Similar functional structure and encroaching dynamics in two Juniperus species with contrasting distribution patterns

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Similar functional structure and encroaching dynamics in two *Juniperus* species with contrasting distribution patterns

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Abstract

In the valley of Espot, central Iberian Pyrenees, two congeneric species coincide in the same habitats, showing a similar encroaching ability as prostrate shrubs expanding on former rangeland. Out of this valley, however, while *Juniperus communis* is widespread from regional to Holarctic levels, *J. sabina* is only sparsely found in distant Palearctic regions, where it remains restricted to scarce locations.

We wanted to document and understand the dynamics of both species as drivers of landscape transformation, and to unveil which differences may explain their contrasting biogeographic performance. To do so, we analysed them in terms of plant traits, phenology, primary growth, population structure, and role in the landscape.

Juniperus communis and *J. sabina* showed similar strong investment to stress resistance, according to the functional traits measured (SLA, LDMC, leaf lifespan), although both species expanded laterally at similarly noticeable ratios. The populations of *J. communis* included more balanced age classes and showed good recruiting ability, which would enhance the active role of this species along secondary succession. In contrast, *J. sabina* showed a poor recruiting ability, with populations lacking juveniles and including notably old shrubs. Longevity and clonal performance in this species would explain its persistence only in particular locations scattered over distant regions. Therefore, contrasting plant distribution and ecology – i.e. widespread and active colonizer vs. secluded and relict– would be based on a few interspecific developmental and reproductive differences.

Keywords Plant traits, primary growth, population structure, relict species, shrub encroachment, Pyrenees.

Introduction

The expansion of shrubs into mountain grasslands has increased in many European regions in recent decades. This has been documented in a wide scope of mountain ranges, such as the Pyrenees, Guadarrama, the Cantabrian range, the Alps, the Apennines, the Carpathians, the Balkans and the Scandes (MacDonald et al. 2000; Dullinger et al. 2003; Sanz-Elorza et al. 2003; Roura-Pascual et al. 2005; Montané et al. 2007; Hallinger et al. 2010; Pedashenko et al. 2015). Shrub encroachment mostly responds to the abandonment of traditional practices, chiefly the extensive livestock grazing and the use of fire to create summer pastures (Lasanta-Martínez et al. 2005; Komac et al. 2013). Moreover, ongoing global warming is expected to promote the growth and expansion of shrubs and trees in temperature-limited mountain ecosystems (Körner and Paulsen 2004). Nevertheless, land use change is the main driver of encroachment and reforestation in anthropogenic treeless landscapes of Alpine mountain systems (Palombo et al. 2013; Ameztegui et al. 2016; Cudlín et al. 2017).

In the patched landscapes typical of the Pyrenees and similar ranges, shrub encroachment leads to subsequent woodland advance in secondary succession processes leading to the expansion of pine or deciduous forests, particularly noticeable over the last decades (Ninot et al. 2011; Álvarez-Martínez et al. 2014; Ameztegui et al. 2021). Shrublands are therefore mostly transient vegetation units at decadal scales, at least below the treeline.

Shrub encroachment is known to cause important changes in soil and litter properties in Alpine mountain ranges (Montané et al. 2010; Urbina et al. 2020; Gao et al. 2021) and in the structure and function of fungal communities (Wardle 2004; Lauber et al. 2008; Eldridge et al. 2011; Grau et al. 2019). Essential soil nutrients (N, P, K) mostly decrease under mature shrubland with respect to grassland, since they become sequestered in the biomass and necromass along secondary succession. This, together with low concentrations of N and P in the plant-soil compartments, high soil C:N ratio, and more relevance of arbuscular mycorrhizae and ectomycorrhizae, would favour the dominance of long-lived plant species, such as that of *Juniperus* and *Pinus* genera (Grau et al. 2019).

Shifts in soil properties and processes and the sharp light extinction at the ground level in these phases of secondary succession lead to reduction in plant species richness. A wide scope of stress-related, heliophilous and opportunistic grassland strategists become replaced by competitor or tolerant strategists, which promote community convergence across sites and habitats (Anthelme et al. 2009; Kesting et al. 2015; Boch et al. 2019; Gómez-García et al. 2023; Liu et al. 2023). On the other side, these encroaching plant communities, although species-poor, involve particular biodiversity and thus have an interesting conservation value (Anthelme

et al. 2009). For instance, birds and invertebrates related to *Juniperus* shrubland include specific taxa, as well as generalists of open habitats (Thomas et al. 2007; Giménez Benavides 2009; Montesinos and García 2009). Also fungal communities along the secondary succession occurring in this shrubland have been found specific and highly diverse (Grau et al. 2019). Therefore, the encroaching scrubs and the habitats occurring along succession include valuable biodiversity components and unique ecological processes, which justifies the inclusion of many of them in the European conservation directives (Giménez Benavides 2009; Montesinos et al. 2009). Acquiring functional knowledge on encroaching scrubs is thus a sine qua non condition to better understand and manage biodiversity and natural landscapes.

The Pyrenean valley of Espot provide the opportunity for analysing two congeneric species, *Juniperus communis* L. and *J. sabina* L., which foster active encroaching processes on the same open habitats (Ninot and Carrillo 2019). Despite their morphological and functional similarity, and the evidence of similar successional role in the area, these species differ markedly in their overall ecological and biogeographical distribution. While *J. communis* is a Holarctic widespread species, commonly building active populations in a wide range of habitats, *J. sabina* is restricted to a few scattered Palaearctic regions, where it forms apparently relict poor populations in open, dry mountain ecosystems. The analysis in parallel of these congeneric species is of particular interest to understand the vegetation dynamics dominated by widespread expanding shrub species within the worldwide encroaching processes, and the persistence strategy of rare relicts –useful in designing conservation policies.

The primary aim of this study is to understand the mechanisms driving the dynamics of the scrubs dominated by two congeneric species, *J. communis* and *J. sabina*, under the ongoing landscape changes in a mountain area, through a wide analysis of their plant traits, phenology, primary growth, population structure, and role in the landscape. Secondly, we want to unveil which differences may explain the contrasting biogeographic and ecological performance between these species, taking advantage of the fact that they play a similar ecological role in the study area. We hypothesise that, in spite of sharing a similar habit, performance and vegetation patterns, the wider distribution and colonization ability of *J. communis* could be driven by small advantages given by higher vegetative plasticity, better colonizing ability or stronger competitiveness. Conversely, the apparently lower performance of *J. sabina* should be compensated by other developmental or higher persistence traits, which would explain its success in the area studied.

Materials and Methods

The study species and sites

The two species studied are similar in most ecological and functional features, being both xeromorphic, slow-growing conifers which, in the area here considered, develop as diffuse or prostrate shrubs in the form of near-circular, expanding patches. Both species are dioecious and, as it is characteristic in the genus *Juniperus*, they produce berry-like cones (fruits hereafter) remaining on the plant for months. They are both resiniferous, heliophilous, and tolerant to contrasting temperatures. However, they clearly differ in their biogeographic status and also in the precise expression of morpho-functional traits (Adams 2004).

Juniperus communis L. is widely distributed through Eurasia and North America and from the Arctic to subtropical mountains. In the Pyrenees it is typically an erect shrub at lower or moderate elevations, but grows as a diffuse or even cushion-shaped shrub at higher elevations. The later forms are identified as subsp. *nana* (Wild.) Syme, while intermediate growing forms, found in the montane and the lower subalpine belts, correspond to var. *intermedia* (Schur) Sanio (Bolòs and Vigo 1984). The shrubs here investigated belong to this variety, which will be referred to as *J. communis* hereafter. It is particularly noticeable in encroaching pasture landscapes, where individuals encompass a gradation of habits and sizes, rarely exceeding 6 m in diameter. Although their branches are mostly prostrate, one or more stems grow upwards or oblique. Their leaves are acicular, and thus light is progressively filtered through the canopy.

Juniperus sabina L. is found sparsely from South-Western Europe to Mongolia, and from North Africa to the Alps. It occurs mainly in dry, continental mountains around the Mediterranean basin, being very rare or scattered in the Pyrenees (Adams and Schwarzbach 2006; Bolòs and Vigo 1984). However, it is common in the Espot valley and neighbouring areas (Central Iberian Pyrenees), where it plays a noticeable role on rocky slopes (Ninot and Carrillo 2019). Its growth form is clearly creeping, the main branches producing adventitious roots, and forming medium to large sized spots (i.e., 8-12 m diameter) tightly adapted to the topography. The younger shoots are tightly covered by scale-like leaves.

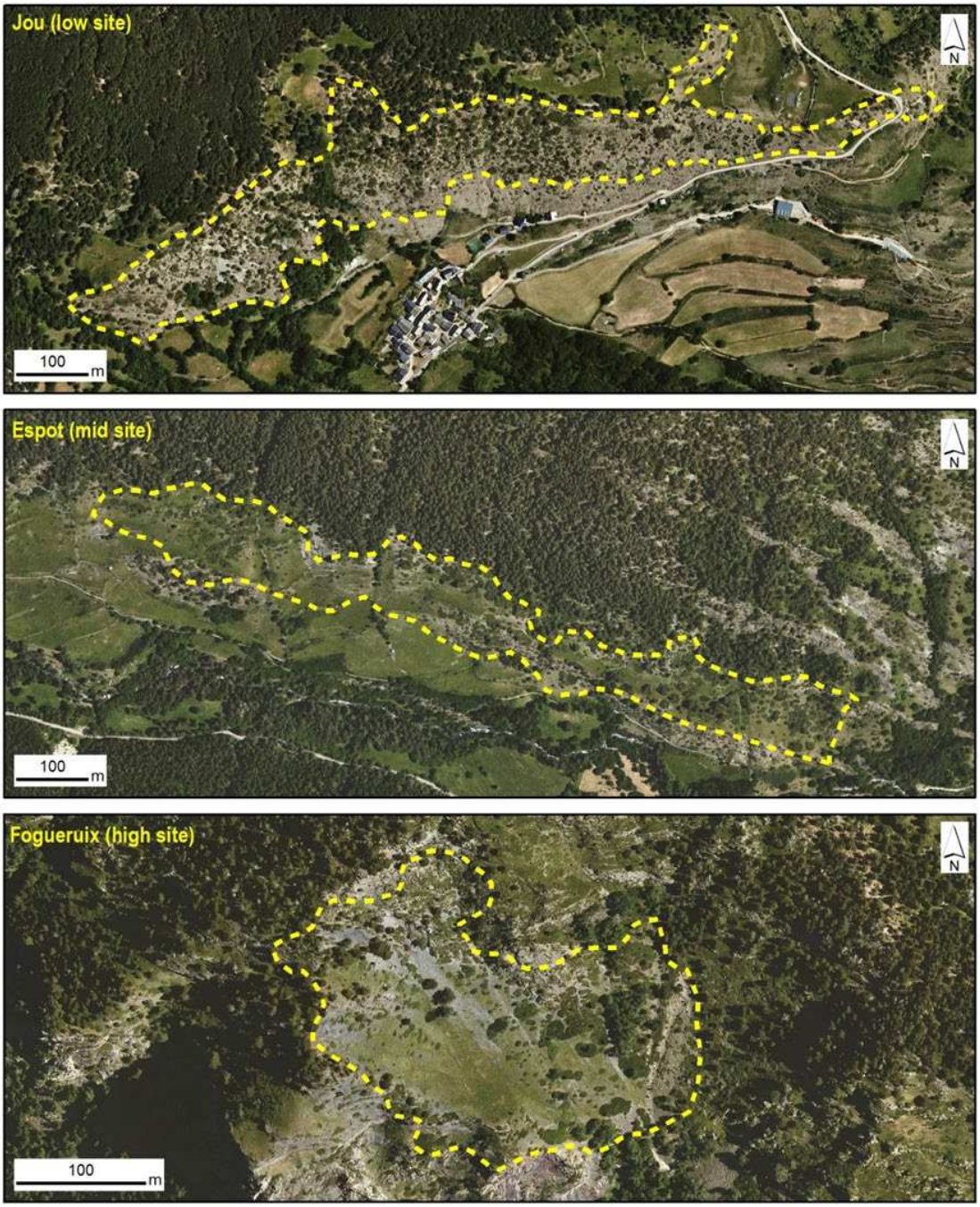

Fig. 1 Aerial ortophotographs of the three sites sampled (taken from Institut Cartogràfic i Geològic de Catalunya – Vissir3 (http://srv.icgc.cat/vissir3/, accessed until November 2023). The polygons outlined correspond to the landscape units considered, where we located the sampling shrubs and evaluated the significance per species.

This study was performed in the valley of Espot and surrounding areas, where *J. sabina* and *J. communis* coexist in open or patched landscapes built on slopes of varying parent material, inclination and rockiness –though always roughly south-facing. The area spans from 1300 to 2100 m a.s.l., which encompasses a clear bioclimatic gradient. This is expressed through key bioclimatic indicators, such as the length of the growing period, which shortens along the

elevation gradient from 7.5 to 5 months (Ninot et al. 2013); the annual rainfall average, increasing from 690 to some 1200 mm (Meteocat 2023); and the ratio between summer potential evapotranspiration and summer rainfall (mediterraneity index), which decreases from 1.3 to 0.9 (Ninot et al. 2013). In any case, the shrubs investigated mostly thrive on poorly developed soils, where the water supply is clearly lower than that assumed in general models for regular soils.

We sampled the shrubs in three locations along the elevation gradient described, named low, mid and high hereafter (Fig. 1). The low location was near the Jou village (1300-1450 m a.s.l.; 1.117140963 E, 42.601159343 N), where xerophilous grasslands (Xerobromion erecti (Br.-Bl. et Moor 1938) Zoller 1954) dominate the landscape, together with rocky shelves and *Pinus sylvestris* L. spots (Supplementary material, Fig. 9). The mid location lies over the Espot village (1500-1620 m a.s.l.; 1.061838142 E, 42.586179588 N) on a slope built from lime schists, and ranges from small rocky outcrops to eroded sloping patches, to gentle grassland relieves with xerophilous and meso-xerophilous pasture (Xerobromion erecti, Bromion erecti Koch 1926), and to expanding *P. sylvestris* dry forest (Supplementary material, Fig. 10). In both locations, traditional land use had enhanced extensive pasture and sown fields until few decades ago. Nowadays, the abandonment of rural activity has allowed *Juniperus* and other shrubs to encroach the open areas as part of the secondary succession leading to pinewoods of *Pinus sylvestris*. The high location, Fogueruix, is found upwards on the same main slope (1980-2100 m a.s.l.; 1.079102551 E, 42.591072140 N), where the shrubby patches alternate with schist rocky relieves, eroding dry pastures (Bromion erecti) and open stands of *Pinus uncinata* Ramond ex DC. (Supplementary material, Fig. 11). At these high elevations the traditional land use was extensive pasture and clear cutting, which decreased from mid XXth century on to the present abandonment.

Therefore, the three study sites were found in forest dominions, where encroachment of open areas by *Juniperus* and other shrubs is part of the secondary succession leading to pinewoods (Supplementary material, Fig. 12). In fact, most *Juniperus* spots included other shrub species or tree juveniles, which may indicate facilitation effects (Verdú et al. 2004). In the same line, young pinewood spots grown over older juniper layers are also part of these landscapes.

Functional structure

We measured relevant plant traits at different gradient positions, together with the mass distribution into leaves, stems and roots, to obtain a comprehensive knowledge of the functional structure of both *Juniperus* species. We assessed these plant functional traits (PFT)

177 in July 2014 from two populations per species, namely one at the high and other at the mid
178 sites for both.

179 As functional plant traits we measured canopy height, specific leaf area (SLA, mm^2/mg), leaf
180 dry mater content (LDMC, mg/g), and seed mass, following the protocols set by Pérez-
181 Harguindeguy et al. (2013). From 20 individuals per population, we measured canopy height
182 and collected twig samples. We used sets of five leaves per individual to obtain their fresh
183 weight, and scanned them to measure their area with the software *ImageJ* (Abràmoff et al.
184 2004). In the case of *J. sabina*, we considered as 'functional leaf' each small leafy twig, namely
185 the apical segment of each stem axis plus the scale-like leaves covering it; and calculated the
186 leaf area as twice the twig lateral projection. The dry weight was obtained after drying the leaf
187 subsamples at 60 °C for 48 hours. We calculated seed mass as the air-dried seed weight
188 average obtained from 10 sets of 20 (in *J. sabina*) or 40 (in *J. communis*) seeds each, of 10
189 individual plants per population and species.

190 We clipped the aerial biomass at the ground level within five distinct small square areas (40 ×
191 40 cm each) representative of the local shrub community in two locations per species (at the
192 mid and high elevation sites) to characterize the functional structure at population level. From
193 a significant part of each five field samples, we sorted separately leaves and stems of the focal
194 species. Specifically, from each 40 × 40 cm sample we analysed random shoots amounting at
195 least its 20% weight. Then we extrapolated the leaves and stems dry weight to the whole
196 sample to obtain average values for each shrub community.

197 We evaluated the root density and structure of both *Juniperus* species, taking advantage of a
198 comprehensive experiment that had been conducted the year before at the high elevation
199 site. For each species, in July 2013 we took four soil cores with a steel cylinder of 4 cm
200 diameter per 12 cm depth, in four distinct dense stands of scrub. In the laboratory, we dried
201 out the soil samples and, by means of sieves and gravity sorting, we gathered the root
202 segments from each soil core. Since this procedure neglected many tiny fragments of finer
203 roots, too difficult to be properly sorted for all the samples, we evaluated the neglected
204 fraction of fine roots from subsamples analysed under stereomicroscope. We ensured these
205 subsamples to be representative by homogenizing each sample and taking as subsample at
206 least 20% of each sample. Then, we washed all the root subsamples with distilled water to
207 release clay or other mineral debris, and separated all the root fragments obtained into two
208 size classes: coarse roots (> 2 mm in diameter) and fine roots (< 2 mm).

209 We obtained the dry mass of the subsamples of stems, leaves and roots after keeping them
210 during 48 h at 70 °C, and calculated their dry mass per ground area.

211

212 Root growth

213 We evaluated the production of new roots by an infilling experiment. We infilled the same
214 holes made when obtaining the soil cores for root measurements with new soil obtained from
215 the vicinity, after sieving it to avoid gravels, roots and other plant structures. This free soil was
216 placed in cylindrical sheaths made of 1 mm plastic mesh, 4-5 cm diameter and 12 cm length.
217 During their installation, we made sure the mesh filled the soil hole as much as possible. In
218 October 2013, we recovered these cylindrical sheaths with soil and the summer production of
219 new rootlets, and filled the holes with new cylindrical sheaths containing new free soil in the
220 same way as described above. These last cylinders were recovered one year later, in October
221 2014, and thus represented the root production in one year time.

222 We led all the soil samples air-drying in the laboratory, and then we sorted, washed and
223 obtained the dry weight of the root fragments as described in the previous section. All these
224 new root segments were fine (< 2 mm in diameter).

225

226 Canopy expansion

227 At the three sampling sites, we analysed the two study species for a size and age assessment
228 between autumn 2016 and spring 2017. We sampled eight shrubs per species, sex and site,
229 representative of the shape diversity found in each case (i.e., including small, medium sized
230 and large individuals in similar proportions). In each shrub we measured two diameters of the
231 canopy –one following the slope gradient, the other perpendicular–, its maximum height and
232 the basal diameter of the thickest stem. We then selected two ultimate prostrate branches
233 illustrative of the centrifugal expansion of the shrub –one upslope, the other downslope– to
234 analyse their primary growth as the elongation ratio per year. For this, we took one small stem
235 segment at 100 cm from the branch tip to the centre of the shrub. Complementarily, we noted
236 relevant site features per shrub sampled, namely slope, habitat –categorized as mesophilous
237 grassland, xerophilous grassland or rocky area– and the occurrence of other woody species
238 within the shrubby spot.

239 In the laboratory, we prepared the stem sections and counted the annual xylem rings of each
240 stem segment under stereomicroscope. From these data, we calculated the stem elongation
241 ratio or shrub radial growth per year in each case (hereafter primary growth of the shrubs).

242

243

244 Phenomorphology

245 We monitored the phenological performance of the two study species in the mid-elevation
246 site, where seasonality may be taken as intermediate among the elevation span of both
247 species in the area. Among the shrubby spots, we selected five male and five female
248 individuals per *Juniperus* species, representative of the morphological and size diversity in each
249 case. From spring to fall of years 2013 and 2014, we recorded roughly each 20 days the status
250 of the individuals selected through field notes, detailed photos and abundant samples of plant
251 material. This dataset was complemented profiting a few additional visits from spring 2017
252 until spring 2022. We then standardized the information gathered using the criteria and
253 phenophases drawn by Orshan (1989), while keeping other data (e.g., branching, leaf
254 shedding, herbivory damage, etc.) as complementary information. The analysis of herbarium
255 samples and detailed photos were used to refine or complement the field reports and to
256 evaluate the average leaf life span. This last trait was assessed according to the procedure set
257 by Pérez-Harguindeguy et al. (2013) for conifers, i.e. counting the number of leaf cohorts that
258 maintained more than half original leaves on ten distinct individuals per species, twice in the
259 middle of the growing seasons of 2013 and 2014.

260 In addition, we assessed fruit production in seven female individuals per species in the same
261 mid elevation site. In fall 2021, we clipped the fruiting branches included in three
262 representative square areas of 40 x 40 cm per individual, and counted the maturing fruits.
263 Then we obtained an individual value of fruit production in the form of fruits per square metre
264 of projected shrub canopy. Since *J. communis* is reported as masting species (Thomas et al.
265 2007), our evaluation has to be taken as a rough approximation, at least for this species.

266

267 Population structure

268 We characterized the population structure through the data collected from the shrubs
269 sampled for the analysis of canopy expansion, together with data from other shrubs measured
270 in the field, to an amount of 30-36 shrubs per species in each local population. The newly
271 measured shrubs were chosen to represent the variety of size and habitat occupied in each
272 case, and were characterized in terms of size –two perpendicular diameters–, sex, habitat, and
273 co-occurring species of trees or taller shrubs.

274 Moreover, we analysed the three sites sampled through aerial ortophotographs taken in 2016,
275 available from the on-line facilities of the Institut Cartogràfic i Geològic de Catalunya (Fig. 1;
276 ICGC 2020). After locating the shrubby spots sampled in the field, we evaluated the extension
277 of the whole populations of the two species studied within each local landscape.

Statistical analyses

For the comparison of PFT, aboveground structure, belowground structure and fruit production between the two species, we fitted simple linear models and used ANOVA. For the analysis of PFT and aboveground structure, we used shrub species (*J. communis* and *J. sabina*), site (high and mid) and their interaction as fixed effects. For the analysis of the belowground structure and dynamics, since there was a unique site per species, we only used shrub species as explanatory variable. We used log-transformations to reach the assumptions of normality and homoscedasticity. Where transformations did not work, we used the varIdent structure (Zuur et al. 2009) to account for heteroscedasticity. For the analysis of fruit production we used a non-parametric Wilcoxon sum rank test.

Finally, we fitted a linear mixed-effects model to assess the effect of several biotic and abiotic parameters on shrub canopy expansion and used ANOVA to test for significant effects. We fitted the model including primary growth of the branches as the response variable; stem diameter, shrub height and slope as covariates; and site (high, mid and low), shrub species, sex and their interactions as fixed effects. We included habitat (mesophilous grassland, xerophilous grassland and rocky slope) as a random factor. We checked that the random factor contributed to the model fits with AICc. We also tested whether the covariates contributed to the model fits with AICc. The only covariate included in the final model was slope. We visually checked for normality and homoscedasticity of residuals, and used Tukey post-hoc tests to test the differences between factor levels. We performed all statistical analyses with R v. 4.3.0 (R Core Team 2023), using the packages nlme (Pinheiro et al. 2019) for linear mixed-effects models, and emmeans (Lenth 2020) for post-hoc tests. We used ggplot2 (Wickham 2016) for graphical display.

Results

Functional structure

Referring to leaf traits, both *J. communis* and *J. sabina* had similarly low SLA (averaging 7.0 and 5.7, respectively) and high LDMC (416 and 470; Fig. 2a, b; Supplementary material, table 2). More in detail, there were significant differences in SLA and LDMC between species and sites. While *J. communis* had larger SLA than *J. sabina* at the mid site, they did not differ at the high site. For LDMC, we found site differences (higher values at the high site), which was particularly true for *J. communis*. At the mid site, the pattern was opposite than SLA, with *J. communis* showing lower values than *J. sabina*. Again, there were no differences between species at the high site.

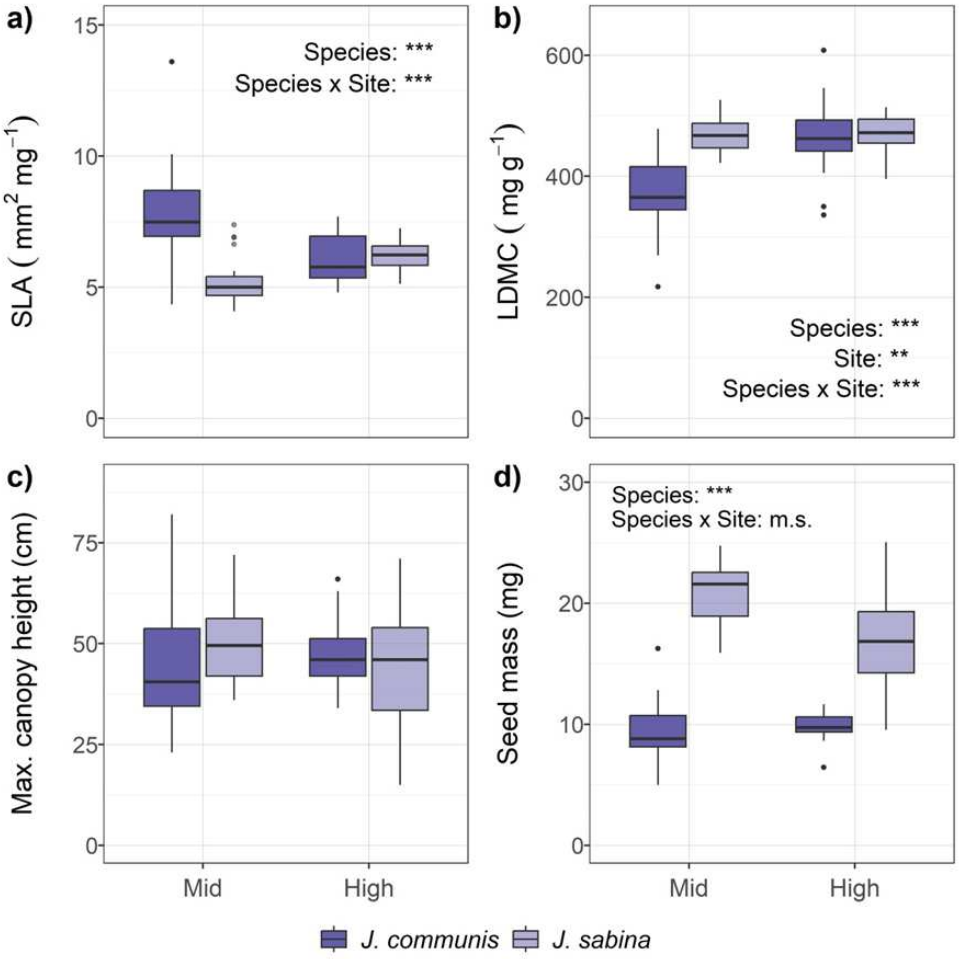

Fig. 2 Plant functional traits studied for each *Juniperus* species at each site (high and mid elevation; obtained from 20 individuals per site, 10 in the case of seed mass). Asterisks indicate significant differences at P<0.01 (***) and at 0.05>P>0.01 (**); m.s. indicate marginally significant differences at 0.10>P>0.05.

There were no significant differences between sites or species for canopy height, ranging in average from 45 cm to 51 cm (Fig. 2c; Supplementary material, table 2). Seed mass differed between species, with *J. sabina* producing seeds twice bigger than *J. communis* at both sites (19.1 mg *vs*. 9.6 mg in average; Fig. 2d; Supplementary material, table 2). Differences between species were larger at the mid site than at the high site.

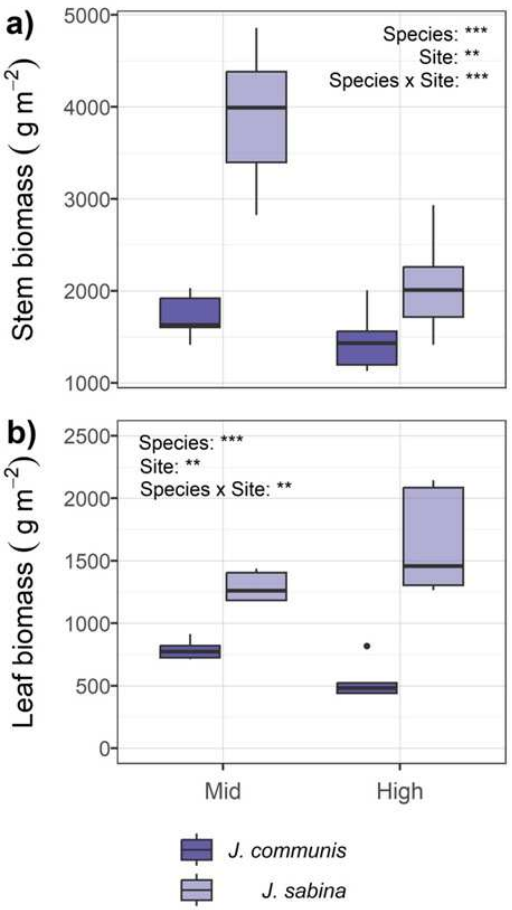

Fig. 3 Aboveground biomass of the two *Juniperus* species at each site (high and mid elevation), expressed as dry weight of stems (a) and leaves (b) per square metre covered. Asterisks indicate significant differences at P<0.01 (***) and at 0.05>P>0.01 (**).

Table 1 Results of the LME ANOVA testing for differences in functional traits (PFT), aboveground structure, belowground structure and dynamics, and fruit production. Significant p-values (P<0.05) are shown in bold.

	Species			**Site**			**Species × Site**		
	df	**F**	**P**	**df**	**F**	**P**	**df**	**F**	**P**
1. PFT									
Height	1,76	0.44	0.508	1,76	0.23	0.636	1,76	2.23	0.140
SLA	1,76	23.58	**<0.001**	1,76	0.81	0.372	1,76	27.07	**<0.001**
LDMC	1,76	26.29	**<0.001**	1,76	4.99	**<0.029**	1,76	18.49	**<0.001**
Seed mass	1,34	71.41	**<0.001**	1,34	0.80	0.379	1,34	3.67	0.064
2. Aboveground structure									
Stem biomass	1,16	21.41	**<0.001**	1,16	8.15	**0.012**	1,16	10.59	**0.005**
Leaf biomass	1,16	89.69	**<0.001**	1,16	4.92	**0.041**	1,16	7.98	**0.012**
3. Belowground structure and dynamics									
Coarse root biomass	1,6	0.19	0.680	-	-	-	-	-	-
Fine root biomass	1,6	0.04	0.853	-	-	-	-	-	-
Summer root growth	1,6	3.04	0.132	-	-	-	-	-	-
Annual root growth	1,6	1.42	0.287	-	-	-	-	-	-
4. Fruit production									
Number of fruits	W=49		**0.002**	-	-	-	-	-	-

Both species built up dense shrubby communities considering their low canopy height (dry weight of aboveground biomass averaging 2,256 g/m^2 in *J. communis,* and 4,451 g/m^2 in *J. sabina*), with leaves playing a noticeable role (25-44% of dry weight; Fig. 3; Supplementary material, table 3). The differences between species, with *J. sabina* forming denser shrubs –referring to both stems and leaves–, were significant; and so were differences between sites, with lower biomass values at the high site (Table 1, Fig. 3). Root system was similarly dense in both species (dry weight in the upper 12 cm soil level 2,801 g/m^2 in *J. communis,* and 2,512 g/m^2 in *J. sabina*) and it was similarly partitioned in both species into coarse and fine roots (Fig. 4a,b; Supplementary material, table 3).

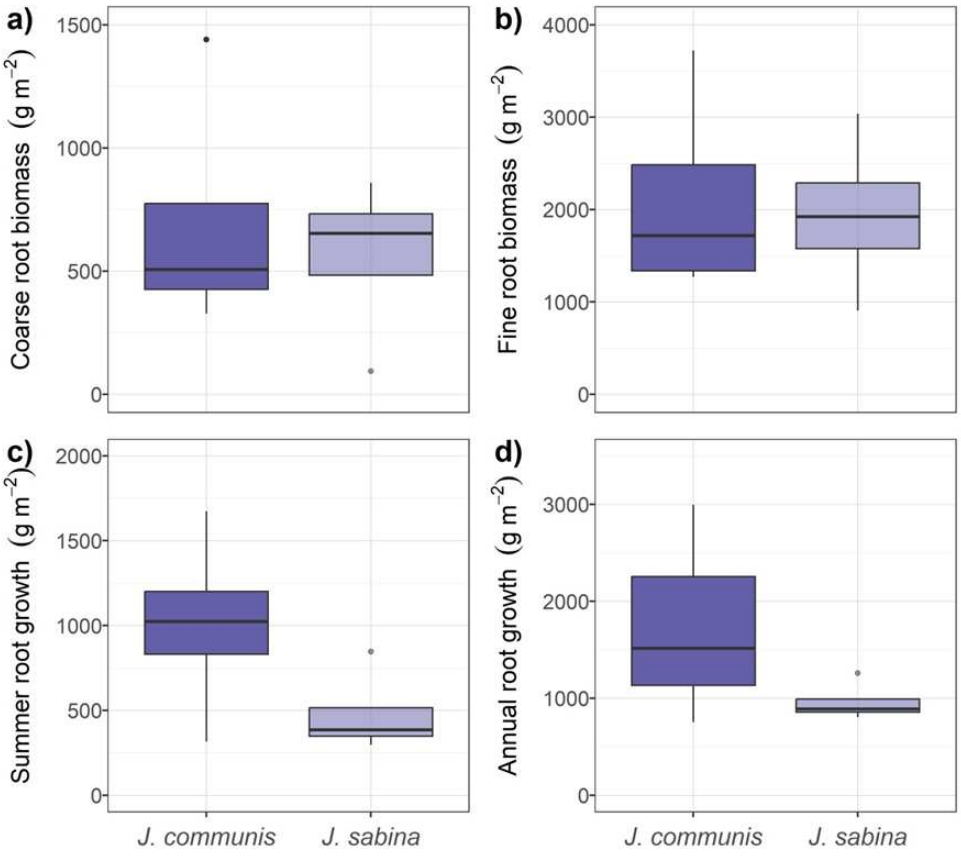

Fig. 4 Belowground biomass of the two *Juniperus* species at the high site, expressed as dry weight per square meter ground, found in the shallow (0-12 cm) soil level. The upper half of the figure corresponds to coarse (a) and fine (b) roots found in undisturbed shrubs, whereas the lower half shows the fine roots produced in an infilling experiment during summer (c) and yearly (d). We did not find differences between species (see main text and Table 1).

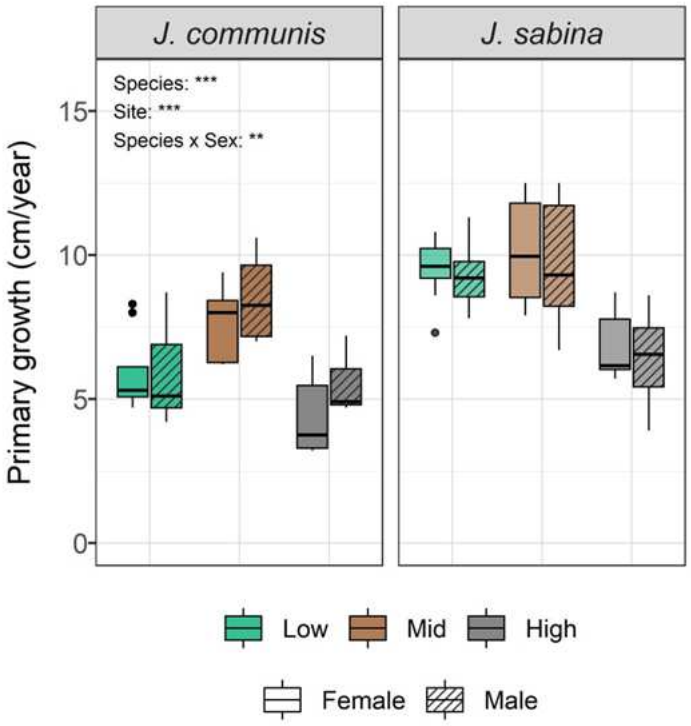

Fig. 5 Expansion of the two *Juniperus* species at each site, expressed as cm of shoot elongation per year. Data for female individuals are shown at the left side of each boxplot pair, and data for male individuals at the right. Significant differences obtained with the model including both species are shown at the left panel. Asterisks indicate significant differences at P<0.01 (***) and at 0.05>P>0.01 (**).

Shrub aboveground and belowground growth

The growth during the last decades of the shrubs measured (n = 88) varied according to species and sites. Centred in the canopy expansion, i.e. the primary growth or elongation of the prostrate branches, our model showed significant effects for site ($F_{2,70}$ = 40.21, P < 0.001), species ($F_{1,70}$ = 66.67, P < 0.001) and a for the species × sex interaction ($F_{1,70}$ = 5.93, P = 0.017; Fig. 5). Primary growth was larger in *J. sabina* than in *J. communis* (mean ± SD values of 8.71 ± 0.31 cm/year and 6.48 ± 0.30 cm/year, respectively; more data in Supplementary material, table 4), both for males and females. Within each species, we found only a trend for higher growth in females than in males of *J. sabina* (post-hoc Tukey = 0.051). Post-hoc tests showed that primary growth at the high site was significantly lower than at the mid (P < 0.001) and at the low sites (P < 0.001), but there were no significant differences between the mid and the low sites (P = 0.963).

Regarding belowground growth, *J. communis* seemed to be more active in producing new roots than *J. sabina*, although it also showed higher variability. In the high elevation site, *J. communis* produced in one year new fine roots equivalent to 83% of the average biomass of fine roots per soil volume found in established shrubs, mostly during summer (57%), while *J. sabina* produced half (49%) the fine root density found in established shrubs, half of which

398 being grown in summer (Fig. 4c, d; Supplementary material, table 3). However, there were no
399 significant differences between species due to the large data spread within each of them
400 (Table 1).

401 According to the field measurements and observations, the performance of the two species
402 differed in a few key aspects. Referring to the size of the shrubs and the thickness of their main
403 stem, in *J. communis* the larger shrubs included one or more thick main stems (e.g., larger than
404 40 mm in diameter, eventually larger than 60 mm). Contrastingly, the larger *J. sabina* shrubs
405 were based on medium-sized stems (mostly 20-40 mm in diameter; Supplementary material,
406 Fig. 13). This difference reflects that most *J. communis* shrubs maintained main stems along
407 their lifespan, whereas in larger *J. sabina* shrubs the older stems had vanished. In a few of
408 them this resulted into an empty gap in the central part of the shrub.

409

410 Phenomorphology

411 The integration of the data gathered has been synthesised as a simplified phenogram for each
412 species (Fig. 6). Both species performed similarly through long phases, which was particularly
413 apparent for shoot elongation. This process took place from early spring to the beginning of
414 winter, with some delay in *J. sabina* with respect to *J. communis*. Leaves were similarly long-
415 lived in both species, with an estimated average lifespan of 42 months for *J. sabina* and 38
416 months for *J. communis*. Regarding flowering, while *J. communis* performed a rather massive
417 blossom in spring, *J. sabina* produced cones from mid-summer to the following early spring –
418 but not in true winter–, although at low density and showing substantial variation between
419 individuals and years. Fruit ripening occurred earlier in *J. communis* than in *J. sabina*, but in the
420 first species fruits remained on the plant for longer time.

421 As for fruit production, *J. communis* produced significantly more fruits than *J. sabina* in 2021
422 (W=49, P=0.002). In average, we recorded more than ten-fold fruits in the former than in the
423 later (2681 ± 1012 fruits/m^2 *vs.* 161 ± 208 fruits/m^2, mean and standard deviation,
424 respectively; Fig. 7). In fact, during the two years of phenomorphological monitoring, a similar
425 lower fruit production and higher individual heterogeneity were observed in *J. sabina* with
426 respect to *J. communis*.

427

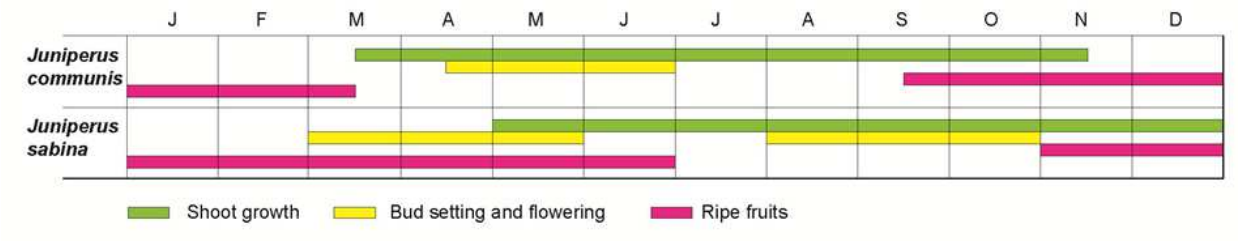

Fig. 6 Phenology of the two *Juniperus* species, regarding vegetative and reproductive main phases.

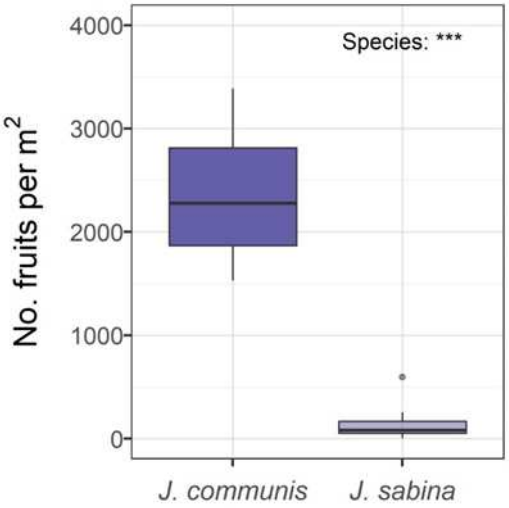

Fig. 7 Fruit production (2021) by the two study species, expressed as number of fruits per square metre covered by the shrubs. Asterisks indicate significant differences between the species (P<0.01).

Population structure

The populations of *J. communis* found in the three sites included higher proportion of shrubs of small to medium sizes than those of *J. sabina* (Fig. 8a, c, e). On the contrary, the populations of *J. sabina* stood up by larger shrubs, particularly at the mid and high elevation sites. There, one third of the *J. sabina* shrubs covered more than 100 m^2 each –an area not reached by any of the *J. communis* shrubs measured (Supplementary material, Fig. 13). Indeed, in a few cases we had to discard analysing some groups of poorly detached *J. sabina* spots because we could not ensure their distinctiveness, i.e., they might correspond to a single very large clone progressively fragmented.

When transforming the size-based population structure into an age structure (according to the average radial growth for each species and site given above), we observed similar patterns, with *J. communis* showing a larger proportion of young shrubs and *J. sabina* showing more old shrubs –although the difference is less apparent than that based on size (Fig. 8b,d,f). In any case, the age structure of both species revealed noticeable recruitment during the last decades. In the case of *J. communis*, the maximum recruitment would have occurred 20-30

years ago in the low and mid elevation sites, whereas at the higher site the maximum would have peaked 40-50 years ago. Recruitment in *J. sabina* showed less pronounced maxima, although the pattern was similar.

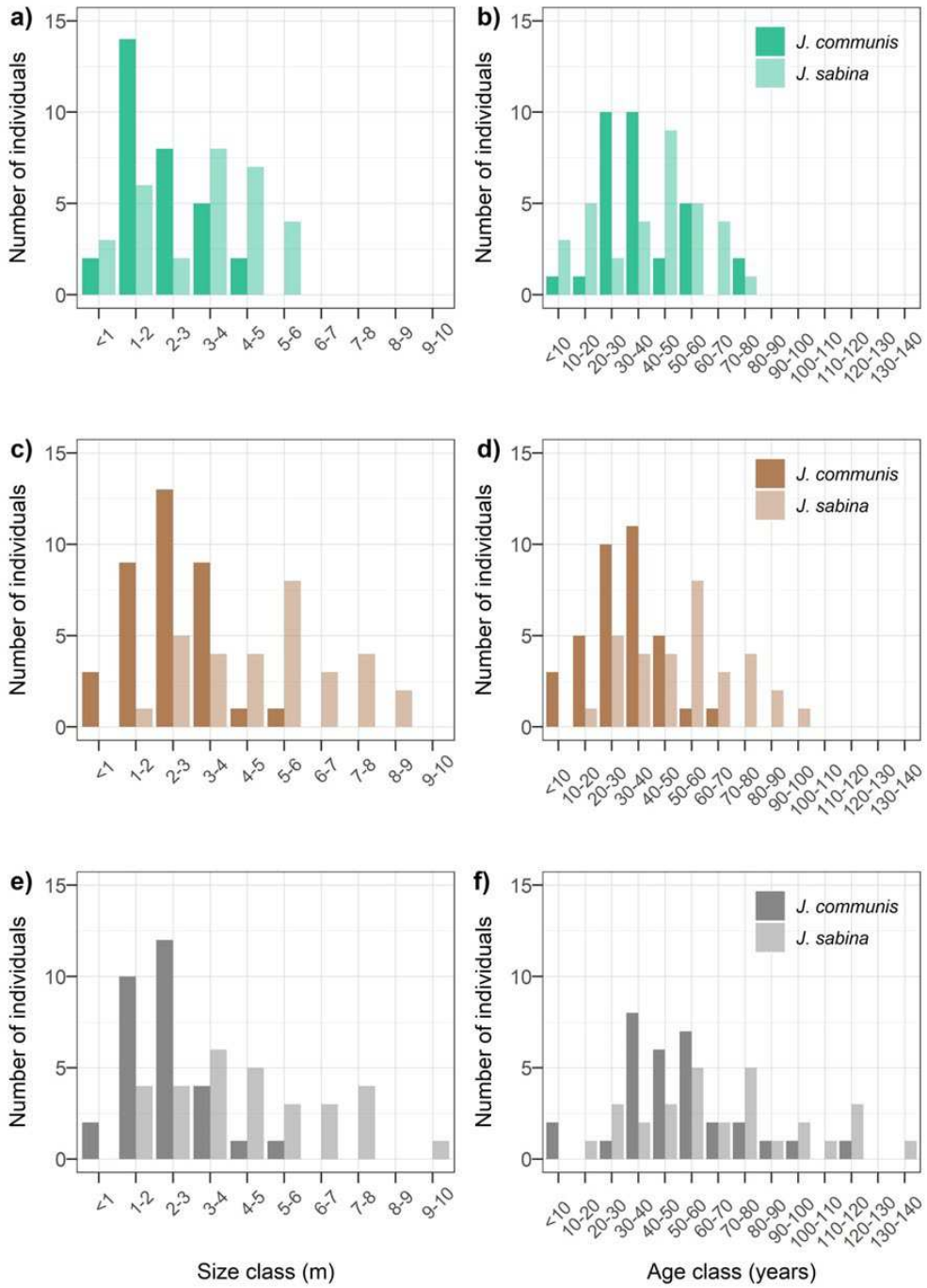

Fig. 8 Population structure of the two *Juniperus* species across the three locations sampled (from top to bottom, low, mid and high elevation sites), expressed as number of individuals distributed into size classes as average of radiuses (left panels, a,c,e), and into decadal age intervals (right panels, b,d,f).

Both *Juniperus* species showed a varying cover in the three landscapes considered. From the low to the high sites, *J. communis* occupied 2.2%, 3.0% and 5.9% of each local area, and *J. sabina* occupied 3.7%, 6.7% and 15.7% of each area (Fig 1; Supplementary material, table 5). Their cover would have sharply increased during the last decades, as illustrated in section 3.2. Namely, considering the radial expansion rates calculated, the shrubs of *J. communis* analysed would have increased their local cover 82.1%, 123.6% and 53.7% during one decade (from 2007 to 2017) in the low, mid and high sites, respectively; while the values for *J. sabina* would have been 62.9%, 45.8% and 39.1%. Note that, although *J. sabina* showed higher relative cover and individual expansion rates than *J. communis* everywhere, the second species achieved higher percentage increases of global cover, since it was represented by smaller shrubs, where relative growth was higher.

Through the distinct structure of these landscapes, *J. sabina* was more frequently found on rocky areas or on xerophilous grassland –including outcrop spots– while *J. communis* was more frequent in mesophilous grasslands (Supplementary material, table 5).

Discussion

Subtle functional differences may result into contrasting species behaviour

Both *J. communis* and *J. sabina* strongly invest in leaves resistant to stress through distinct morphology and structure, according to the leaf trait values obtained (SLA, LDMC, lifespan; Westoby et al. 2002; Pérez-Harguindeguy et al. 2013). The small differences found between species stand for stronger investment in stress resistance in *J. sabina*, and may be related to the distinct leaf morphology between both species. Also, higher variance in SLA and LDMC in *J. communis* (Fig. 2; Supplementary material, table 2) may reflect slightly higher developmental accommodation ability in this species, which is known for its noticeable phenotypic plasticity (Thomas et al. 2007). In any case, the distinct leaf functional structure allows both species to cope with summer drought stress (Grime 2006; Alonso-Forn et al. 2020), which is more noticeable in the low location, and with frost events, which are relevant in the high location. Both stressing drivers would have shaped the *Juniperus* functional morphology, since most species in this genus are associated to environments defined by these factors (Adams 2014).

In the populations studied at the mid elevation site, plants maintained growth –shoot elongation and production of new leaves– through long seasons, i.e. eight months. This necessarily means a slow growth ratio –i.e., 1 and 1.2 cm per month in average in *J. communis* and in *J. sabina*, respectively–, which goes in line with the costly production of such leaves along primary growth (Westoby et al. 2002; Onoda et al. 2017). However, the long growth

period allowed a noticeable annual growth for the shrubs, amounting from 4.8 to 9.9 cm of primary growth (i.e., radial expansion) depending on species and sites. These values were higher in *J. sabina* across all sites, apparently due to its distinct growth pattern. In this species, primary growth occurred much noticeably in centrifugal expanding branches, which become progressively rooted that ultimately produce a layering shrub. In *J. communis*, growth was more diverted between diffuse or vertical main trunks and radially expanding branches, which depend on those trunks (Supplementary material, Fig. 9, 10 and 12). This would reflect a strategic morphology in *J. sabina* more focused on clonal growth and eventually on vegetative multiplication, compared to *J. communis.* Thus, the former species would exhibit better adaptation to the effects of disturbance on the aerial parts, such as fire, cutting or extreme drought, which would compensate its lower recruiting ability (Bellingham and Sparrow 2000).

The phenological delay in the growth of *J. sabina* with respect to *J. communis* suggests that the former needs warmer temperatures than *J. communis* to start vegetative growth and reproduction phases. However, the expanding rates showed a similar pattern in both species along the elevation gradient, i.e. similar growth rates in the mid and low elevation sites (Fig. 5). Thus, *J. sabina* growth was not enhanced by warmer conditions at the low site, likely due to the stronger effect of summer drought events occurring there, which would limit or interrupt growth (Arzac et al. 2016). Flowering phenology and fruit yield differed sharply between the two species. Contrasting with the seasonal blooming in *J. communis*, *J. sabina* showed a diffuse seasonal distribution in producing male and female cones (i.e., from late summer to the following spring, with winter interruption) and irregular behaviour between individuals, at least in the mid location. This would contribute to the much lower fruit yield observed in *J. sabina*, which has been also reported from other regions (Benito Matías et al. 2012b).

Dioecy seemed not to affect the fitness of female individuals in the *Juniperus* here studied; i.e., no clear growth differences have been found between sexes in any location, even in *J. communis*, where female individuals produced so many fruits. This is in contrast with the majority of woody dioecious species, although in some cases there is no evidence for better vegetative performance in male plants (Marion and Houle 1996; Obeso 2002; Bañuelos and Obeso 2004). For instance, in *Juniperus thurifera* different responses between sexes to experimental fertirrigation suggest that females compensate the cost of sexual reproduction via differential plastic responses, without affecting vegetative growth (Montesinos et al. 2012).

The two *Juniperus* species studied are known for the low rates of seedling emergence, which has been related to diverse dysfunctions of seed production and to fruit predation by vertebrate and invertebrate fauna (García et al. 2000; Wesche et al. 2005; Benito Matías et al.

2012a, 2012b). Nevertheless, the much higher fruit production of *J. communis* may translate into a higher ability in expanding its populations. In the study sites, the populations analysed included noticeable proportions of young and moderately aged shrubs of *J. communis*, as it has been described in other Pyrenean and Atlantic locations (García et al. 1999). Moreover, this species settled on distinct habitats, from rocky spots to grassland and scrub; indeed, young individuals were frequently found within *J. sabina* scrubs. This may have favoured the higher ecological ubiquity observed in the *J. communis* populations, both at local and regional scales (Carrillo and Ninot 1992; Florapyr 2023).

Contrastingly, young individuals of *J. sabina* were very scarce (apparently absent from the mid and high sites). Similar population age structure has been reported from other regions, where it has been assumed to respond to low fertility of the seeds and to competence from herbaceous vegetation and herbivory (Wesche et al. 2005; Benito Matías et al. 2012b). Thus, heavier seeds in *J. sabina* would not result, through more vigorous seedlings, in higher settling ability. In fact, most *J. sabina* individuals had their origin in rocky outcrops (pers. obs.; Supplementary material, table 5) –from where they currently progress across pasture. This suggests a lower competitive ability of the seedlings of this species within established vegetation, which would turn the rocky spots to be the most suitable microhabitat where to set on.

The lower recruiting ability in *J. sabina* may be compensated by higher longevity provided by clonal development. Ageing of shrubs in this species is generally accompanied by the loss of the oldest aerial parts, thus persisting for decades or even centuries through slow renewal of the entire plant and layering growth (Wesche et al. 2005; De Witte and Stöcklin 2010). The rich rooting system formed by their creeping branches counterbalances the senescence of the older parts of the stems, which would facilitate the horizontal expansion and the re-colonization of eventual gaps within the shrub. In *J. communis*, the combination of keeping the main axes for long and lacking adventitious rooting leads to ageing of the entire shrub and senescence (García et al. 1999), which may be compensated by higher fruit production and new plant recruitment.

Settling on open habitats and encroaching landscapes

The occurrence and spread of *Juniperus* spots in the study area is apparent when comparing orthophotographs taken along the last 70 years (Ninot and Carrillo 2019). The sharp abandonment of the traditional land use during the last decades has promoted the re-colonization of open habitats by remnant populations of the two *Juniperus* species, resulting in

570 the decrease or fragmentation of these open areas. The re-colonization most likely originated
571 from sparse individuals remaining in the rocky spots found in these mountain landscapes.
572 This process must have progressed differently in the distinct localities analysed. The lower and
573 mid elevations formerly corresponded to mosaics of grassland and sown fields, interspersed
574 with rocky spots. These agro-pastoral mosaics were still present until the mid XXth century,
575 when they became extensive rangeland with noticeable ovine grazing until a few decades ago.
576 In contrast, the high elevation area was never cultivated, and was kept as a patchwork
577 rangeland of pasture, shrubs and trees until some decades ago (Fig. 1). Then, vanishing
578 domestic herbivores has involved much lower herbivory by wild ungulates only (Ninot et al.
579 2011; Ameztegui et al. 2021). The three localities studied also differ in various ecological
580 factors correlated with the elevation gradient (Ninot et al. 2013). From the lower to the higher
581 sites, the growing period becomes shorter –thus vegetation dynamics may have proceeded
582 slower– and biotic interactions such as plant-plant interactions and faunal fruit dissemination
583 may be less pronounced. This is in accordance with the lower shrub growth and expansion
584 found in both *Juniperus* species at the high elevation site (Fig. 5; Arzac et al. 2016) and with the
585 age structure of the populations studied (Fig. 8).
586 At the landscape level, the two species expanded during the last decades at varying rates, but
587 cumulative growth led them to reach a significant ground cover, depending on the sites
588 (Supplementary material, table 5). The expanding shrubs have facilitated the occurrence of
589 taller shrubs or tree saplings protruding from their canopy, especially in the low and mid sites,
590 and more in the case of *J. sabina* than in *J. communis* –in spite of the denser canopies of the
591 former species (Fig. 3). Most of the facilitated plants may have arrived by ornithochory, since
592 birds use these shrubs as a perch on open landscapes at medium to long distance (Verdú et al.
593 2004; Adams 2014), and may have succeeded establishing within the moderately shaded
594 environment of the shrub. Even for *J. sabina*, taller recruits of other species do not appear to
595 be detrimental to the nurse individuals at the midterm, as was also found by Verdú et al.
596 (2004). However, growing tree canopies of *Pinus sylvestris, P. uncinata* or *Betula pendula*
597 reduce the former dense scrub into vanishing etiolated understory populations in both species
598 (Supplementary material, Fig. 12). Along the process of shrub expansion and densification
599 across grasslands, the *Juniperus* species investigated filter the occurrence of understory
600 species, preventing or limiting the importance of those most abundant in open habitats, and
601 promoting those of shrubland or forest (Ninot and Carrillo 2019; Gómez-García et al. 2023). As
602 for the fungal soil communities, Grau et al. (2019) showed that those of encroaching *Juniperus*
603 shrubs in the high site become more related to forest communities than to grassland

communities along succession. The OTUs composition under mature shrubs becomes similar between *J. communis* and *J. sabina,* although showing higher OTUs richness and space heterogeneity in the former. In any case, shrub encroachment by *Juniperus* spp. definitively leads to afforestation in a clear example of secondary succession, through the facilitation of forest-building shrubs and trees and substantial changes in soil characteristics and processes, and in associated biodiversity.

Shrub persistence over dynamic landscapes

In Pyrenean landscapes under traditional use, scrubs such as those built by *Juniperus* species may have persisted on rocky outcrops of the slopes, such as shelves or steeper areas, with grazing and occasional fires preventing their expansion over pasture (Lasanta-Martínez et al. 2005; Roura-Pascual et al. 2005; Ninot et al. 2017). These habitats may have been also their primeval location, when woodland dominated most mountain areas prior to 3.5 millennia ago (Rodríguez-González et al. 2023). The decline in traditional land use would have given way to scrub densification in these rocky relieves and to its expansion through grassland on gentler areas (Gómez-García et al. 2023; Komac et al. 2013). However, landscape dynamics during the last decades in the Pyrenees and similar mountains have kept low land cover of shrub units. This is due to the fact that shrub gains in cover due to grassland encroachment are counterbalanced by losses through shrubland forestation (Monje 2003; Ferré et al. 2005; Roura-Pascual et al. 2005). Thus, although shrubland cover undergoes spatial changes, shrub communities may remain a minority in these mountain landscapes.

Juniperus communis scrubs widely encroach distinct ecosystems across temperate Europe and in Mediterranean mountains (García et al. 1999; Thomas et al. 2007; Benito Matías et al. 2012a). Over these areas, this species follows landscape changes thanks to good colonization ability and space occupancy, as discussed above. These dynamics counterbalance the vanishing populations under growing trees. At the local scale, the case of *J. sabina* seemed to follow similar dynamics, but the whole geographic distribution of this species –only common in the study area but very sparse in the Pyrenees, and irregularly distributed along its entire range– may be related to its contrasting population behaviour. Concretely, the dominance of medium-sized to very large adults and the scarcity or lack of regeneration classes of *J. sabina* in the area studied and in other regions (Wesche et al. 2005; Benito Matías et al. 2012b) suggests that the populations of this species follow the model of persistence through longevity and vegetative regeneration. This would be consequence of its clonal development, an adaptive ability very scarce within the genus *Juniperus* and in conifers in general (Adams 2014). Thus, the

acquisition of clonality in *J. sabina* may have played a relevant role in the persistence of its populations. In fact, the ability of adventitious rooting and eventual clonality has been described at population level for other conifers, and even for *J. communis,* when found in suboptimal habitats (Houle and Babeaux 1994; Payette et al. 1994; Thomas et al. 2007). In this way, *J. sabina* populations may have persisted on a few Pyrenean abrupt south-facing slopes through environmental stress tolerance and facing moderate disturbance –goat and sheep browsing, fire, erosion– anchored in rocky places. In spite of its low success in recruitment and in colonizing new habitats, these remnant populations should have acted as a source for eventual recruitment and expansion into neighbour habitats. This would occurred through appropriate ecological windows (Eriksson 1996; Bellingham and Sparrow 2000; García and Zamora 2002), as evidenced in the populations of *J. sabina* here studied and in those scattered in the Pyrenees (FLorapyr 2023). As in other relict or rare species, this strategy has proven to be effective in conferring inertia to populations that persist locally, despite their apparent reproductive collapse (Picó and Riba 2002; García 2008). This can allow many plant species to persist regionally for long time spans, even up to several millennia (Médail and Quézel 1997; García and Zamora 2002), which significantly contributes at explaining the maintenance of high plant diversity on mountain regions such as the Pyrenees.

Credit authorship contribution statement

Josep M. Ninot: Conceptualization, Methodology, Investigation, Resources, Writing – original draft, Writing – review & editing, Project administration. **Alba Anadon-Rosell:** Conceptualization, Methodology, Formal analysis, Investigation, Writing – original draft, Writing – review & editing. **Aldo Molino:** Investigation, Resources, Formal analysis. **Oriol Grau:** Conceptualization, Methodology, Investigation, Resources. **Moisès Caminal:** Investigation, Resources. **Amanda Casanovas:** Investigation, Resources. **Empar Carrillo:** Investigation, Resources.

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