et al. 2013), and it regenerates well in Mediterranean and Alpine mountains (Alberti et al. 2009, Chauchard et al. 2010; Garbarino et al. 2014; Bebi et al. 2017). Further, at this altitude, fir should, along with beech (*Fagus sylvatica* L.), be the locally codominant species according to the tree age structure found in old stands (Chauchard et al. 2013).

## 2 Materials and methods

### 2.1 Study area

The study area (1300–1416 m above sea level, 43° 42′ N, 6° 38′ E) is located in the Malay massif, in the foothills of the southern French Alps. The climate of the Malay massif is typical of Mediterranean mountain areas, i.e. warm and dry in summer and cold and snowy in winter, with mean ( $\pm$  sd) precipitation of  $988 \pm 262$  mm year<sup>-1</sup> (1907–2004, meteorological station at Comps-sur-Artuby, 2.5 km away at 943 m a.s.l.). The mean annual temperature is  $9.9 \pm 0.7$  °C (1952–2004) with mean monthly temperatures ranging from 2.4 °C in January to 26.8 °C in July. No precipitation trend was determined over the twentieth century, but the mean temperature has increased significantly by 1 °C since 1985 (Chauchard et al. 2007).

The study site is a large summit plateau, unfenced and situated within the Mediterranean montane vegetation belt. The Malay massif has been part of the *Natura 2000* network since 2010. This mountain summit includes several wide shrubby grassland and rocky habitats of conservation importance which have been colonised by trees, notably Scots pine (*Pinus sylvestris* L.). These upper grasslands constitute the habitat of the meadow viper (*Vipera ursinii* Bonaparte), a European protected species. Secondary succession, which began through tree encroachment during the late nineteenth century following abandonment of the land (Chauchard et al. 2007), represents the main threat to the meadow viper. The conservation plan for the meadow viper included clear felling of pioneer Scots pine forest and of mixed conifer-beech forests plus grazing by sheep to mimic the former large population of wild ungulates. This area was grazed until the late nineteenth century when trees started to regenerate (Chauchard et al. 2013). Since then, the forest has not been exploited. The summit plateau is now covered by a forest with an average pine density of around 317 individuals per hectare, representing  $34.6 \text{ m}^2 \text{ ha}^{-1}$  (94.9% of the total basal area), whereas fir and

beech cover the understory with a basal area of 1.7 and  $< 1 \text{ m}^2 \text{ ha}^{-1}$ , respectively (5.1% of the total basal area). Today, pine is almost absent from the understory and does not regenerate anymore (Chauchard et al. 2013).

## 2.2 Historical and current grazing pressure

Livestock levels fell drastically in the early twentieth century following land use abandonment and rural exodus (Chauchard et al. 2007). Grazing by sheep was very low in the area between the First World War and 1995. From 1970 to 1995, only a single herd of around 250–300 individuals grazed the study area each year, during two seasons: spring (April to June) and autumn (October to November). After 1995, the number of sheep (*Ovis aries* L.) increased, to about 600 in 2000, grazing all year round except for the snowy months from January to March (Fig. 1). Grazing pressure today is about 0.1 livestock unit per hectare ( $\text{LU ha}^{-1}$ ).

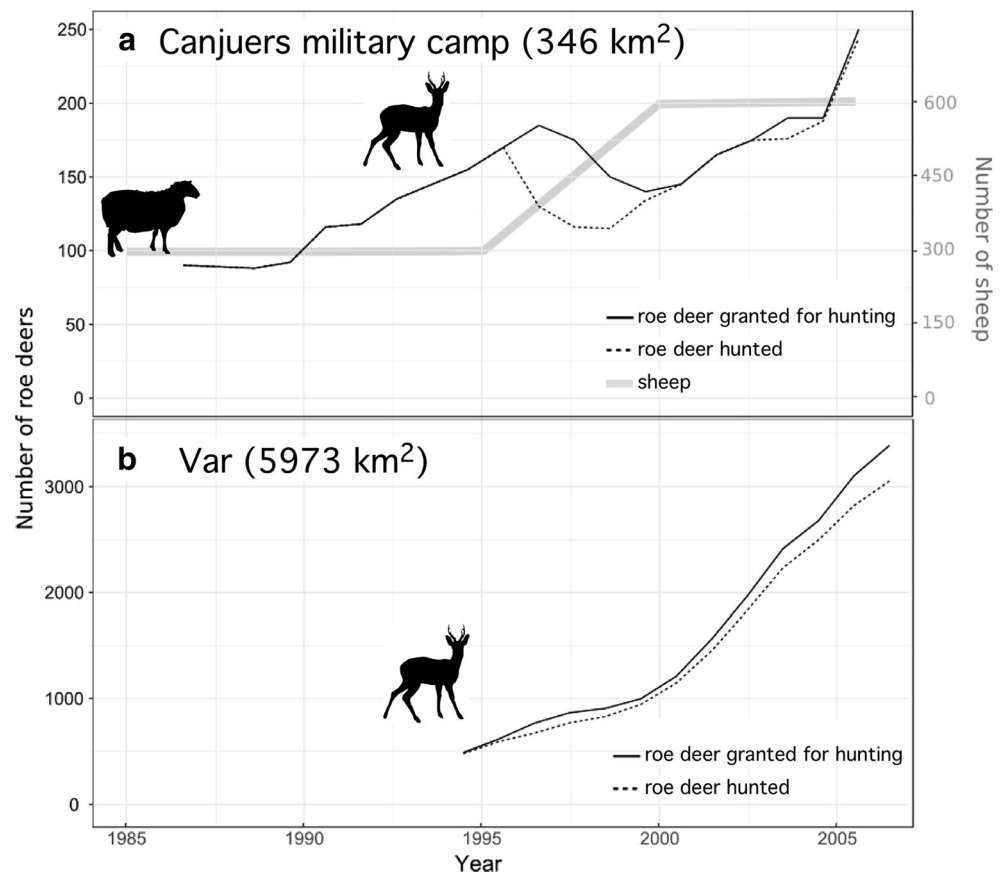
If sheep are mostly grazers, red deer (*Cervus elaphus* L.) is an intermediate mixed feeder and roe deer (*Capreolus capreolus* L.) a browser (Storms et al. 2008; Redjadj et al. 2014). Both deer species had disappeared during the nineteenth century and were re-introduced about 25 km northwest of the study site, in 1946 and 1954 respectively. In 2005, densities are estimated at four to six individuals for roe deer

and less than one individual for red deer per square kilometre in this massif. Although there is no accurate data on deer populations in the Malay massif, the wildlife administration has concluded that their densities were similar or higher, because since the establishment of the military training camp in 1970, deer abundances have been effectively promoted by the exclusion of rural practices such as gathering, forestry and trekking from this area and severe restriction of hunting. The ratio between harvested and available hunting quotas is a proxy for the status of the deer population. Between 1986 and 2005, the number of roe deer hunted has increased by 271% in the military camp that includes the Malay massif (source: *Fédération Départementale des Chasseurs*; Fig. 1). Between 1986 and 1996, hunting quotas generally increased due to the increasing density of roe deer, but decreased between 1997 and 1999 due to difficulties by hunters to attain the quotas (Fig. 1). Since 2000, the number of roe deer hunted has matched the quotas, indicating a strong actual increase of roe deer population density.

## 2.3 Sampling design

Two rectangular plots or transects were selected within the pine forest: the elongated form of the study plots, running along the slope, aims to capture all the possible ecological

**Fig. 1** Number of hunted (“realised”) roe deer (*Capreolus capreolus*) per year (a) in the district of Canjuers military camp (34,652 ha) and (b) the Var department (several districts per department; 5973 km<sup>2</sup>). The size of the sheep (*Ovis aries*) herd is illustrated by a grey curve (a): this is an estimation based on a survey of land managers. In France, deer populations have been hunted since the 1970s in accordance with quotas which are calculated yearly according to the estimated deer numbers present in each district. In the study area, red deer (*Cervus elaphus*) hunting is not allowed. Because of uncertainty in population size estimates, we used the number of roe deer hunted as a proxy for population (data from the *Regional Agriculture and Forest Administration, Direction Départementale de l’Agriculture et de la Forêt*)



heterogeneity associated with the variations in elevation and terrain of the area. The transect Tr1 measured  $20 \times 775$  m and the second, Tr2,  $20 \times 320$  m. Pine basal area was  $34.3 \text{ m}^2 \text{ ha}^{-1}$  and  $35.3 \text{ m}^2 \text{ ha}^{-1}$  respectively, representing 99.5% and 86.6% of the total basal area (Table 1). Individual fir trees were scarce in Tr1 (32 individuals  $\text{ha}^{-1}$ ) and abundant in Tr2 (955 individuals  $\text{ha}^{-1}$ ). The cumulative basal area of *Abies alba* was low in both transects ( $< 1 \text{ m}^2 \text{ ha}^{-1}$  for the first transect and  $4.74 \text{ m}^2 \text{ ha}^{-1}$  for the second; Table 1).

Tr1 was sampled in 2003 and 2004, whereas Tr2 was sampled in 2005. All living and dead fir trees (height:  $h > 1.5$  m), saplings ( $0.1 < h < 1.5$  m) and seedlings ( $h < 0.1$  m) were identified and labelled. One-year-old seedlings were not considered. Trunk diameters were measured at their base to assess the basal area. Most trees and saplings were sawed to collect a basal disc or an entire stem in order to assess their height growth, whilst the others were cored with an increment borer. In total, we examined 232 seedlings and 399 saplings and trees: 77 individuals were cored and 322 were sawed.

## 2.4 Age estimates and age structure

The age of fir seedlings was estimated by counting the terminal bud leaf scale remains. The age of fir saplings and trees was based on counting tree-rings. The wooden samples (cores, discs or entire stems) were first air-dried and then polished using progressively finer sandpaper. Tree-rings were counted under a dissecting microscope (Schweingruber 1988) and cross-dated using skeleton plot technique (Fritts 1976). To assess fir age structure that would illustrate the net demography (regeneration *minus* mortality), we plotted trees' ages in 10-year classes.

## 2.5 Individual growth pattern

After air-drying, discs were cut at regular intervals (ca. 9–10 cm; Fig. 2) between each branch whorl along each entire

**Table 1** Topographic and structural characteristics of the expanding forest on the summit of the Malay massif, Mediterranean mountains, southeastern France

	Transect 1	Transect 2
Area (ha)	1.55	0.64
Aspect	North-facing	North-facing
Total basal area ( $\text{m}^2 \text{ ha}^{-1}$ )	34.3	40.7
Tree composition (% of total basal area)		
<i>Abies alba</i>	0.13%	11.7%
	$< 1 \text{ m}^2 \text{ ha}^{-1}$	$4.7 \text{ m}^2 \text{ ha}^{-1}$
<i>Fagus sylvatica</i>	$< 0.10\%$	1.5%
	$< 1 \text{ m}^2 \text{ ha}^{-1}$	$< 1 \text{ m}^2 \text{ ha}^{-1}$
<i>Pinus sylvestris</i>	99.5%	86.6%
	$34.3 \text{ m}^2 \text{ ha}^{-1}$	$35.3 \text{ m}^2 \text{ ha}^{-1}$

stem (254 firs). The length of the stem between two consecutive discs was accurately measured to determine height growth (Gutsell and Johnson 2002). Annual radial growth was measured from cross sections with a precision of  $10^{-3}$  mm along either three or two radii using a Frank Rinn Lintab® measuring device. Radial growth was measured in 36 randomly-selected individuals: 12 individuals recruited after 1980 (1 was unfortunately not efficient for tree-ring measurement) and 24 before 1980, including 12 individuals with an apex above 1.0 m. Annual average ring width was calculated and then transformed into basal increment area using TSAPWIN® software.

Height growth ( $HG_{k-l}$ ) and radial growth ( $RG_{k-l}$ ) were calculated between two heights (Fig. 2),  $k$  and  $l$ , according to the life stage of the tree: for seedlings whose height ( $h$ ) was  $< 10$  cm,  $k=0$  and  $l=0.1$  m; for small saplings whose height was between 10 and 50 cm,  $k=0.1$  and  $l=0.5$  m; for tall saplings stage whose height was below  $< 100$  cm,  $k=0.5$  and  $l=1.0$  m; and for trees stage whose apex was  $> 100$  cm,  $k=1.0$  and  $l=1.5$  m. The years following 2003 were excluded from the height and radial growth computation for individuals sampled in 2004 or 2005 to avoid the effects of conservation-related clear cutting that began during winter of 2003–2004.

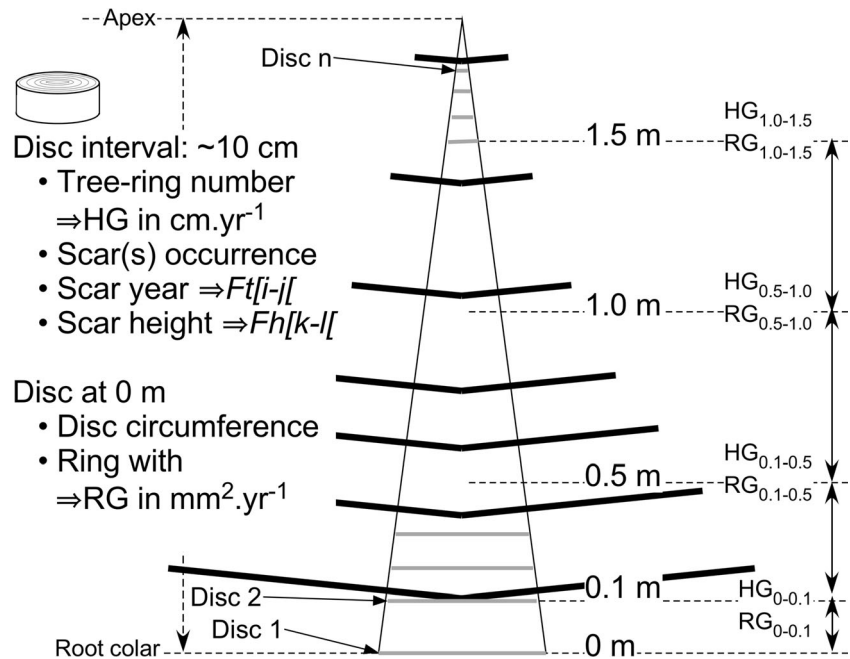
Seedling and small sapling height and radial growths ( $HG_{0-0.1 \text{ m}}$  and  $HG_{0.1-0.5 \text{ m}}$ ,  $RG_{0-0.1 \text{ m}}$  and  $RG_{0.1-0.5 \text{ m}}$ ) in different decades were compared to test the increasing effect of ungulate populations. Linear mixed effect models (hereafter “LME model”) were used on log-transformed data, the decade of regeneration being a fixed effect and transect a random effect. After LME models, type-II analysis-of-variance tables were calculated, and Wald chi-square tests were used to test fixed effects. To compare height and radial growth below and above 100 cm ( $HG_{0.5-1.0 \text{ m}}$  and  $HG_{1-1.5 \text{ m}}$ ,  $RG_{0.5-1.0 \text{ m}}$  and  $RG_{1-1.5 \text{ m}}$ ), a LME model was used and data log transformed: below versus above 1.0 m, the sapling's life stage and their interaction were treated as fixed effects and the transect and sapling's individual as a random effect. All sampled saplings that overshoot the height of 1.0 m regenerated between the 1950s and the 1970s (Table 2). No individual from the 1990s and 2000s attain this height chosen for the comparison of height and radial growth between 0.5–1.0 and 1.0–1.5 m. The analyses were performed using *nlme*, *car* and *multcomp* packages in R software.

## 2.6 Scars and wounded stems

Browsing and fraying can both leave marks, conspicuous or inconspicuous, on the stem (Kaennel and Schweingruber 1995). These marks, referred to as scars (Fig. 3b), can be used to assess ungulate impact on tree population or forest stands and can be dated by dendrochronology because radial growth stops at the lesion spot but continues around it (Vila et al. 2004). Scarring events were searched in each stem disc and



**Fig. 2** Sampling design on seedlings, saplings and trees of fir (*Abies alba*) for height and radial growth assessment and temporal and height frequencies of scars on trunks due to grazing, browsing or fraying by ungulates



dated using tree-ring counts and cross-dating (Fig. 2). The height and diameter of the stem at the position of the scar were measured. The temporal frequency of scarred trees in each age class  $[i-j]$ , i.e.  $Ft_{[i-j]}$ , was calculated as follows:

$$Ft_{[i-j]} = \text{Nsc}_{[i-j]} / N_j \times 100 \quad (1)$$

where  $\text{Nsc}_{[i-j]}$  is the number of trees with at least one scar in the age class  $[i-j]$ , and  $N_j$  is the total number of tree in all age classes growing in year  $j$  (Fig. 2). The frequency of scarred trees in the height class  $[k-l]$ , i.e.  $Fh_{[k-l]}$  was calculated as follows:

$$Fh_{[k-l]} = \text{Nsc}_{[k-l]} / N_l \times 100 \quad (2)$$

where  $\text{Nsc}_{[k-l]}$  is the number of trees with at least one scar in the height class  $[k-l]$  and  $N_l$  is the total number of tree number higher than height  $l$ . There was no maximum height for scar searching; however, scars above 2 m were not expected given the ungulate species in the area.

**Table 2** Number per decades of sampled trees with an apex > 1.0 m and used for comparisons of radial and height growth under and above 1.0 m: 0.5–1.0 m versus 1.0–1.5 m. Only one sampled tree has regenerated in the 1980s but was not considered in the analysis

Decades	Height growth (HG)	Radial growth (RG)
1950s	7	3
1960s	18	5
1970s	29	4

### 3 Results

Data are publicly available in Chauchard et al. (2018).

#### 3.1 Age structure of silver fir

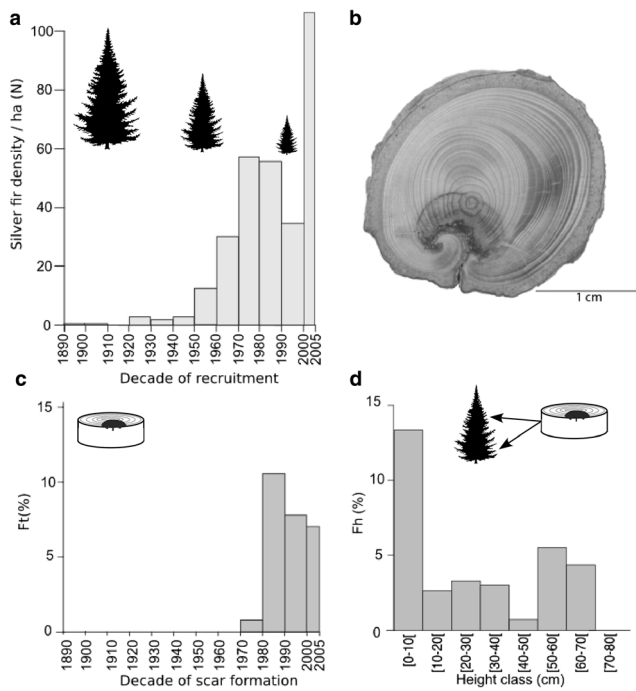
*Abies alba* regeneration has been particularly active since the 1970s although the oldest fir was dated to 1909 (Fig. 3a). Two regeneration maxima were apparent, the first during the 1970s and 1980s and the second during the 2000s. No correlation was found between the dates of fir regeneration and precipitation or temperature (based on total, spring and summer precipitation and annual mean, spring and summer temperature; Spearman test), indicating that climate was not a driver of the dynamics of fir regeneration.

#### 3.2 Scar distributions

The oldest scar was dated to 1976 (Fig. 3c): this was the only scar recorded before 1980, comprising < 1% of total scar numbers. The frequency of scars first increased in the 1980s (Fig. 3c) and then remained more or less constant until the end of the 1990s. Most of the affected trees displayed scars below a height of 70 cm (Fig. 3d). Scars were only found on small-diameter stems, i.e.  $0.64 \pm 0.54$  cm.

#### 3.3 Growth pattern

Height growth at seedling stage ( $HG_{0-0.1 \text{ m}}$ ) was similar among the individuals recruited irrespective of the decade (Fig. 4a, LME model,  $F$  value (4; 248) = 1.85,  $p = 0.11$ ).



**Fig. 3** **a** Age-class distribution of silver fir, *Abies alba* ( $n = 630$  individuals), depicted as individuals per hectare. One-year-old seedlings are not considered. **b** Example of browsing scar on a fir cross section. **c** Distribution of scarred firs per age class,  $F_{t_i-l}$  (%) plotted against time. **d** Height-frequency of scarred trees per height class of scars,  $F_{h_{k-l}}$  (%)

However, height growth at sapling stage ( $HG_{0.1-0.5\text{ m}}$ ) differed significantly between decades (LME model,  $F$  value (4; 177) = 4.65,  $p < 0.001$ ). At sapling stage, height growth has generally decreased throughout the decades of regeneration from the 1950s to the 1990s (Fig. 4b).

Radial growth at seedling stage ( $RG_{0-0.1\text{ m}}$ ) differed significantly between the decades of regeneration (Fig. 4c; LME model,  $F$ -statistic (2; 31) = 6.71,  $p = 0.001$ ): seedlings recruited in the 1950s and 1960s grew faster than those recruited between the 1970s and the 1990s. However, radial growth at sapling stage ( $HG_{0.1-0.5\text{ m}}$ ) was similar between the decades of regeneration (Fig. 4d, LME model,  $F$ -statistic (2; 31) = 1.06,  $p = 0.35$ ).

For saplings  $> 1\text{ m}$ , height growth was significantly different (LME model,  $F$ -statistic (1; 42) = 19.31,  $p < 0.001$ ) between the time when the apex was under 1.0 m ( $HG_{0.5-1\text{ m}}$ ) and when it overshoot 1.0 m ( $HG_{1-1.5\text{ m}}$ ), with growth rates being higher when trees were above 1.0 m ( $HG_{0.5-1\text{ m}}$   $9.1 \pm 3.6\text{ cm year}^{-1}$ ;  $HG_{1-1.5\text{ m}}$   $15.0 \pm 6.6\text{ cm year}^{-1}$ ; Fig. 5a). Radial growth of individuals also varied according to the height of the apex, under or above 1.0 m (LME model,  $F$ -statistic (1; 10) = 7.01,  $p = 0.02$ ), with higher radial growth rates when individuals had their apex above 1.0 m ( $RG_{1-1.5\text{ m}}$   $162.7 \pm 96.3\text{ mm}^2\text{ year}^{-1}$ ) than when it was under 1.0 m ( $HG_{0.5-1\text{ m}}$   $95.7 \pm 86.1\text{ mm}^2\text{ year}^{-1}$ ; Fig. 5b). For saplings taller than 1.0 m,

decade of regeneration (single or interaction effects) had no effect on values of height or radial growth.

## 4 Discussion

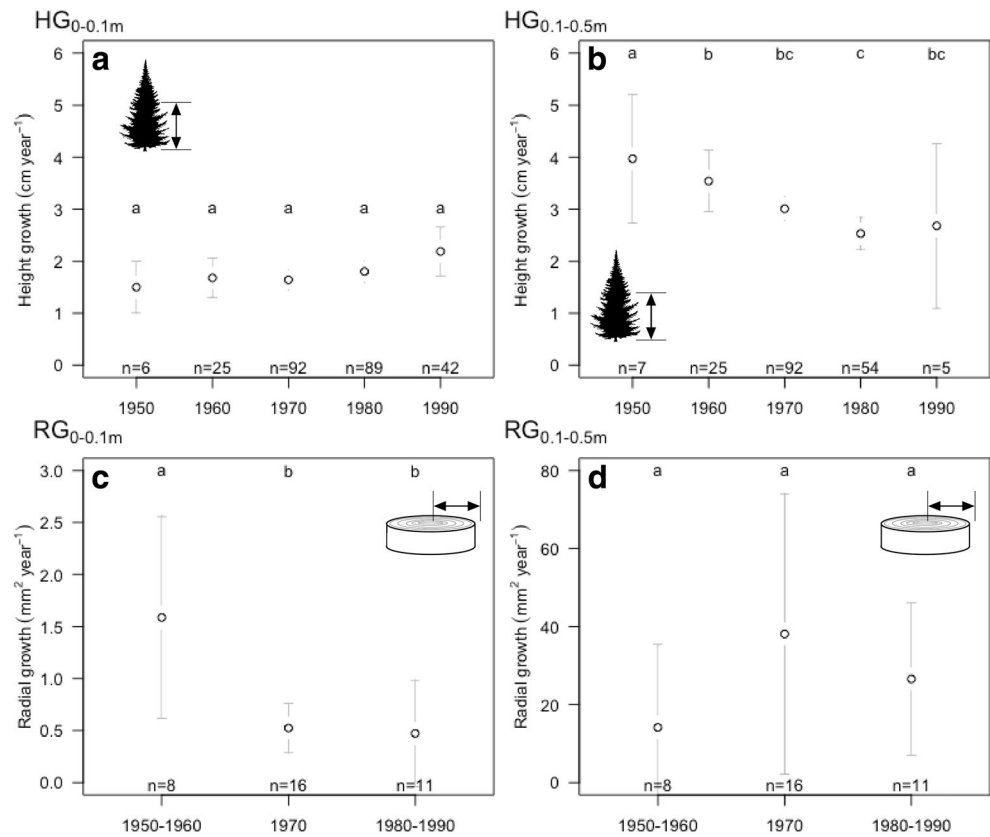
Our results show that, despite the presence of wild and domestic ungulates, the age structure of silver fir shows sustained population dynamics even when it is subject to high levels of browsing. Although seedling and sapling growth is reduced, tree recruitment is hindered but not prevented by browsing by wild or domestic ungulates. These results do not support the hypothesis that ungulates could prevent tree encroachment in such Mediterranean montane ecosystems, because encroachment by the most browsed species in the forest is not prevented. This hypothesis was tested in the context of a conservation management plan for natural grassland colonised by trees, i.e. areas which in Europe are often managed through low-intensity agricultural practices or extensive grazing (Ostermann, 1998). We discuss the conservation and management of these areas further below.

### 4.1 Ungulate densities and change in scars and growth of firs

Wild and domestic ungulate densities have progressively increased in the Malay massif and the surrounding areas over the past 40 years, although domestic ungulate densities have remained below those of the nineteenth century (Chauchard et al. 2007). The local increase in wild ungulates (Fig. 1a) is consistent with the general pattern observed in most Mediterranean areas of Europe (Maillard et al. 1999), partly due to the reintroduction of deer in relevant habitats, i.e. the young woody ecosystems which have resulted from land abandonment. This has been accompanied by a recent local increase in sheep herd densities, contributing to the overall increase in ungulate density.

Following the increase in ungulate densities, scar dating has shown a clear increase in browsing and fraying of firs since the 1970s (Fig. 3c). This result could be expected, considering the deer behaviour in relation to trees (e.g. Motta 1995, 1996), the diet of red or roe deer (Storms et al. 2008; Redjadj et al. 2014) and the grazing effect and diet preference of sheep (e.g. McEvoy and McAdam 2008, Speed et al. 2011a). Although there are no significant effect of ungulates on the height growth of seedlings ( $< 10\text{ cm}$  tall; Fig. 4a), height growths of small saplings ( $HG_{0.1-0.5\text{ m}}$ ) decrease with each decade of recruitment, thus suggesting an increase in stress intensity, primarily since the 1970s (Fig. 4b) which coincides with the rise in incidences of scarring (Fig. 3c). Interestingly, no change in the radial growth of small saplings ( $RG_{0.1-0.5\text{ m}}$ ) was observed from decades 1950–1960 to 1980–1990 (Fig. 4d). This could be a compensatory effect of the

**Fig. 4** Height (HG) and radial growth (RG) of silver fir (*Abies alba*) in the Malay massif, Mediterranean mountains, southeastern France; measurements of seedlings (**a**,  $HG_{0-0.1}$  cm; **c**,  $RG_{0-0.1}$  cm) and small saplings (**b**,  $HG_{0.1-0.5}$  cm; **d**,  $RG_{0.1-0.5}$  cm) versus decade of recruitment. Different letters indicate significant differences in growth between decades of recruitment ( $p < 0.05$ )



decrease in their height growth. Indeed, removal of apical buds by browsing can favour radial growth (McNaughton 1983). The lack of clear impact of ungulates on seedlings may be explained by the fact that the majority of affected seedlings have since disappeared and were therefore neither observed nor recorded.

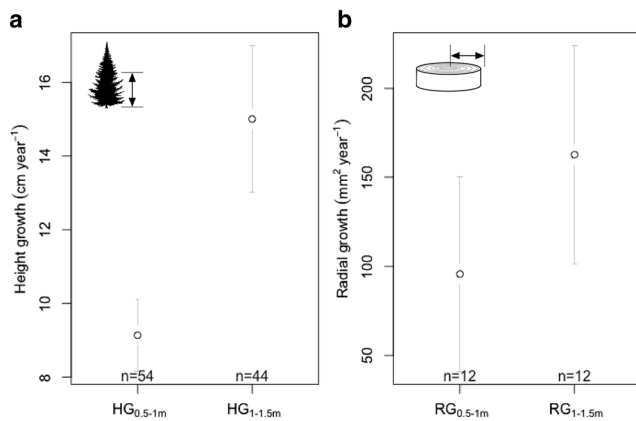
The coincidence between the patterns of increasing deer and sheep density and decreasing apical growth of saplings suggests that changing ungulate density is the main cause of these progressive growth changes in young firs (Fig. 4b). The rough cuts that we observed, characterised by a shredded edge, are typical of ungulate damage rather than rabbit or hare damage. The differences in height and radial growth above and below 1 m further support the assumption that roe deer and sheep are responsible for this impact, and not red deer. Indeed, the maximum height of twigs or apical shoots that ungulates can browse, i.e. the browsing limit, is around 1.80 m for red deer, 1.10 m for roe deer (Mayle 1999) and between 1 and 1.30 m for sheep (Brown 1985, Speed et al. 2011a).

Although the observed decrease in height growth of saplings and radial growth of seedlings could result from forest maturation (e.g. competition for light, water or nutrients, changes in soil organic matter) or from a warming climate (+1 °C since 1985, without precipitation changes during the twentieth century; Chauchard et al. 2007) which has increased evapotranspiration and plant stress in the Mediterranean, the

rate of scars on saplings (Fig. 3) and the abundance of browsed twigs point strongly towards the effect of ungulates (Senn and Suter 2003). We cannot rule out the possibility that climate may have interacted with ungulates to reduce height or radial growth (Speed et al. 2011b), but our sampling was not designed to test this climate-related question. However, trees that have regenerated from decades 1950s to 1970s showed notably higher apical and radial growth after reaching 1 m between 1980 and 2003 ( $HG$  and  $RG_{1.0-1.5}$  m,  $HG \sim 15$  cm year<sup>-1</sup>;  $RG \sim 164$  mm<sup>2</sup> year<sup>-1</sup>) than before 1980 when trees were < 1.0 m ( $HG_{0.5-1.0}$  m  $\sim 9$  cm year<sup>-1</sup>;  $RG_{0.5-1.0}$  m  $\sim 96$  mm<sup>2</sup> year<sup>-1</sup>; Fig. 5). These results indicate that herbivory by roe deer and sheep was the main driver, offsetting the longer-term impact of warming. When apex escaped to browsing height of ungulates, the growth was released significantly whatever the climate conditions.

#### 4.2 Respective impacts of wild and domestic ungulates on individual firs

The impact of wild and domestic ungulates cannot be easily differentiated on the basis of scars or ungulate densities because data regarding wild ungulate populations are proxies derived from the hunting census. However, scar height distribution does tell us something (Motta 1995). The fact that scars occur exclusively below 1 m (Fig. 3d) and the small mean



**Fig. 5** Height growth (**a**, HG) and radial growth (**b**, RG) of silver fir (*Abies alba*) in the Malay massif, Mediterranean mountains, southeastern France, with measurements below ( $HG_{0.5-1\text{ m}}$ ,  $RG_{0.5-1\text{ m}}$ ) and above ( $HG_{1-1.5\text{ m}}$ ,  $RG_{1-1.5\text{ m}}$ ) 1.0 m for sampled individuals with an actual apex above 1.0 m.  $HG_{0.5-1\text{ m}}$  and  $HG_{1-1.5\text{ m}}$  are significantly different (LME model,  $F$ -statistic (1; 42) = 19.31,  $p < 0.001$ ), as are  $RG_{0.5-1\text{ m}}$  and  $RG_{1-1.5\text{ m}}$  (LME model,  $F$ -statistic (1; 10) = 7.62,  $p = 0.02$ ); details on effectiveness cf. Table 2

diameter of scarred stems exclude the possibility of extensive impacts by red deer suggests browsing by roe deer and sheep. Unfortunately, scar distributions do not make it possible to differentiate between roe deer and sheep, and their effects are cumulative (Brookshire et al. 2002). However, the two species preferences for specific feeding habitats do lead to distinct spatial distributions as sheep prefer grass-rich habitats inside the forest, where grass is highly available, whilst roe deer prefer habitats with greater canopy cover (Myserud et al. 1999). We thus assume that most impact result from roe deer and that sheep had minor effect on silver fir.

### 4.3 Fir population dynamics and management implications

Since the 1960s, firs have regenerated well (Fig. 3a) in the understory of pines that began to recruit in what were previously pastures at the end of the nineteenth century. As in other southern European mountain areas (Doležal et al. 2004; Paluch 2005; Chauchard et al. 2010; Bebi et al. 2017), expanding fir populations attest the very strong dynamics associated with, firstly, the abandonment of low-productivity areas which started at the end of the nineteenth century and intensified during the 1950s (e.g. Chauchard et al. 2007), and secondly, changes in forestry practices: for instance, systematic removal of fir seedlings or selective cutting of fir saplings to preserve tree species that do not limit too much the grass growth and offer a shadow for domestic ungulates in summer (e.g. Scots pine in the montane belt, larch in subalpine) or that were not the target for wood production (Motta and Garbarino 2003; Doležal et al. 2004). In this area, fir was a target species only in north-facing steep slopes to produce timber beams for

roofing constructions (farms, house, castle), and beech in gentle slopes devoted for charcoal production (coppice system). Overall, the impact of ungulates on Mediterranean mountain trees, including fir, is just one part of the complex processes of forest management and conservation, in which species (plants, animals), altitude, seasonal effects, etc., also play a role (Ameztegui and Coll 2015).

Although increased ungulate populations since 1980 have limited individual firs' height growth (Fig. 4b), the overall density of ungulates ( $\sim 0.1\text{ LU ha}^{-1}$ ) has not hindered the population fir dynamics (Fig. 3a) despite a fall in the pine population caused by lack of recruitment and regeneration (Chauchard et al. 2007). The J-shape distribution of fir (Fig. 3), i.e. the grazed and browsed species, is conflicting with the lack of pine regeneration since the 1970 (Chauchard et al. 2007), because, generally, fir consumption by ungulates favours the unpalatable species that is here Scots pine (e.g. Bernard et al. 2017). The lack of pine recruitment is independent on fir dynamics, and fir dynamics is obviously not affected by the presence of herbivores despite their presence at least, their increasing abundances at most. Here, fir recruitment is extremely active whatever the level of grazing activity. Interestingly, similar densities of between 0.05 and  $0.20\text{ LU ha}^{-1}$  have been shown to hinder tree growth without strictly controlling recruitment or demography (Cierjacks and Hensen 2004; Casasús et al. 2007). Generally speaking, a low-density presence of herbivores in forests does not prevent tree encroachment and expansion as long as other disturbances (e.g. fire, windthrow, clear-cut) are not associated with it (Peringer et al. 2017; Boulanger et al. 2018; Jeffers et al. 2018), whilst high-density of herbivores can be extremely efficient, including on unpalatable species like pines (e.g. Chauchard et al. 2006).

### 4.4 Management implications

The conservation of Mediterranean mountain grasslands is thus compromised if the presence of herbivores is not combined with significant human intervention or other natural disturbances which prevent tree recruitment. To inhibit tree regeneration and growth in the study area, we suggest that a significant increase in ungulate densities should be promoted up to the levels that prevailed in the nineteenth century, ideally alongside with other disturbances such as fires or clear-cut. An increase in ungulate density should prevent regeneration of preferentially browsed species such as *Abies alba* but would also benefit less browsed species such as *Pinus sylvestris*. Other management methods have been tested in similar areas: for instance, low-severity fires which can realistically reduce encroachment by woody plants and promote palatable species and sustain food networks (e.g. Hancock et al. 2011). Unfortunately, such prescribed burning has a strongly negative impact on meadow viper survival (Lyet et al. 2009), which is a key species to



protect. Hence, the method of clear-cut, if associated with a higher density of ungulate grazing, appears to be the main management strategy for such mountain grasslands in the context of ongoing forest expansion in Mediterranean areas. Here, since our sampling and first results, the clear-cut has been chosen in addition to sheep grazing as management plan to protect this mountain grassland.

## 5 Conclusion

Whilst livestock has maintained herb-dominant landscapes by grazing and browsing over centuries, current ungulate densities are insufficient to control tree expansion effectively. Our observations raise questions about the sustainable management of these former grasslands and of cultural landscapes more generally. By offering an empirical case study with moderate ungulate herbivory, our study contributes to ongoing debate about the most suitable approach to long-term management of grass-dominated habitats in Mediterranean mountains.

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**Data availability** The datasets generated and/or analysed during the current study are available in the Portail Data Inra repository (Chauchard et al. 2018). Dataset not peer-reviewed. Chauchard S, Guibal F, Carcaillet C (2018) data from: Fir expansion not controlled by moderate densities of large herbivores: a grassland conservation issue. V1. Portail Data Inra. [Dataset]. <https://doi.org/10.15454/NBFE4F>.

## Compliance with ethical standards

**Conflict of interest** The authors declare that they have no conflict of interest.

## References

Avecedo P, Delibes-Mateos M, Escudero MA, Vicente J, Marco J, Gortázar C (2005) Environmental constraints in the colonization sequence of roe deer (*Capreolus capreolus* Linnaeus, 1758) across the Iberian Mountains, Spain. *J Biogeogr* 32:1671–1680

- Alberti G, Peressotti A, Piussi P, Zerbi G (2009) Structure and stand development of secondary forests in eastern PreAlps (Italy). *Dendrochronologia* 27:173–181
- Ameztegui A, Coll L (2015) Herbivory and seedling establishment in Pyrenean forests: influence of micro- and meso-habitat factors on browsing pressure. *For Ecol Manag* 342:103–111
- Archer S, Boutton TW, Hibbard KA (2000) Trees in grasslands: biogeochemical consequences of woody plant expansion. In: Schulze ED, Harrison SP, Heimann M, Holland EA, Lloyd J, Prentice IC, Schimel D (eds) *Global biogeochemical cycles in the climate system*. Academic Press, San Diego, pp 115–137
- Bakker ES, Ritchie ME, Olf H, Milchunas DG, Knops JMH (2006) Herbivore impact on grassland plant diversity depends on habitat productivity and herbivore size. *Ecol Lett* 9:780–788
- Bebi P, Seidi R, Motta R, Fuhr M, Firm D, Krumm F, Conedera M, Ginzler C, Wohlgemuth T, Kulakowski D (2017) Changes of forest cover and disturbance regimes in the mountain forests of the Alps. *For Ecol Manag* 388:43–56
- Bernard M, Boulanger V, Dupouey JL, Laurent L, Montpied P, Morin X, Picard JF, Saïd S (2017) Deer browsing promotes Norway spruce at the expense of silver fir in the forest regeneration phase. *For Ecol Manag* 400:267–277
- Birks HJB (2005) Mind the gap: how were open European primeval forests? *Trends Ecol Evol* 20:154–156
- Blondel J, Aronson J (1999) *Biology and wildlife of the Mediterranean region*. Oxford University Press, USA
- Boulanger V, Baltzinger C, Saïd S, Ballon P, Picard JF, Dupouey JL (2015) Decreasing deer browsing influenced understory vegetation dynamics over 30 years. *Ann For Sci* 72:367–378
- Boulanger V, Dupouey J-L, Archaux F, Badeau V, Baltzinger C, Chevallier R, Corcket E, Dumas Y, Forgeard F, Mârell A, Montpied P, Paillet Y, Picard JF, Ulrich E (2018) Ungulates increase forest plant species richness to the benefit of non-forest specialists. *Glob Chang Biol* 24:e485–e495
- Bradshaw RHW, Hannon GE, Lister AM (2003) A long-term perspective on ungulate-vegetation interactions. *For Ecol Manag* 181:267–280
- Brookshire ENJ, Kauffman JB, Lytjen N, Otting N (2002) Cumulative effects of wild ungulate and livestock herbivory on riparian willows. *Oecologia* 132:559–566
- Brown RF (1985) The growth and survival of young mulga (*Acacia aneura* F. Muell) trees under different levels of grazing. *Rangel J* 7:143–148
- Casasús I, Bernués A, Sanz A, Villalba D, Riedel JL, Revilla R (2007) Vegetation dynamics in the Mediterranean forest pastures as affected by beef cattle grazing. *Agric Ecosyst Environ* 121:365–370
- Chauchard S, Pille G, Carcaillet C (2006) Large herbivores control the invasive potential of nonnative Austrian black pine in a mixed deciduous Mediterranean forest. *Can J For Res* 36:1047–1053
- Chauchard S, Carcaillet C, Guibal F (2007) Patterns of land-use abandonment control tree-recruitment and forest dynamics in Mediterranean mountains. *Ecosystems* 10:936–948
- Chauchard S, Beilhe F, Denis N, Carcaillet C (2010) An increase in the upper tree-limit of silver fir (*Abies alba* Mill.) in the Alps since the mid-20<sup>th</sup> century: a land-use change phenomenon. *For Ecol Manag* 259:1406–1415
- Chauchard S, Guibal F, Carcaillet C (2013) Land-use legacies: multi-centuries years-old management control between-stands variability at the landscape-scale in Mediterranean mountain forests, France. *J For Sci* 59:1–7
- Chauchard S, Carcaillet C, Guibal F (2018) Data from: Fir expansion not controlled by moderate densities of large herbivores: a grassland conservation issue. V1. Portail Data Inra. [Dataset]. <https://doi.org/10.15454/NBFE4F>
- Cierjacks A, Hensen I (2004) Variation of stand structure and regeneration of Mediterranean holm oak along a grazing intensity gradient. *Plant Ecol* 173:215–223

- Cocca G, Sturaro E, Gallo L, Ramanzin M (2012) Is the abandonment of traditional livestock farming systems the main driver of mountain landscape change in alpine areas? *Land Use Policy* 29:878–886
- Doležal J, Št'astná P, Hara T, Šrutek M (2004) Neighborhood interactions and environmental factors influencing old-pasture succession in Central Pyrenees. *J Veg Sci* 15:101–108
- Estes JA, Terborgh J, Brashares JS, Power ME, Berger J, Bond WJ, Carpenter SR, Essington TE, Holt RD, Jackson JBC, Marquis RJ, Oksanen L, Oksanen T, Paine RT, Pikitch EK, Ripple WJ, Sandin SA, Scheffer M, Schoener TW, Shurin JB, Sinclair ARE, Soulé ME, Virtanen R, Wardle DA (2011) Trophic downgrading of planet Earth. *Science* 333:301–306
- Faluccci A, Maiorano L, Boitani L (2007) Change in land-use/land-cover patterns in Italy and their implications for biodiversity conservation. *Landsc Ecol* 22:617–631
- Fortuny X, Carcaillet C, Chauthard S (2014) Land use legacies and site variables control the understorey plant communities in Mediterranean broadleaved forests. *Agric Ecosyst Environ* 189: 53–59
- Fortuny X, Chauthard S, Carcaillet C (2017) Confounding legacies of land uses and land-form pattern on the regional vegetation structure and diversity of Mediterranean montane forests. *For Ecol Manag* 384:268–278
- Fritts HC (1976) *Tree rings and climate*. Academic Press, London
- Garbarino M, Sibona E, Lingua E, Motta R (2014) Decline of traditional landscape in a protected area of the southwestern Alps: the fate of enclosed pasture patches in the land mosaic shift. *J Mt Sci* 11:544–554
- Gill RMA (1992) A review of damage by mammals in north temperate forests—3. Impact on trees and forests. *Forestry* 65:363–388
- Güthorl V (1994) Zusammenhänge zwischen der Populationsdichte des Rehwildes (*Capreolus capreolus*, Linné 1758) und dem Verbißdruck auf die Waldvegetation. *Z Jagdwiss* 40:122–611
- Gutsell SL, Johnson EA (2002) Accurately ageing trees and their height-growth rates: implications for interpreting forest dynamics. *J Ecol* 90:153–166
- Halada L, Evans D, Romão C, Petersen JE (2011) Which habitats of European importance depend on agricultural practices? *Biodivers Conserv* 20:2365–2378
- Hancock MH, Amphlett A, Proctor R, Dugan D, Willi J, Harvey P, Summers RW (2011) Burning and mowing as habitat management for capercaillie *Tetrao urogallus*: an experimental test. *For Ecol Manag* 262:509–521
- Hobbs NT (1998) Modification of ecosystems by ungulates. *J Wildl Manag* 60:695–713
- Höchtel F, Lehringer S, Konold W (2005) “Wilderness”: what it means when it becomes a reality—a case study from the southwestern Alps. *Landsc Urban Plan* 70:85–95
- Jeffers ES, Whitehouse NJ, Lister A, Plunkett G, Barratt P, Smyth E, Lamb P, Dee MW, Brooks SJ, Willis KJ, Froyd CA, Watson JE, Bonsall MB (2018) Plant controls on Late Quaternary whole ecosystem structure and function. *Ecol Lett* 21:814–825
- Kaennel M, Schweingruber FH (1995) Multilingual glossary of dendrochronology. Terms and definition in English, French, Spanish, Italian, Portuguese and Russian. Paul Haupt Publishers, Stuttgart
- Kunstler G, Chadoeuf J, Klein EK, Curt T, Bouchaud M, Lepart J (2017) Tree colonization of sub-Mediterranean grasslands: effects of dispersal limitation and shrub facilitation. *Can J For Res* 37:103–115
- Kupferschmid AD, Zimmermann S, Burgmann H (2013) Browsing regime and growth response of naturally regenerated *Abies alba* saplings along light gradients. *For Ecol Manag* 310:393–404
- Lyet A, Cheylan M, Prodon R, Besnard A (2009) Prescribed fire and conservation of a threatened mountain grassland specialist: a capture–recapture study on the Orsini’s viper in the French Alps. *Anim Conserv* 12:238–248
- Maillard D, Duncan P, Gaillard JM, Gaultier P (1999) Le retour des grands mammifères sauvages dans la région méditerranéenne française. *For Mediterr* 20:9–16
- Mayle B (1999) *Managing deer in the countryside*. Forestry commission, Edinburgh 12 p
- McEvoy PM, McAdam JH (2008) Sheep grazing in young oak *Quercus* spp. and ash *Fraxinus excelsior* plantations: vegetation control, seasonality and tree damage. *Agrofor Syst* 74:199–211
- McNaughton SJ (1983) Compensatory plant growth as a response to herbivory. *Oikos* 40:329–336
- Mitchell FJG (2005) How open were European primeval forests? Hypothesis testing using paleoecological data. *J Ecol* 93:168–177
- Motta R (1995) Dendroecology in ungulate forest damages: 1. Fraying scars. *Dendrochronologia* 13:33–41
- Motta R (1996) Impact of wild ungulates on forest regeneration and tree composition of mountain forests in the western Italian Alps. *For Ecol Manag* 88:83–98
- Motta R, Garbarino F (2003) Stand history and its consequences for the present and future dynamic in two silver fir (*Abies alba* Mill.) stands in the high Pesio Valley (Piedmont, Italy). *Ann For Sci* 60:361–370
- Mysterud A, Larsen PK, Ims RA, Østbye E (1999) Habitat selection by roe deer and sheep: does habitat ranking reflect resource availability? *Can J Zool* 77:776–783
- Navarro LM, Pereira HM (2012) Rewilding abandoned landscapes in Europe. *Ecosystems* 15:900–912
- Osem Y, Fogel T, Moshe Y, Ashkenazi M, Brant S (2017) Understorey structure and function following release from cattle grazing and overstorey thinning in Mediterranean conifer plantations. *Ann For Sci* 74:22. <https://doi.org/10.1007/s13595-017-0622-5>
- Ostermann OP (1998) The need for management of nature conservation sites designated under Natura 2000. *J Appl Ecol* 35:968–973
- Paluch J (2005) Spatial distribution of regeneration in West-Carpathian uneven-aged silver fir forests. *Eur J For Res* 124:47–54
- Peringer A, Buttler A, Gillet F, Patru-Stupariu I, Schulze KA, Supariu MS, Rosenthal G (2017) Disturbance-grazer-vegetation interactions maintain habitat diversity in mountain pasture-woodlands. *Ecol Model* 359:301–310
- Preiss E, Martin JL, Debussche M (1997) Rural depopulation and recent landscape changes in a Mediterranean region: consequences to the breeding avifauna. *Landsc Ecol* 12:51–61
- Redecker B, Härdtle W, Finck P, Riecken U, Schröder E (2002) *Pasture landscapes and nature conservation*. Springer, Berlin
- Redjadj C, Darmon G, Maillard D, Chevrier T, Bastianelli D, Verheyden H, Loison A, Saïd S (2014) Intra- and interspecific differences in diet quality and composition in a large herbivore community. *PLoS One* 9:e84756
- Romero-Calcerrada R, Perry GLW (2004) The role of land abandonment in landscape dynamics in the SPA ‘Encinares del rio Alberche y Cofio, Central Spain, 1984–1999. *Landsc Urban Plan* 66:217–232
- Rooney TP, Waller DM (2003) Direct and indirect effects of white-tailed deer in forest ecosystems. *For Ecol Manag* 181:165–176
- Schweingruber FH (1988) *Tree rings: basics and applications of dendrochronology*. D. Reidel Publishing Co, Dordrecht
- Senn J, Suter W (2003) Ungulate browsing on silver fir (*Abies alba*) in the Swiss Alps: beliefs in search of supporting data. *For Ecol Manag* 181:151–164
- Sitzia T, Semenzato P, Trentanovi G (2010) Natural reforestation is changing spatial patterns of rural mountain and hill landscapes: a global overview. *For Ecol Manag* 259:1354–1362
- Speed JD, Austrheim G, Hester AJ, Mysterud A (2011a) Growth limitation of mountain birch caused by sheep browsing at the altitudinal treeline. *For Ecol Manag* 261:1344–1352
- Speed JD, Austrheim G, Hester AJ, Mysterud A (2011b) Browsing interacts with climate to determine tree-ring increment. *Funct Ecol* 25: 1018–1023

- Storms D, Aubry P, Hamann JL, Saïd S, Fritz H, Saint-Andrieux C, Klein F (2008) Seasonal variation in diet composition and similarity of sympatric red deer *Cervus elaphus* and roe deer *Capreolus capreolus*. *Wildl Biol* 14:237–250
- Vanderberghe C, Freléchoux F, Moravie MA, Gadallah F, Buttler A (2007) Short-term effect of cattle browsing on tree sapling growth in mountain wooded pastures. *Plant Ecol* 188:253–264
- Vera FWM (2000) *Grazing ecology and forest history*. CABI Publishing, Wallingford
- Vila B, Guibal F, Torre F, Martin JL (2004) Assessing spatial variation in browsing history by means of fraying scars. *J Biogeogr* 31:987–995
- WallisDeVries MF, Baker JP, Van Wieren SE (1998) *Grazing and conservation management*. Conservation Biology Series. Kluwer Academic Publishers, Dordrecht
- Walther P (1986) Land abandonment in the Swiss Alps: a new understanding of a land use problem. *Mt Res Dev* 6:305–314