1 Uncoupled changes in tree cover and field layer vegetation at two

2 Pyrenean treelines over 11 years

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9 *Abstract*

10 **Background:** The alpine treeline ecotone has been regarded as a sensor of the effects of global
11 changes on alpine plant communities. However, little is known about how treeline dynamics influences
12 the diversity and composition of alpine plant communities. Such information is necessary to forecast
13 how ascending subalpine forests may affect the composition of alpine flora.

14 **Aims:** We analysed the temporal variations in tree cover, plant diversity and composition, and the
15 influence of tree cover dynamics on field layer vegetation over a period of 11 years, in two alpine
16 treeline ecotones in the Spanish Pyrenees.

17 **Methods:** Tree and field layer vegetation was sampled in permanent transects in 1998 and 2009
18 using the point-intercept method. Temporal changes in tree cover, plant species richness and
19 abundance were characterized along the ecotone by using a randomisation approach, rarefaction
20 curves, and a non-parametric multivariate test, respectively.

21 **Results:** Tree cover increased significantly at one of the sites, whereas plant species richness only
22 increased at the other site where tree cover had not changed. The vegetation composition changed
23 significantly at both sites, but it was not spatially coupled with changes in tree cover along the ecotone.

24 **Conclusions:** An increase of tree cover does not necessarily trigger changes in the alpine community
25 during study periods of a decade. The results challenge our ability to infer short-term biodiversity

26 impacts from upslope advance of subalpine forests. Integrated tree field layer monitoring approaches
27 are necessary to produce a better understanding of the impact of ongoing global change on treeline
28 ecotones.

29

30 Keywords: alpine diversity; climate change; forest limit; land-use change; monitoring; multivariate
31 analysis; national parks; *Pinus uncinata*; species accumulation curves; subalpine forest; treeline
32 ecotone

33

34 *Introduction*

35 Climatic warming is causing the extension of plant species' ranges towards higher altitudes
36 and shifts in community composition in mountain regions during the last decades (Grabherr et al.
37 1994; Peñuelas and Boada 2003; Walther 2003; Walther et al. 2005; Cannone et al. 2007; Lenoir et al.
38 2008, Kullman 2010, Pauli et al. 2012). The alpine treeline ecotone has been regarded as a reliable
39 sensor of such climatic effects worldwide (e.g. Hessler and Baker 1997; Baker and Moseley 2002;
40 Kullman 2002; Daniels and Veblen 2003; Camarero and Gutiérrez 2004; Gehrig-Fasel et al. 2007;
41 Mathisen et al. 2013), because its highest altitudinal limit is expected to be climatically determined by
42 a thermal threshold (Jobbagy and Jackson 2000; Körner and Paulsen 2004; Holtmeier and Broll
43 2005). Therefore, treelines should respond to ongoing climate warming by shifting upwards (Grace et
44 al. 2002). However, some studies have reported tree cover increases within the ecotone while minor
45 or no upward advances occurred, especially during the second half of the twentieth century (Camarero
46 and Gutiérrez 2004). The reduction of grazing activities has also promoted forest re-growth (Motta and
47 Nola 2001; Holtmeier 2003; Ninot et al. 2008; Ameztegui et al. 2010), and it might override the effects

of climate warming in treeline ecotones that have been historically below their natural threshold due to grazing (e.g. Gehrig-Fasel et al. 2007; Batllori and Gutiérrez 2008; Palombo et al. 2013).

Regardless of the main drivers of treeline responses, if the uppermost limits of subalpine forests ascend, adjacent alpine communities should undergo changes. An overall assumption is that treeline advancement and the increase of tree density should trigger diversity losses and ecosystem degradation in adjacent grasslands and in the field layer of the ecotone, respectively (Dirnböck et al. 2003; Walther 2003; Tinner and Kaltenrieder 2005; Illerbrun et al. 2011). An increase of tree cover would modify light availability, moisture regime, soil properties and resource availability (Holtemeier and Broll 2005), creating forest-like microclimatic conditions in the treeline ecotone. Vegetation composition would therefore shift towards a greater dominance of shade-tolerant species, and the original shade-intolerant alpine flora at the treeline ecotone would be gradually replaced by taxa of the lower subalpine forest (Hofgaard and Wilmann 2002; Grytnes 2003; Sundqvist et al. 2008). However, the consequences of canopy closure are often based on studies of forest succession (e.g. Dahlgren et al. 2006; Laughlin et al. 2011), rather than on direct evidences from treeline ecotones (but see Illerbrun et al. 2011).

In particular, despite the large amount of literature dealing with treeline dynamics, little attention has been paid to the real consequences of treeline ascension on field layer plant diversity (but see Camarero and Gutiérrez 2002; Hofgaard and Wilmann 2002; Camarero et al. 2006; Sundqvist et al. 2008; Batllori et al. 2009a). The scarce available information is from interpretations of chronosequences that use a space-for-time approach for assessing temporal dynamics, which has recently been criticised (Johnson and Miyanishi 2008). Therefore, empirical data are required for a reliable evaluation of the consequences of the treeline dynamics on the alpine flora. Here, we provide a detailed description of the dynamics of tree cover and associated field layer vegetation in treeline ecotones of *Pinus uncinata* in two national parks in the Spanish Central Pyrenees between 1998 and

72 in 2009. The aim of this study was to assess the short-term influence of tree cover dynamics on field
73 layer vegetation in the treeline ecotones. Specifically, we addressed the following questions: (i) did
74 tree cover in two undisturbed treeline ecotones increase between 1998 and 2009? (ii) did field layer
75 plant diversity and composition change significantly? and if so, (iii) were changes in tree and field
76 vegetation layers coupled in space?

77 *Material and methods*

78 *Study area*

79 Here we defined the alpine treeline ecotone as the transition zone between the closed
80 subalpine forest (hereafter forest limit) to the uppermost scattered and stunted trees (following
81 Holtmeier 2003). The two examined treeline ecotones were located in two protected areas in the
82 Spanish Central Pyrenees. The Ordesa site (hereafter abbreviated as O) was located in the buffer
83 zone of Ordesa-Monte Perdido National Park. The Tessó site (hereafter abbreviated as T) was located
84 within the buffer zone of Aigüestortes-Estany de Saint Maurici National Park. On the basis of available
85 historical data and comparison of aerial photographs taken in 1946 and 1988, these ecotones have
86 been considered as undisturbed during the past century (Camarero and Gutiérrez 2002, 2004;
87 Camarero et al. 2006). We have visually examined orthophotos taken in 1998 and 2009 to confirm that
88 tree dynamics was not subjected to wildfires, outbreaks, snow avalanches or logging during this
89 period. Repeated historical photographs also evidence reduced or null altitudinal ascent of both
90 treelines during the twentieth century (Camarero et al. 2000; Camarero and Gutiérrez 2004).

91 Sites O and T were selected to represent the main contrasting structures of *Pinus uncinata*
92 alpine treeline ecotones (Camarero and Gutiérrez 2002). Site O was defined as a krummholz abrupt
93 treeline, where the size and growth form of *P. uncinata* individuals changed abruptly upslope along the
94 ecotone, from tall trees near the forest limit to a dense belt of krummholz individuals near the treeline

(Camarero and Gutiérrez 1999) (Figure 1). In contrast, the density and height of *P. uncinata* individuals decreased gradually with increasing altitude at site T (Camarero and Gutiérrez 1999), which can be characterized as a diffuse treeline ecotone (*sensu* Harsch et al. 2009). The two studied sites also differed in terms of climate, geology, and vegetation composition. Site O (47° 37' N, 0° 02' W, 2110 m a.s.l.) was located on a south-facing gentle slope (5-10°), under continental and Mediterranean influence (1660 mm annual precipitation and 5 °C mean temperature), with a yearly maximum snowpack of 1.0-2.0 m (Góriz Meteorological Station, 42° 39' N, 00° 01' E, 2215 m a.s.l.; 1981-1989 and 1992-1995 data [Camarero and Gutiérrez 2002]). The bedrock type was mainly limestone, although it contained many sandstone patches (Camarero and Gutiérrez 2002). Rock outcrops were abundant in the uppermost part. Field layer was dominated by hemicryptophytes such as *Calluna vulgaris*, *Carex sempervirens* and *Festuca rubra*. Site T (42° 28' N, 01° 03' E, 2330 m) was located on a north-east-facing steep slope (20-30°), under continental local climate (ca. 1600 mm of annual precipitation; 3 °C of mean temperature), and with a yearly maximum snowpack of 1.5-3.0 m (Esterri Meteorological Station, 42° 37' N, 01° 07' E, 1054 m a.s.l., 1970-1997 data [Camarero and Gutiérrez 2002]). The bedrock was shale, and the soil contained abundant organic matter (Camarero and Gutiérrez 2002). In this site, *Rhododendron ferrugineum* and *Vaccinium myrtillus* dwarf shrubs were dominant near the forest limit, whereas patchily distributed species such as *Dryas octopetala* were more abundant in gaps near the treeline.

The mean temperature has increased at a rate of +0.3 °C per decade on average between 1950 and 2006 in the Pyrenees (López Moreno et al. 2010), causing a decrease of snow depth in the Pyrenees (López-Moreno 2005). The same increasing rate of temperature has been recorded during the last decade in a nearby Meteorological Station to O site (R. García-González, unpublished data). This rate constitutes an accelerated warming in comparison to historical available temperature records before 1950 (Bücher and Dessens 1991). On the other hand, grazing activities have diminished along

the central Pyrenees during the twentieth century, due to the phasing out of transhumance and the depopulation of rural areas (García-Ruiz et al. 1996; Lasanta-Martínez et al. 2005). For instance, the estimated number of sheep in the Ordesa-Monte Perdido National Park was reduced by 75% since 1960 to recent decades (Komac 2010). The mean stocking rate in 1990s was below 1.5 and 1 LAU (1 Large Animal Unit = 8 sheep or 1 cow) per month ha⁻¹ in the Ordesa-Monte Perdido and Aigüestortes-Estany de Saint Maurici National Parks, respectively (Aldezábal et al. 1992; Bas et al. 1994). However, we assumed that grazing pressure was much lower at O and T site, based on their distant location away from the main summer pastures and livestock itineraries. Only a few goats were observed in the surroundings of O site (Aldezábal et al. 1992), being their impact on *P. uncinata* individuals negligible (Camarero and Gutiérrez 1999).

Sampling methods

In summer 1998, Camarero and Gutiérrez (2002) laid 6 line transects (140 m long each) along the maximum slope from the alpine grassland to the closed subalpine forest at both study sites. Transects were separated from each other by 6 m (Figure 1), and their position was permanently marked by using anchors, metal sticks and milestones. The abundance of *P. uncinata* and vascular plant taxa was estimated by using the point-intercept method (Barbour et al. 1987). A metal rod (diameter: 2 mm) was placed every 1 m along each transect, and all taxa contacted by the rod and its vertical projection were recorded (Camarero and Gutiérrez 2002). In 2009 transects were relocated and the tree and field layer vegetation was sampled in the same way as 11 years earlier (see Camarero and Gutiérrez 2002). As we were only interested on the dynamics of the treeline ecotone, we shortened transect length to the distance between the treeline and forest limit (i.e. the uppermost 80 and 100 m at sites O and T, respectively), to exclude the sampling points located within the forest (see Figure 1). More than 600 and 1000 plant records were gathered each year at O (486 sampling

points) and T sites (606 sampling points), respectively. The botanical nomenclature of field layer species follows de Bolòs et al. (2005).

Statistical analyses

Both tree layer and field layer vegetation changes over time were first examined by considering each transect as a statistical unit after confirming the lack of spatial autocorrelation in preliminary analyses (Mantel test; $R = 0.41$ and 0.25 for Ordesa and Tessó sites, respectively; $P > 0.05$). Additionally, we explored temporal changes along the altitudinal gradient by splitting transects into 10 m long altitudinal segments (see Figure 1).

Temporal changes in tree cover

We estimated the cover of *P. uncinata* individuals (hereafter tree cover) per transect ($n = 6$) by dividing the number of hits by the total number of sampling points in each survey (1998 and 2009). According to our previous knowledge of the treeline ecotones, we would expect an increase of tree cover rather than a decrease over 11 years. Therefore, we tested only for the increase of tree cover both at each treeline ecotone and along altitudinal segments using one-tailed Wilcoxon tests.

To assess whether forest limit significantly shifted upwards over the 11-year study period, we tested whether the tree cover values along the altitudinal gradient in 2009 were the same as those observed below the forest limit in 1998. Different subjective criteria have been used to determine a threshold that separates the treeline ecotone from the forest below (e.g. Jobbagy and Jackson 2000; Holtmeier 2003). To overcome such limitations, we computed a site specific threshold value on the basis of the tree cover values recorded below each forest limit in 1998 (Camarero et al. 2006) (Figure 1), and tested if the forest limit had shifted by using a randomisation procedure proposed by Harper and MacDonald (2001). First, we randomly selected one altitudinal segment below the forest limit

(segments 12 to 14 in 1998, see Figure 1) in each transect and calculated the mean tree cover ($n = 6$) in the forest limit at each treeline ecotone. These steps were repeated 10,000 times for each ecotone, and the 2.5th percentile (two-tailed test, $\alpha = 0.5$) of the resulting distribution of mean cover values was selected as the threshold value. Finally, the tree cover values observed in 2009 were compared to such threshold values. We considered that the forest limit had shifted upwards when a set of consecutive segments of 2009 presented tree cover values that did not significantly differ ($P > 0.05$) from those observed in the subalpine forest in 1998.

Temporal changes in plant diversity

Changes in plant diversity were analysed by considering the variation of species richness and evenness. We compared species richness between 1998 and 2009 by using sample-based rarefaction curves with 95% confidence intervals (Gotelli and Colwell 2001; Colwell et al. 2004). Both curves and confidence intervals were calculated by using the EstimateS software (Colwell 2005), and following the analytical formulas detailed in Mao et al. (2005) (see also Colwell et al. 2004). The differences between sample-based rarefaction curves could be due to differences in species richness, but also to differences in the number of individuals recorded (Gotelli and Colwell 2001; Colwell et al. 2004). As we were only interested in the former and given that we recorded a different number of plant individuals in 1998 and 2009, we re-scaled the rarefaction curve to the number of plant individuals (Gotelli and Colwell 2001). Once rarefaction curves were plotted, significant differences ($P < 0.05$) between 1998 and 2009 were established when confidence intervals did not overlap (Colwell et al. 2004). Additionally, we tested the changes of species richness between years at each altitudinal segments by using a two-tailed Wilcoxon test.

As an estimation of evenness we chose the Simpson index (1-D) (Legendre 1998) which is preferable over other indices because it stabilises at small sample sizes (Magurran 2004). Differences of evenness between surveys were tested by using a two-tailed *t*-test with the Welch correction for

groups with unequal variances (onwards Welch test), once normal distribution of the data was confirmed. We applied the same procedure for testing the changes in evenness along the altitudinal gradient.

Temporal changes in vegetation composition

We tested the change in the composition and abundance of field layer species (henceforth vegetation composition) in the treeline ecotone by using a non-parametric multivariate analysis of variance based on distance matrices (Anderson 2001; McArdle and Anderson 2001). This multivariate analysis was done by using the *adonis* function (Oksanen et al. 2010) in R (version 2.10.1; 2009), after excluding all unidentified species (see Appendix S1). This test is analogous to a MANOVA (Anderson 2001) and compares the variability in composition dissimilarity among transects from the same observation year to the variability in composition dissimilarity among transects from 1998 and 2009. We first calculated the dissimilarity among transects within and between observation years (the distance matrix), based on the Chao distance. This distance minimizes the contribution of rare species (defined as those with one or two hits in only one of the sampling years) to the estimated dissimilarity (Chao et al. 2005, Chao et al. 2006), even so, the vegetation turnover might still be biased by our sampling limitation to track the changes of species occurring at low densities. To further exploration of this, we additionally calculated distance matrices by excluding less abundant species (defined as those species with abundance values lower than the observed third quartile; see further details on Appendix S2). Next, the pseudo *F*-ratio statistic was computed for each distance matrix. A large value of this test statistic means that transects sampled in 1998 and 2009 differ in vegetation composition. The significance of this statistic was tested by using a permutation test ($n = 10,000$) carried out under the true null hypothesis of no difference between observation years. Each permutation transect was reassigned to one of the two possible observation years at random, and a new pseudo *F*-ratio was calculated. Finally, the significance of the test ($\alpha = 0.05$) was obtained by comparing the pseudo *F*-

ratios obtained under permutation and those pseudo F -ratios corresponding to collected data (Anderson 2001; McArdle and Anderson 2001). As in similar analyses of variance, the *adonis* test is sensitive to data heterocedasticity (Anderson 2001; 2006). To confirm that significant differences between years were not caused by data heterocedasticity across sampling years, we carried out a permutation test (10,000 randomisations), based on a multivariate dispersion analysis (Anderson 2006) by using the *permutest.betadisper* function (Oksanen et al. 2010).

The temporal turnover of vegetation was examined along the altitudinal gradient by using a dissimilarity index. We merged species abundances recorded at each altitudinal segment and computed the Chao index by using the *vegdist* function (Oksanen et al. 2010). High values indicate large changes in the composition of the assemblage between years. We also re-calculated Chao index by excluding the less abundant species (see Appendix S2) to determine whether the turnover pattern was affected by sampling limitations (i.e. insufficient capture of species occurring at low densities). Finally, temporal changes in the abundance of each particular taxon were tested by using a χ^2 test.

Results

Between 1998 and 2009, tree cover significantly increased from 20% to 35% at site O (one-tailed Wilcoxon test; $W = 4$, $P = 0.013$). Near the forest limit, the tree cover in 2009 was not statistically different from the threshold values (randomisation test; $P > 0.05$), indicating that tree cover in these altitudinal segments of the treeline ecotone was similar to that observed within the subalpine forest (Figure 2). We also detected significant increases of tree cover in the third and fifth altitudinal segments (one-tailed Wilcoxon test, W -statistic ranges from 5.5 to 7, $P < 0.05$; Figure 2). The tree cover at T site was below 21% in 2009, and unlike at O, statistically the same as 11 years earlier (one-

235 tailed Wilcoxon test; $W = 7.5$, $P = 0.054$). Tree cover did not significantly increase in any of the
236 altitudinal segments either ($P > 0.05$ in all segments; Figure 2).

237 Regarding field layer vegetation, plant richness did not significantly differ between 1998 and
238 2009 at site O (Figure 3), but evenness significantly increased from 0.82 to 0.86 through time (two-
239 tailed Welch test; $t = 3.60$, d.f. = 8.18, $P = 0.005$). At site T, plant richness increased significantly from
240 23 to 36 recorded taxa ($P < 0.05$; Figure 3), whereas evenness was the same as in 1998 (0.78; two-
241 tailed Welch test; $t = 0.63$, d.f. = 9.8, $P = 0.54$). Neither richness nor evenness changed significantly
242 (two-tailed Wilcoxon test and two-tailed Welch test, respectively, $P > 0.05$) along the altitudinal
243 gradient at the T site (Appendix S3).

244 Vegetation composition changed significantly between 1998 and 2009 at both sites (O *adonis*;
245 $F = 9.03$, $R^2 = 0.44$, $P = 0.003$; T *adonis*; $F = 17.01$, $R^2 = 0.63$, $P < 0.001$). None of these results were
246 biased due to data heterocedasticity (*permutest.betadisper*; $F = 1.31$, $P = 0.283$ in O and $F = 0.53$, $P =$
247 0.487 in T). These shifts in composition were mostly driven by the species with cover less than 5% at
248 site O (Figure 4). In particular, we only found significant increases (χ^2 test; $P < 0.05$) in the abundance
249 of alpine grasses such as *Nardus stricta*, *Danthonia decumbens* and *Agrostis capillaris* at this treeline
250 ecotone (Figure 5), whereas the new detected species at T site contributed the most to the observed
251 change (Figure 4). However, when we repeated analyses excluding less abundant species ($n = 14$ and
252 12 , O and T sites respectively), we also found a significant shift in vegetation composition between
253 years (O *adonis*; $F = 15.18$, $R^2 = 0.60$, $P = 0.002$; T *adonis*; $F = 36.05$, $R^2 = 0.78$, $P < 0.001$). Four
254 species accounted for more than two-thirds of the hits both in 1998 and 2009 in the field layer at O:
255 *Calluna vulgaris*, *Festuca rubra*, *Carex sempervirens* and *Hippocrepis comosa*, and only the
256 abundance of the latter shifted significantly between 1998 and 2009 (Figure 5). At site T
257 *Rhododendron ferrugineum*, *Vaccinium myrtillus*, *Dryas octopetala*, *Festuca gautieri* and *Carex*

sempervirens occupied the 90% of the cover hits in 1998 and 89% in 2009, and only the abundance of two of the species changed significantly (Figure 5).

The highest values of vegetation turnover were patchily distributed along the altitudinal gradient at site O, whereas at site T, the highest turnover was restricted to the upper half altitudinal segments (Figure 2). The same altitudinal pattern, though with lower turnover values, was found in both sites when the less abundant species were excluded, except for the uppermost segment in T (see Appendix S2). At both treeline ecotones, the patterns of highest vegetation turnover and significant increases of tree cover were spatially uncoupled along the altitudinal gradient (Figure 2).

Discussion

We found that changes in *Pinus uncinata* canopy cover and field layer plant communities were spatially uncoupled in Pyrenean treeline ecotones at a decadal scale. Even if the upwards shift of the forest limit at site O might have drastically modified field layer microsite conditions (e.g. by reducing the light reaching the field layer) (Smith et al. 2003), the vegetation turnover was not higher there than in other part of the treeline ecotone. Moreover, contrary to overall expectation, the species involved in the turnover were not shade-adapted, but they belong to grasslands. Taken together, these results suggest that canopy closure did not trigger short-term changes in the composition of the field layer vegetation in the treeline ecotone.

The main driver of vegetation dynamics is unclear after removing the effect of the tree cover. Unlike some subarctic forest tundra ecotones (Pajunen et al. 2012), the shift in field layer vegetation cannot be attributed to denser shrub canopies, because none of the shrubs species (*Calluna vulgaris*, *Rhododendron ferrugineum* and *Vaccinium myrtillus*) enhanced their cover values over the 11 years. At O site, *Nardus stricta*, *Agrostis capillaris* and *Danthonia decumbens* significantly increased at the

281 expense of other grasses and non-dominant forbs between 1998 and 2009. These species are
282 abundant on acid grassland in the Pyrenees (Fillat et al. 2008), thereby their increase in abundance
283 may be linked to the past decline in grazing intensity. Indeed, increasing abundance of *N. stricta* and
284 *A. capillaris* has also been observed after two decades of grazing exclusion in nearby grasslands (D.
285 Gómez and R. García-González, pers. comm.). However, climate-mediated responses cannot be
286 discarded either, because such graminoids might have responded rapidly to the rising temperatures
287 due to their modular organisation and high tissue turnover (Shaver et al. 1997). Whatever the driver
288 was, the higher values of vegetation turnover was associated to the distribution of graminoids,
289 whereas the lower turnover would correspond to the sites where biotic conditions were little altered
290 such as rock outcrops or locations where shrubs and tussock forming sedges (*Carex sempervirens*)
291 were already established in 1998 (Camarero and Gutiérrez 2002). By contrast, the higher vegetation
292 turnover was found on the upper half of the treeline ecotone at site T. In the uppermost altitudinal
293 segment, we found a discordance between the turnover based on all species and that based on a
294 subset of most abundant ones (Appendix S2), which indicates a higher contribution of new detected
295 species to vegetation turnover there. The fact that higher turnover values matched the distribution of
296 the dwarf shrub that changed the most, i.e. *D. octopetala* (Camarero and Gutiérrez 2002), led us to
297 suggest that a competition ease (i.e. recession of *D. octopetala*) allowed the increase in abundance of
298 less competitive species (*Plantago monosperma*, *Primula elatior* subsp. *intricata*, *Soldanella alpina*)
299 near the treeline (Choler et al. 2001, Virtanen et al. 2010). We cannot directly infer causality from our
300 descriptive study, however, the low grazing since 1990s, and the resemblance of the *D. octopetala*
301 response to experimental warming (Klanderud and Totland 2005) may suggest a major role of raising
302 temperature on the reduction of this dwarf shrub. On the other hand, the microclimatic conditions
303 imposed under the dense layer of ericoid shrubs (*R. ferrugineum* and *V. myrtillus*) could explain the
304 lack of vegetation change in the lower half of the altitudinal segments at T site.

It is important to note that the reported changes in vegetation composition and species richness could also reflect stochastic processes that cannot be detected in our study, such as year-to-year fluctuations in the abundance of each species (Magurran et al. 2010). However, given that all recorded species were long-lived that buffer stochastic fluctuations to a great extent (García et al. 2008) the contribution of temporal variability to the observed vegetation changes may be regarded as minor. Another important caveat in our study was the number of sampling points per transect established in 1998 (Camarero & Gutiérrez 2002), which could be insufficient to capture species occurring at very low densities. To overcome such potential bias, we reduced the weight of species with lower cover on vegetation analysis by using the dissimilarity distance that yielded the most conservative results (see Material and methods). More importantly, we demonstrated that the observed results were robust to the removal of species with lower cover from analyses (except the aforementioned case in the uppermost altitudinal segment at T site; see Appendix S2). Finally, the detection of species not recorded in 1998 should be interpreted as an increase of their abundance in the treeline ecotone, rather than as a migration from adjacent belts. Taken together, the results indicate moderate changes in field layer plant diversity, supporting previous conclusions raised from high-latitude treelines (Virtanen et al. 2010).

Regarding the dynamics of *P. uncinata*, we only found a change in tree cover at O site. This result deviates from general predictions of lower sensitivity to raising temperature of abrupt treeline ecotones as compared with diffuse ones, which are less subjected to additional constraints (e.g. wind, snow) (Harsch et al. 2009; 2011). It must be noted that tree pine recruitment and growth were limited by wind stress at the O site (Camarero et al. 2000). Therefore, potential wind effects (winter desiccation, summer cooling, needle abrasion by snow particles or mechanical damage to exposed stems [Scott et al. 1993; Smith et al. 2003; Holtmeier and Broll 2010]) should be lessened. In this context, a positive interaction between *P. uncinata* adults (Batllori et al. 2009b) may explain the

upwards shift of the forest limit, whereas in the krummholz band (see Figure 1), the increased tree cover may be associated to more favourable microsite conditions under krummholz individuals (Germino et al. 2002; Smith et al. 2009, Holtmeier and Broll 2010), some field layer species (e.g. *Calluna vulgaris*) (Bartolomé et al. 2008), or/and the shelter of surface geomorphologic features (Resler 2006). By contrast, the dense patches of *R. ferrugineum* might have played the opposing role, preventing the recruitment of *P. uncinata* at site T (Pornon and Doche 1996; Ninot et al. 2008; Batllori et al. 2009b).

Conclusions

Our study provides support for a weak influence of tree cover on field layer vegetation at the alpine treeline ecotone in the short-term. Moreover, plant diversity and vegetation composition could have changed regardless of the dynamics of tree cover: grazing abandonment, climate change and microsite variability could shape field layer vegetation more than canopy closure. So far, this local uncoupling between tree and field layer vegetation has not been evidenced because similar datasets are rarely available. Thus, there is still little basis for robust generalisations based on comparative approaches. Further long-term monitoring studies that include field layer vegetation are required for assessing the full impact of ongoing global change on treeline dynamics and related plant diversity. Meanwhile, alpine biodiversity losses inferred from rough dynamics of treeline ecotones at large or regional spatial scales should be cautiously interpreted.

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517

518 **Notes on contributors:** MBG and JJC designed the study. IP, JJC and EG carried out vegetation
519 sampling. IP performed all analyses. IP and MBG wrote the manuscript with contributions from all
520 authors.

521 Iker Pardo investigates the spatial distribution and temporal dynamics of plant diversity in mountain
522 areas. Major research question addresses the consequences of climate and land-use change on
523 alpine plant communities.

524 J. Julio Camarero is a forest and plant ecologist interested on growth and regeneration patterns in
525 harsh environments such as the alpine treeline.

526 Emilia Gutiérrez is an ecologist carrying out research on phenology of Mediterranean woody species.
527 She also retrieves information from tree-ring data to study ecological process, e.g. climate - tree
528 growth relationships, forest disturbances regime, and the pace and pattern of long-term complex
529 ecological processes such as succession (stand dynamics) and treeline shifts.

530 Maria B. García's research interests include plant population dynamics; plant-animal interactions;
531 assembling rules of plant communities; and diversity responses to climate and land-use change at
532 species, population and community level.

533 Figure 1. Schematic representation of the sampling design and tree layer structure in the Ordesa and
534 Tessó treeline ecotones. *Pinus uncinata* individuals were located and their height was measured in
535 1995 (modified from Camarero and Gutiérrez, 1999). Vegetation transects were set from the treeline
536 to the upper part of the subalpine in 1998, whereas in 2009 the study was restricted to the treeline
537 ecotone (TLE), excluding the forest limit (FL). The position of FL was established according to
538 Camarero et al. (2006).

539 Figure 2. *Pinus uncinata* cover in 1998 (empty circles) and 2009 (filled circles) (means and standard
540 errors), and vegetation turnover estimated as Chao index (grey line), at the Ordesa and Tessó treeline
541 ecotones. Altitudinal segments are numbered from the treeline to the forest limit. Asterisks indicate
542 significant (one-tailed Wilcoxon test, $P < 0.05$) increase of tree cover after 11 years. The dotted line
543 corresponds to the threshold value of tree cover that separates the treeline ecotone from the forest
544 limit.

545 Figure 3. Individual-based rarefaction curves of the Mao-Tau expected species richness in 1998
546 (empty circles) and 2009 (filled circles) in Ordesa and Tessó treeline ecotones. Bars correspond to
547 95% confidence intervals.

548 Figure 4. Non-metric multidimensional scaling ordination of field layer vegetation composition in the
549 Ordesa and Tessó treeline ecotones. Convex hulls joint vegetation samples from 1998 (solid lines)
550 and 2009 (dashed line). Circles indicate the position of the less abundant (grey circles) and most
551 abundant (black circles) plant species in the multivariate space. Note that most distant species in the
552 ordination axes load more the position of samples. The size of the circles represents their absolute
553 change in abundance from 1998 to 2009. For clarity only the names of most abundant species are
554 shown (see Appendix S1 for species' code).

555 Figure 5. Changes in the cover of dominant (on the left) and non-dominant species (on the right) at
556 Ordesa and Tessó treeline ecotones between 1998 and 2009. Asterisks indicate significant temporal
557 changes in abundance (χ^2 test, $P < 0.05$, $n = 6$ transects). Grey negative and positive symbols
558 indicate species only recorded in 1998 or 2009, respectively. Codes for species are in Appendix S1.

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560